Brief Communication

MOLECULAR METABOLISM

The role of kidney in the inter-organ coordination of endogenous glucose production during fasting

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ABSTRACT

Objective: The respective contributions to endogenous glucose production (EGP) of the liver, kidney and intestine vary during fasting. We previously reported that the deficiency in either hepatic or intestinal gluconeogenesis modulates the repartition of EGP via glucagon secretion (humoral factor) and gut—brain—liver axis (neural factor), respectively. Considering renal gluconeogenesis reportedly accounted for approximately 50% of EGP during fasting, we examined whether a reduction in renal gluconeogenesis could promote alterations in the repartition of EGP in this situation.

Methods: We studied mice whose glucose-6-phosphatase (G6Pase) catalytic subunit (G6PC) is specifically knocked down in the kidneys (K-G6pc$^{-/-}$ mice) during fasting. We also examined the additional effects of intestinal *G6pc* deletion, renal denervation and vitamin D administration on the altered glucose metabolism in K-G6pc$^{-/-}$ mice.

Results: Compared with WT mice, K-G6pc$^{-/-}$ mice exhibited (1) lower glycemia, (2) enhanced intestinal but not hepatic G6Pase activity, (3) enhanced hepatic glucokinase (GK encoded by *Gck*) activity, (4) increased hepatic glucose-6-phosphate and (5) hepatic glycogen spared from exhaustion during fasting. Increased hepatic *Gck* expression in the post-absorptive state could be dependent on the enhancement of insulin signal (AKT phosphorylation) in K-G6pc$^{-/-}$ mice. In contrast, the increase in hepatic GK activity was not observed in mice with both kidney- and intestine-knockout (KI-G6pc$^{-/-}$ mice). Hepatic *Gck* gene expression and hepatic AKT phosphorylation were reduced in KI-G6pc$^{-/-}$ mice. Renal denervation by capsaicin did not induce any effect on glucose metabolism in K-G6pc$^{-/-}$ mice. Plasma level of 1,25 $(OH)_2$ D_3, an active form of vitamin D, was decreased in K-G6pc$^{-/-}$ mice. Interestingly, the administration of 1,25 $(OH)_2$ D_3 prevented the enhancement of intestinal gluconeogenesis and hepatic GK activity and blocked the accumulation of hepatic glycogen otherwise observed in K-G6pc$^{-/-}$ mice during fasting.

Conclusions: A diminution in renal gluconeogenesis that is accompanied by a decrease in blood vitamin D promotes a novel repartition of EGP among glucose producing organs during fasting, featured by increased intestinal gluconeogenesis that leads to sparing glycogen stores in the liver. Our data suggest a possible involvement of a crosstalk between the kidneys and intestine (via the vitamin D system) and the intestine and liver (via a neural gut-brain axis), which might take place in the situations of deficient renal glucose production, such as chronic kidney disease.

Keywords Endogenous glucose production; Gluconeogenesis; Hypoglycemia; Glycogen; Chronic kidney disease; Vitamin D

1. INTRODUCTION

Endogenous glucose production (EGP), encompassing glycogenolysis and gluconeogenesis, is a fundamental physiological function to keep blood glucose level constant during the periods of food deprivation. Once glycogen is exhausted in the early phase of fasting, gluconeogenesis plays a central role in glucose supply. Only three organs, liver, kidney and intestine, perform gluconeogenesis since they have a specific enzyme for that, glucose-6-phosphatase (G6Pase). G6Pase dephosphorylates glucose 6-phosphate (G6P): a reaction that releases glucose and inorganic phosphate. Altered repartition of EGP among gluconeogenic organs takes place in various nutritional situations, such as during long-term fasting and under protein-enriched diet [1]. For example, the liver accounts for 70—75% of EGP in post-absorptive state, while renal gluconeogenesis contributes to up to 50% of EGP after 24 h of fasting in rats [2]. Consistent with the animal studies, results in humans showed that about 50% of EGP are contributed by the kidney after long-term fasting [3,4]. Intestinal glucose production, making a small contribution to EGP at the fed to fasted transition, accounts to about 20%—25% of EGP after 24 h of fasting in the rat [5,6]. These reports suggest that gluconeogenesis from the kidney and intestine is increasingly important during fasting. While hepatic gluconeogenesis from lactate and alanine is an endergonic process that consumes energy, renal and intestinal gluconeogenesis, utilizing

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glutamine as the main substrate, is exergonic and produces 4 ATP per mole of synthetized glucose [7]. This repartition of EGP allows the body to first maintain plasma glucose constant and simultaneously preserve the energetic status of the body for anabolic purposes [1]. The difference in glucose precursors according to gluconeogenic organs would also imply that the regulatory mechanisms of glucose production are not the same in the liver and/or kidney/intestine. Moreover, we previously found that the deficiency in hepatic glucose production induces upregulation of both renal and intestinal gluconeogenesis [8], while upregulation of intestinal gluconeogenesis decreases hepatic glucose production via a neural gut—brain—liver axis [9,10]. These findings indicate the existence of inter-organ coordination of EGP to maintain glucose and energy homeostasis.

