Stimulatory effect of glucokinase activation on hepatic glycogen turnover as measured by 13C-MRS

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Introduction

Glucokinase (GK), a rate controlling enzyme present in both the liver and the pancreas, acts as a glucose sensor by facilitating the phosphorylation of glucose to glucose-6-phosphate (G6P). In response to rising levels of plasma glucose, GK activation triggers insulin secretion by the pancreatic β-cells and causes a shift in glucose metabolism towards storage and utilization in the liver. Given this knowledge, a drug that stimulates GK activity would present a valid therapeutic option for the treatment of type 2 diabetes. Compound A, a glucokinase activator (GKA) with antihyperglycemic properties, has been shown to have a stimulatory effect on GK activity *in vitro*. The goal of the present study was to measure *in vivo* that compound A stimulates glucose uptake via hepatic GK activation. Acute effects of compound A were tested in diet-induced obese rats continuously infused with glucose for 3 hours to mimic postprandial conditions. ^{13}C magnetic resonance spectroscopy (^{13}C-MRS) was applied to measure kinetic changes in hepatic G6P and glycogen contents as markers of glucose uptake and storage by the liver.

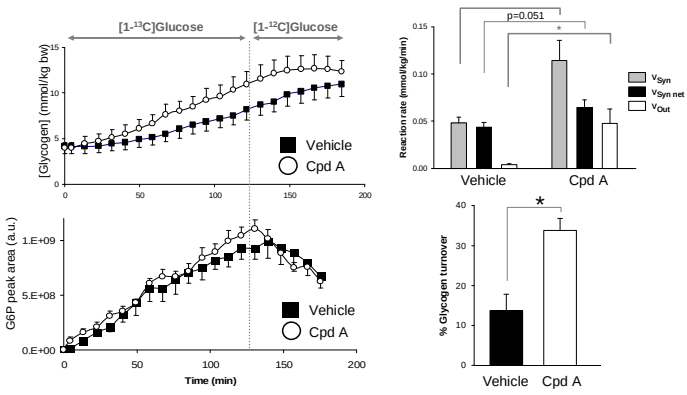
Methods

Measurements were carried out in 12 to 14-week old rats fed with a 60% high fat-enriched (HF) diet (D12492i, Research Diets, Inc., NJ) for 10 weeks prior to the experiment. Each animal was dosed 1 hour before imaging with either vehicle (5ml/kg water, n=10) or treatment (60mg/kg compound A, n=9). Dual cannulation of the carotid artery and jugular vein was performed and infusion lines were set up before baseline MRS scans. [1-^{13}C] labeled glucose was infused for 2 hours, followed by a 1-hour pulse chase infusion of ^{12}C glucose. Blood samples were collected every 20 min upon start of infusion. Immediately after imaging, liver lobes were excised and flash frozen in liquid nitrogen for the determination of glycogen concentration and [1-^{13}C] glycogen enrichment at a later stage. All NMR data were obtained under 1-2% isoflurane using a Bruker Biospec 7T/30cm instrument. A ^{1}H/^{31}C double-tuned surface coil with a 2 cm i.d. was used to collect signal from the liver of the rat. ^{13}C-MRS was preferred over ^{31}P-MRS as it potentially offered higher resolution for the measurement of G6P in the liver [1]. Each ^{1}H-decoupled ^{13}C-NMR spectrum was acquired over 10 min as previously described [1]. The baseline spectrum was acquired (TR 0.9s, 600 scans, 4k data) with broadband Waltz-16 decoupling turned on during the acquisition. Subsequent spectra were accumulated in a similar fashion over 180-min. Hepatic glycogen and G6P were quantified by integrating the C1 glycogen (100.4 ppm), G6Pβ (97.15 ppm) and glucoseβ (96.6 ppm) peaks by using a line fitting procedure. For metabolite confirmation and assignment, G6P was spiked in ^{13}C-NMR spectra of the aqueous liver extracts obtained using a Bruker-600 Avance spectrometer. Plasma glucose and liver glycogen ^{13}C enrichment were determined by LCMS after deproteinization. During the glucose infusion period, the increment in hepatic glycogen concentration ([ΔGly]) during each 20-min interval was calculated using equations derived from the muscle model [2]. To calculate the percent glycogen turnover, the change in [1-^{13}C]glycogen concentration during the unlabeled glucose infusion ($\Delta[^{13}\text{C-Gly}]_0$) was compared with the predicted change in [1-^{13}C]glycogen concentration, with the assumption of a constant flux through glycogen synthase (GS) and no glycogen breakdown [3]. All data are presented as mean±SEM.

Results

In the vehicle-control rats, the flux through glycogen synthase (V_{Syn}) and glycogen phosphorylase (V_{Out}) was 48.1±6.0 and 3.9±1.0 μmol/kg/min, respectively (Fig. 1). The observed rate of net hepatic glycogen synthesis ($V_{Syn\ net}$) was 42.7±4.3 μmol/kg/min. In the compound A-dosed animals, the glycogen synthase flux approximately doubled to 114.4±21.4 μmol/kg/min ($p<0.05$ vs. control). However, due to a much higher glycogen breakdown (5-fold increase *vs.* vehicle, $p<0.05$), only a small, but significant, increase in glycogen storage (i.e., $V_{syn\ net}$) was measured in response to compound A. As a result of the 4-fold elevation in hepatic glycogen turnover (8.3±2.0% vs. 36.0±5.5%, $p<0.05$), a trend towards higher hepatic glycogen concentrations (20% increase vs. vehicle as measured *post-mortem* by biochemistry, NS) was also observed in response to compound A following the 3-hour glucose infusion period. Further increase in glucose-stimulated G6P accumulation could not be observed in response to compound A. Finally, plasma insulin concentrations in both groups started at similar levels and progressively increased to as much as 3-fold the baseline values at the end of the experiment, again with no notable differences between the two groups.

Figure 1 – Effect of compound A on hepatic G6P levels and glycogen metabolism during [1-^{13}C]glucose infusion and [1-^{12}C]glucose pulse chase



Discussion

Here, we examined ^{13}C NMR spectra of the rat liver *in vivo* and successfully observed the incorporation of the non-radioactive ^{13}C label both into hepatic G6P and glycogen. This study showed that extreme glycogen accumulation in response to GK activation was restricted by a sharp increase in the glycogen phosphorylase (GP) reaction. The effect of compound A on glycogen turnover most likely resulted from an allosteric effect of glucose and G6P on glycogen synthesis and GP activities. These data support well recent finding regarding the synergestic effect of combined phosphorylase inactivation and GK activation on net glycogen synthesis in liver (4). In conclusion, non-invasive measurement of hepatic glycogen metabolism may prove particularly useful for understanding in vivo GK activation.

References

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