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The role of skeletal muscle in liver glutathione metabolism during acetaminophen overdose

L.M. Bilinsky [a,*], M.C. Reed [a], H.F. Nijhout [b]

[a] Department of Mathematics, Duke University, United States
[b] Department of Biology, Duke University, United States

HIGHLIGHTS

- We have devised a mathematical model of glutathione (GSH) metabolism in liver.
- We include glutamine (Gln, a GSH precursor) synthesis/export by skeletal muscle.
- We explain the linear decline in muscle Gln accompanying elevated plasma cortisol.
- Sterile inflammation and system x_c^- in liver aid GSH recovery after APAP overdose.
- Giving glutamine (in addition to NAC) may be beneficial during APAP overdose.

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ABSTRACT

Marked alterations in systemic glutamate–glutamine metabolism characterize the catabolic state, in which there is an increased breakdown and decreased synthesis of skeletal muscle protein. Among these alterations are a greatly increased net release of glutamine (Gln) from skeletal muscle into blood plasma and a dramatic depletion of intramuscular Gln. Understanding the catabolic state is important because a number of pathological conditions with very different etiologies are characterized by its presence; these include major surgery, sepsis, trauma, and some cancers. Acetaminophen (APAP) overdose is also accompanied by dramatic changes in systemic glutamate–glutamine metabolism including large drops in liver glutathione (for which glutamate is a precursor) and plasma Gln. We have constructed a mathematical model of glutamate and glutamine metabolism in rat which includes liver, blood plasma and skeletal muscle. We show that for the normal rat, the model solutions fit experimental data including the diurnal variation in liver glutathione (GSH). We show that for the rat chronically dosed with dexamethasone (an artificial glucocorticoid which induces a catabolic state) the model can be used to explain empirically observed facts such as the linear decline in intramuscular Gln and the drop in plasma glutamine. We show that for the Wistar rat undergoing APAP overdose the model reproduces the experimentally observed rebound of liver GSH to normal levels by the 24-h mark. We show that this rebound is achieved in part by the action of the cystine–glutamate antiporter, an amino acid transporter not normally expressed in liver but induced under conditions of oxidative stress. Finally, we explain why supplementation with Gln, a Glu precursor, assists in the preservation of liver GSH during APAP overdose despite the fact that under normal conditions only Cys is rate-limiting for GSH formation.

1. Introduction

The liver is the major site of amino acid metabolism, and the organ in which most glutathione (GSH) synthesis occurs; GSH is often referred to as the "master antioxidant," and has a major role in protection against oxidative stress and removal of xenobiotics. Glutamate (Glu) is one of the three amino acid precursors of GSH and also occupies a central role in the breakdown of dietary amino acids entering the liver via the portal vein by serving as an intermediate in the disposal of amino groups via urea. In the course of this process, Glu is interconverted with α-ketoglutarate, an intermediate in the tricarboxylic acid (TCA) cycle. The liver does not take up Glu directly but obtains it by uptake and deamination of glutamine (Gln). Skeletal muscle is the primary site of Gln synthesis and a major exporter of Gln to plasma in the normal state; in the stressed state, this export is even greater. An amino acid transporter

* Corresponding author.
E-mail address: bilinsky@math.duke.edu (L.M. Bilinsky).

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which may have an important role in Glu metabolism in a number of pathological states is the cystine/glutamate antiporter, which effects an obligate exchange of one hepatic Glu for one plasma cystine. This antiporter is not usually expressed in liver, but is induced during conditions of oxidative stress. Since Glu metabolism is complicated and central in dietary amino acid catabolism, GSH synthesis, and the TCA cycle, understanding how the liver and skeletal muscle work together to regulate its metabolism constitutes an important challenge.

A number of controversies surround Gln–Glu metabolism and the effects of amino acid supplementation in pathological states. Decreased plasma Gln levels are seen in acute catabolic states, in which there is an increased breakdown and decreased synthesis of skeletal muscle protein. Conditions accompanied by a catabolic state include sepsis, burns, trauma (including major surgery), uncontrolled diabetes, and starvation (Ardawi and Jamal, 1990). Droege and Holm (1997) have pointed out that a very large number of diseases and pathological conditions are characterized by both low plasma cystine and glutamine, often accompanied by many of the following: elevated plasma glutamate, increased urea production, low natural killer cell activity, and skeletal muscle wasting. These include late asymptotic stage HIV infection, sepsis, major injury and trauma, cancer, Crohn's disease, and ulcerative colitis. Droege et al. have termed this constellation of abnormalities "low CG syndrome." Although the net release of Gln from skeletal muscle into plasma is greatly increased during a catabolic state, increased demand by splanchnic and immunologic tissue (Cynober and Moore, 2003) results in depleted plasma Gln.