Increasing attention has been paid to the role of kidney in glucose homeostasis [11]. Disordered glucose metabolism is widely recognized in chronic kidney disease (CKD) patients. Fasting hypoglycemia, more common in CKD patients than in the general population [12], is generally attributed to diminished renal gluconeogenesis [13,14]. It is not clear whether the repartition of EGP takes place and even participates in glycemic reduction in CKD with reduced renal gluconeogenesis. A lack of suitable animal model delayed the identification of factors responsible for pathophysiological complexity in CKD. Nephrectomy, often performed in CKD models, not only reduces renal glucose output [15] but surgically impairs almost all other kidney functions. An investigation into more selective pathophysiology caused by reduced renal gluconeogenesis could yield novel insights into the kidney's roles in whole body metabolism. To this end, we generated kidney-specific *G6pc* knockdown mice (K-G6pc$^{-/-}$ mice) in which renal G6Pase activity is reduced [16]. We examined the effect of this reduction in renal G6Pase activity on glucose metabolism during fasting in K-G6pc$^{-/-}$ mice.

In addition to renal gluconeogenesis, kidney-derived neural and/or humoral factors play essential roles in the whole body metabolism [17,18]. For example, renal nerve activity and vitamin D deficiency were previously involved in whole body glucose homeostasis [19,20]. We thus tested the possible involvement of kidney-derived factors, such as renal neural signaling or vitamin D, on the repartition of EGP in K-G6pc$^{-/-}$ mice.

We here report a previously unsuspected kidney's role in the repartition of EGP, which has the capacity to affect glucose metabolism.

2. MATERIAL AND METHODS

2.1. Animals

Male adult (6—8 weeks old) B6.G6pc$^{lox/lox}$.Kap$^{creERT2/w}$, B6.G6pc$^{lox/lox}$.Kap$^{CreERT2/w}$ .Vill$^{CreERT2/w}$ and C57Bl/6 J (Charles Rivers Laboratories, L'Arbresle, France) were intraperitoneally injected once daily with 1 mg of tamoxifen for 5 consecutive days, to obtain K-G6pc$^{-/-}$ mice, KI- G6pc$^{-/-}$ mice, wild-type (WT) mice, respectively. At the time of experiments, mice were 9—11 weeks of age. 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 and standard chow. All procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals (2010/63/EU). The regional animal care committee (CEEA-55, Université Lyon I) approved all experiments.

2.2. Quantitative RT-PCR

Total RNA was extracted from frozen tissues using Trizol reagent (Invitrogen), according to the manufacturer's instructions. Reverse transcription was done on 1 μg of mRNA using the Qiagen QuantiTect Reverse Transcription kit. SsoAdvanced™ Universal SYBR® Green Supermix (BioRad) was used to determine mRNA levels. Ribosomal protein L19 (RPL19) was used as a housekeeping gene. Calculations were made based on the comparative cycle threshold method ($2^{-\Delta\Delta Ct}$). Primer sequences are listed in Supplementary Table 1.

2.3. Western blot analysis

Tissues were rapidly sampled in liquid nitrogen and stored at −80 °C before analysis. Tissues were homogenized in lysis buffer (50 mM Tris pH 7.5, 5 mM MgCl2, 100 mM KCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 1% NP40, protease and phosphatase inhibitors) by FastPrep® system.

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 pAKT Ser473 or AKT (dilution 1:2000, Cell Signaling) and then with goat secondary anti-rabbit IgG linked to peroxidase (dilution 1:10000, BioRad). Membranes were exposed to ClarityTM Western ECL Substrate (BioRad). The visualization and quantification of proteins were performed using the BioRad ChemiDoc™ Touch Imaging system.

2.4. Metabolic studies

Body weight and blood glucose were measured before or 6 h, 10 h, 24 h, 30 h after the beginning of fasting. Blood glucose was measured with an Accu-Check Go glucometer (Roche Diagnostics, Meylan, France). Plasma samples were withdrawn by submandibular bleeding using a lancet and collected into the tube with EDTA after 6 h and 30 h of fasting, just before killing the animal. For the study of renal capsaicin treatment and vitamin D administration, mice were sacrificed at 30 h of fasting. Insulin (Mercodia), glucagon (Alpco Diagnostics), corticosterone (Arbor Assays), adrenaline and 1,25-dihydroxyvitamin D3 (1,25 $(OH)_2$ D_3) (LDN Labor Diagnostika Nord GmbH & Co.KG) were determined with mouse ELISA kits. Beta hydroxybutyrate and non-esterified fatty acids (NEFA) concentrations in plasma were assessed with an Abcam colorimetric kit and a Diasys colorimetric kit, respectively.

Hepatic glycogen and G6P determinations were carried out as previously described [21].

G6Pase activity was directly assayed in the homogenates for 10 min at 30 °C at pH 7.3 in the presence of a saturating G6P concentration (20 mmol/L). The inorganic phosphate release was determined by complexometry [22].

GK activity was measured by using a spectrophotometric method with some modifications, as previously described [23]. Briefly, enzyme activity was assayed at 37 °C using 200 mM Hepes-NaOH, 100 mM KCl, 5 mM MgCl2, 1 mM NADP+, 5 mM ATP, 1 mM DTT, 0.5 g/L BSA, pH 7.6, and excess glucose 6-phosphate dehydrogenase. GK activity in crude homogenates was estimated subtracting the rate of NADPH formation (at 340 nm) in the presence of 1 mM glucose (scoring low-Km HK activity) from that obtained in the presence of 40 mM glucose (scoring total HK activity).

2.5. Renal capsaicin treatment

WT mice and K-G6pc$^{-/-}$ mice were assigned to sham-treated (Sham) or capsaicin-treated (Cap) groups: WT-Sham, WT-Cap, K-G6pc$^{-/-}$-Sham, K-G6pc$^{-/-}$-Cap. Ten days after the tamoxifen injection, sham- or capsaicin-treatments were performed as previously described in rats [24] with some modifications. Treated mice were allowed to recover for 10 days and examined during fasting. Briefly, Cap mice were

