J. Dairy Sci. 89:1062–1071

Hepatic Metabolism of 2-Hydroxy-4-Methylthiobutyrate in Growing Lambs

T. J. Wester,*[1] M. Vázquez-Añón,† J. Dibner,† D. S. Parker,† A. G. Calder,* and G. E. Lobley*[2]
*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK
†Novus International, St. Louis, MO 63141

ABSTRACT

This study was undertaken to determine how, and where, 2-hydroxy-4-methylthiobutyrate (HMTBA) can augment Met metabolism in lambs. Four lambs (initial body weight of 50 kg, SE = 2, and 6 mo of age) prepared with catheters in the mesenteric, portal, hepatic, and jugular veins plus the aorta, were fed at 1.5× maintenance on a grass hay, barley, fish meal, molasses/premix (5:3:1:1, as fed) diet, supplied as hourly meals. Lambs were infused for 10 h with [methyl-$^2H_3$]Met (0.11 mmol/h) in a jugular vein and p-aminohippurate into the mesenteric vein. From 1 h onwards, successive 3-h infusions of saline (control), 0.55 mg/min (3.67 μmol/min), and 4.44 mg/min (29.6 μmol/min) of HMTBA were also infused into the mesenteric vein. Plasma, sampled continuously, was collected every 20 min during the last 60 min of each infusion. All infused HMTBA was recovered at the portal vein with 25% extracted subsequently by the liver. Portal appearance of total Cys and Met was unaltered by HMTBA infusion, but net splanchnic appearance of Cys increased (0.04, 0.08, 0.23 mmol/h, SEM = 0.05), whereas Met decreased (0.14, –0.01, –0.21 mmol/h, SED = 0.05). Despite this, arterial Met increased (27.0, 30.7, 51.5 μ*M*, SEM = 2.1) as did Met irreversible loss rate (27.6, 28.7, 40.1 μmol/h, SEM = 0.51), equivalent to 40% of the HMTBA reentering the plasma after conversion to Met. These data indicate that, in ruminants, HMTBA is probably converted to Met within peripheral tissues; that is, where the metabolic need for Met exists.

Key words: 2-hydroxy-4-methylthiobutyrate, methionine, cysteine, lamb

INTRODUCTION

L-Methionine is an essential AA for production animals, but is limiting in a number of protein sources used commercially (NRC, 2001). In ruminants, supplements in the form of protected Met are often added to diets. Protection is necessary to prevent or reduce microbial degradation of Met in the rumen. Commercial supplements available contain a racemic mixture of DL-Met, and at least part of the increases in plasma Met observed in cattle are due to the accumulation of the D-isomer (Lobley et al., 2001b; Vázquez-Añón et al., 2001). In contrast, the hydroxy analog of Met [2-hydroxy-4-methylthiobutanoic acid (**HMTBA**), Alimet, Novus International Inc., St. Louis, MO], although supplied as the DL racemic mixture, yields only L-Met in ruminants (Lobley et al., 2001b; Vázquez-Añón et al., 2001). One apparent disadvantage with HMTBA, however, is that, following dietary administration, increases in plasma Met concentrations are less than expected (Vázquez-Añón et al., 2001; Koenig et al., 2002). This may be due to 2 reasons, either poorer absorption or differences in how HMTBA and Met are handled by body tissues. In dairy cows, 50% rumen bypass and absorption rates have been reported (Koenig et al., 1999) but this may depend critically on rate of passage (Vázquez-Añón et al., 2001). In terms of tissue metabolism, data from anesthetized chickens indicate that the liver has considerable capacity to remove HMTBA, even at amounts in excess of normal levels of supplementation (Wang et al., 2001). Furthermore, although this extraction did result in increased Met appearance in the hepatic vein, this was much less than when the equivalent amount of DL-Met was supplied (Song et al., 2001). Thus, the anabolic responses observed with HMTBA in pigs (Knight et al., 1998) and poultry (Garlich, 1985) may be because of availability as nonfree Met forms or metabolites; for example, Cys, either free or as glutathione, or Met present in plasma export proteins. Nonetheless, plasma Met does increase in cattle following dietary supplementation with HMTBA (Koenig et al., 1999), indicating that conversion does occur in ruminants, but the site and extent of this metabolism is unknown.

The question of the fate of absorbed HMTBA is addressed in this study and the companion paper (Lobley et al., 2006). In view of the general paucity of information on metabolic interconversions of HMTBA in vivo, use was made of conscious lambs. Furthermore, the

Received April 29, 2005.
Accepted November 2, 2005.
[1]Current address: Institute of Food, Nutrition & Human Health, Massey University, PN 452, Private Bag 11 222, Palmerston North, New Zealand.
[2]Corresponding author: g.lobley@rowett.ac.uk

initial emphasis was on hepatic metabolism in view of the observations in poultry that the liver is the major site of removal (Wang et al., 2001) and metabolism (Dibner and Ivey, 1992) of HMTBA. In addition to monitoring splanchnic movements of HMTBA, Met, and Cys, the impact of HMTBA supplementation on whole-body Met flux was evaluated. This allowed the impact of alterations in body Met metabolism on plasma Met concentration to be investigated. Parts of this study have been presented in abstract and review form (Wester et al., 2000; Lobley et al., 2001b).