The depletion of plasma Gln has been found to be correlated with poor patient outcome, and there is evidence that supplementation with Gln improves outcome in cases including trauma and major surgery (Boelens et al., 2001), as well as burns (Garrel et al., 2003). In fact, it has been suggested (Watford, 2008) that Gln should be considered a conditionally essential amino acid. However, the mechanisms by which such supplementation improves patient condition are still not well understood. For example, Gln supplementation seems to help preserve muscle Gln concentration, usually depleted by about half, and it discourages the breakdown of muscle protein. Also not currently understood is how supplementation with *N* -acetylcysteine (NAC) causes a sturdy increase in plasma Gln levels in HIV patients (Droege and Holm, 1997).

Gln also plays a role in detoxification of exogenous toxins. Gln supplementation of rats undergoing acetaminophen (APAP) overdose acts to preserve GSH levels in liver and reduce the mortality rate relative to controls (Hong et al., 1992). During APAP overdose, the liver's ability to dispose of APAP is overwhelmed, causing it to be metabolized to the hepatotoxic compound NAPQI; NAPQI can be safely removed by conjugation with GSH but, during acute overdose, stores of GSH become depleted. The three amino acid precursors of GSH are cysteine (Cys), glutamate (Glu), and glycine (Gly) and under normal conditions cysteine is rate-limiting as it is present in the smallest concentration in hepatocytes. The results of Hong et al. (1992) suggest that during APAP overdose Glu can become rate-limiting for GSH formation; how this can occur is an interesting and important question.

In this paper we present a mathematical model of Glu–Gln metabolism in liver and skeletal muscle, and use it as a tool to investigate various physiological questions. Where possible, the model is based on information available for rat on amino acid transporter kinetics, the kinetics of biochemical reaction rates, etc. In the first section, we investigate the model's behavior for the case of normal physiologic conditions. We show that it reproduces precisely the empirically observed decline in liver GSH during starvation, as well as its diurnal variation under normal fed conditions. In the second section, we use the model to investigate changes resulting from a 9-day course of dexamethasone treatment. The alterations in amino acid uptake and release that occur in a catabolic state are believed to be mediated by glucocorticoids, potent anti-inflammatory hormones whose secretion into blood is upregulated during times of stress. Dexamethasone is a synthetic glucocorticoid and chronic dexamethasone administration has been used to study the metabolic changes characterizing a catabolic state, in isolation from the various pathologies causing the state (Ardawi and Jamal, 1990; Minet-Quinard et al., 2000). In the third section, we use the model to study APAP overdose and investigate the mechanism by which Gln preserves hepatic GSH levels.

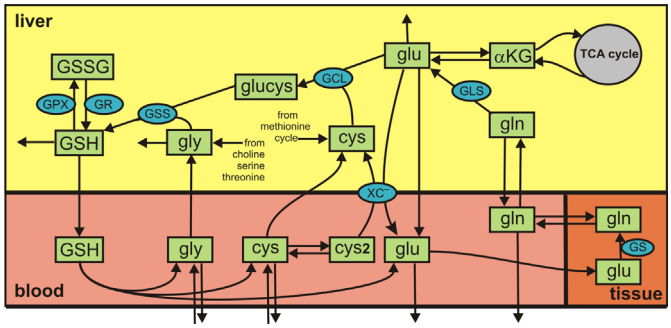

Fig. 1. Glu–Gln metabolism in liver and skeletal muscle tissue. Rectangles enclose the acronyms of substrates that are variables in the model. Arrows at the bottom of the figure represent import of dietary amino acids and import from other organs not explicitly modeled, and losses to organs not explicitly modeled as well as degradation. There is one differential equation for each substrate. The ellipses contain the acronyms of the enzymes that catalyze reactions. Full names for all substrates and a complete description of the mathematical model are given in the text. Full names for all enzymes and the values of all parameters are given in Tables 1–3.

2. Methods

Our model is a system of ordinary differential equations. The dependent variables represent the concentrations (in μM) of various substrates of interest (mainly amino acids and glutathione) in the liver, skeletal muscle, and blood plasma; these are represented by rectangular boxes in Fig. 1, which gives a schematic of the transport processes and biochemical reactions included in the model. Each differential equation is an expression of mass-balance: the time rate of change of a substrate concentration (in μM/h) is the sum of the velocities of reactions producing the substrate (and the velocity of transport into the compartment, where applicable), minus the sum of the velocities of reactions consuming the substrate (and the velocity of transport out of the compartment, where applicable). Enzymes catalyzing biochemical reactions are represented in Fig. 1 by blue ovals enclosing the enzyme's acronym.

The model we present here is a modification of the model for glutathione metabolism in Reed et al. (2008). The methionine and folate cycles are not explicitly featured and only cytosolic concentrations are considered; we do not track mitochondrial concentrations. However, we add the following new features: (1) the inclusion of glutamine as a substrate, (2) biochemical reactions producing and consuming glutamate in the liver, including a glutamate "pool" consisting of the TCA cycle intermediate α-ketoglutarate, and (3) the addition of a compartment for skeletal muscle. Although a large number of biochemical reactions occur in skeletal muscle, for our purposes it suffices to consider the concentrations of glutamate and glutamine there. We assume that the volume of liver (denoted by vL in the model) is 0.0054 L. We assume that the volume of skeletal muscle (vSM) is 0.1008 L and that the volume of blood plasma (vB) is 0.0108 L.