MATERIALS AND METHODS

Animals and Design

This experiment was approved by the Ethical Review Committee of the Rowett Research Institute and conformed to UK legislation of the Animals (Scientific Procedures) Act of 1986. Four Suffolk crossbred lambs (initial BW of 50 kg, SE = 2, and 6 mo of age), obtained from the Rowett Research Institute flock, were surgically prepared with silicone rubber catheters in the hepatic, portal, and mesenteric veins, and the abdominal aorta (Lobley et al., 1995). Lambs were allowed 2 wk of recovery in heated floor pens after surgery before transfer into metabolism crates where they were fed hourly (by automatic feeder) a grass hay/barley-based concentrate diet (50% grass hay, 30% barley, 9% fish meal, 10% molasses, and 1% vitamin and mineral mix; ingredients mixed as fed; 9.3 MJ of ME/kg of DM) at 1.5× maintenance intake (i.e., 675 kJ of ME/kg of $BW^{0.75}$ per d). A temporary polyvinyl chloride catheter (0.8 mm i.d. × 1.2 mm o.d., Critchley Engineering, Auburn, NSW, Australia) was inserted into a jugular vein on the day preceding the infusions.

The experimental protocol is shown in Figure 1. On the day of experiment, 7.3 m*M* L-[methyl-$^2H_3$]Met (99 atom %; Isotec Inc., Miamisburg, OH) was infused for 10 h into the jugular vein (solution infusion rate, 0.25 g/min; 1.85 μmol/min [methyl-$^2H_3$]Met). The [methyl-$^2H_3$] Met was dissolved in physiological saline (154 m*M* NaCl) containing heparin (700 IU/g; Leo Laboratories Ltd., Princes Risborough, UK). Starting 1 h after the initiation of the [methyl-$^2H_3$]Met infusion, 2 solutions, one of 0.267 *M* sodium p-aminohippurate (**pAH**) in 0.05 *M* sodium phosphate, pH 7.4, and the other, 0.15 *M* PBS, pH 7.4, were infused into the mesenteric vein for 3 h, each at a rate of 0.25 g/min. After 2 h, 6-mL blood samples were collected continuously over ice by peristaltic pump for three 20-min periods (Lobley et al., 1995) from the aorta plus the portal and hepatic veins.

After the last blood sample was collected, solutions of 14.7 m*M* HMTBA in 0.15 *M* PBS, pH 7.4, and 0.267 *M* pAH in 0.05 *M* sodium phosphate, pH 7.4, were infused (15 g/h) into the mesenteric vein for 3 h. The HMTBA was infused at a rate of 0.55 mg/min (3.68 μmol/min). Again, three 20-min continuous collections from the aorta, and hepatic and portal veins were taken over the last hour. Then the concentration of HMTBA was increased to 118.4 m*M* (4.44 mg/min, 29.6 μmol/min) for the final 3 h, with three 20-min samples taken continuously during the last hour of infusion.

Analyses

Plasma was prepared from blood by centrifugation at 1,000 × *g* for 15 min at 4°C. Plasma flow was calculated from downstream dilution of pAH by the gravimetric procedure (Lobley et al., 1995). The isotope-dilution technique (Calder et al., 1999) was used to determine concentrations of Met, Cys, and HMTBA in plasma. Briefly, to a known weight (0.9 g) of plasma, was added 0.1 g of a mixture of 0.1 *M* dithiothreitol, 0.2 m*M* [1-^{13}C]Met (99 atom %; Isotec Inc.), 0.8 m*M* [1-^{13}C]Cys (99 atom %; Isotec Inc.), and 0.2 m*M* [1-^{13}C]HMTBA (see below for synthesis). The plasma sample was vortex mixed and allowed to stand at room temperature for 30 min. Plasma proteins were then precipitated with 200 μL of 38% (wt/wt) sulfosalicylic acid. The sample was centrifuged at 7,200 × *g* for 5 min and the supernatant applied to a 0.5-mL AG 50W × 8 (H^+), 100–200 mesh resin column (BioRad Laboratories, Hemel Hempstead, UK). The initial effluent plus the subsequent 0.5-mL water wash was collected for HMTBA analysis. For analysis of Met and Cys, the column was washed with a further 2 × 2 mL of water (discarded), and the AA eluted with 2 mL of 2 *M* NH_4OH followed by 1 mL of water. After freeze-drying, the sample was dissolved in 0.35 mL of 0.1 *M* HCl, transferred to a 1-mL vial, and evaporated to dryness at 90°C under a gentle stream of nitrogen. To the dry residue was added 80 μL of 2 *M* NH_4OH plus 20 μL of 0.1 *M* dithiothreitol; the solution was left to stand at room temperature for 30 min. The sample was then evaporated to dryness, 70 μL of a 1:1 (vol:vol) mixture of N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide and acetonitrile was added, and the t-butyldimethlysilyl derivative was formed by heating at 90°C for 20 min.

For HMTBA analysis, the initial column effluent and 0.5-mL water wash was extracted with 2 × 2 mL of ethyl acetate, centrifuged at 3,000 × *g*, and the pooled organic layers dried over sodium sulfate for 10 min. The pooled ethyl acetate was evaporated to dryness at 40°C under nitrogen. To the dry residue was added 70 μL of N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide:acetonitrile, as above, and the t-butyldimethlysisilyl derivative of HMTBA was formed by heating at 90°C