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Clofibrate treatment up-regulates novel organic cation transporter (OCTN)-2 in tissues of pigs as a model of non-proliferating species

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Abstract

Recent studies have shown that treatment of rodents with agonists of peroxisome proliferator-activated receptor (PPAR)-α causes an up-regulation of novel organic cation transporter (OCTN)-2, a carnitine transporter, and increases carnitine concentration in the liver. This study was performed to investigate whether such effects occur also in pigs which like humans have a lower expression of PPARα and are less responsive to treatment with PPARα agonists than rodents. An experiment with 18 pigs was performed which were fed a control diet or the same diet supplemented with 5 g clofibrate/kg for 28 days. Pigs treated with clofibrate had higher relative mRNA concentrations of OCTN2 in liver (3.1-fold), skeletal muscle (1.5-fold) and epithelial cells from small intestine (1.8-fold) than control pigs ($P<0.05$). Pigs treated with clofibrate had also higher concentrations of free and total carnitine in the liver and a higher concentration of free carnitine in skeletal muscle than control pigs ($P<0.05$). Concentrations of γ-butyrobetaine, the precursor of endogenous formation of carnitine, in liver, muscle and plasma did not differ between both groups; the activity of γ-butyrobetaine dioxygenase, the rate limiting enzyme of carnitine synthesis, in the liver was lower in pigs treated with clofibrate than in control pigs ($P<0.05$). This study shows for the first time that treatment with a PPARα agonist causes an up-regulation of OCTN2 in liver, muscle and enterocytes from small intestine of pigs. This in turn increases carnitine concentrations in liver and muscle probably by enhancing carnitine uptake into cells.

Keywords: Clofibrate; Novel organic cation transporter; Carnitine; Peroxisome proliferator-activated receptor-α; (Pig)

1. Introduction

Carnitine is an essential metabolite, which has a number of indispensable functions in intermediary metabolism. The most prominent function lies in its role in the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix where β-oxidation takes place (McGarry and Brown, 1997; Brass, 2002; Steiber et al., 2004). Carnitine is derived from dietary sources and endogenous biosynthesis (Rebouche and Seim, 1998). Carnitine biosynthesis involves a complex series of reactions involving several tissues. Lysine provides the carbon backbone of carnitine. Lysine in protein peptide linkages undergoes methylation of the ε-amino group to yield trimethyllysine, which is released upon protein degradation. The released trimethyllysine is further oxidised to γ-butyrobetaine by the action of trimethyllysine dioxygenase, 3-hydroxy-*N*-trimethyllysine aldolase and 4-*N*-trimethylaminobutyraldehyde dehydrogenase. γ-Butyrobetaine is hydroxylated by γ-butyrobetaine dioxygenase to form carnitine. In humans, this last reaction occurs primarily in liver and kidney (Vaz and Wanders, 2002).

Distribution of carnitine within the body and intracellular homeostasis of carnitine are controlled by novel organic cation transporters (OCTN) which belong to the solute carrier 22A family (Lahjouji et al., 2001; Tein, 2003). Three OCTN have been identified so far, OCTN1, OCTN2 and OCTN3, localised in the plasma membrane of cells (Tamai et al., 1997; Tamai et al., 1998; Tamai et al., 2000). OCTN are polyspecific; they transport several cations and L-carnitine (Ohashi et al., 1999; Ohashi et al., 2001). Carnitine transport by OCTN1 and OCTN2

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is sodium dependent whereas that by OCTN3 is sodium independent (Tamai et al., 2000). OCTN1 and OCTN2 are expressed in several tissues such as kidney, intestine, skeletal muscle, heart, liver and brain (Wu et al., 1999; Tamai et al., 2000; Slitt et al., 2002). In contrast, OCTN3 is expressed exclusively in testes and kidney (Tamai et al., 2000). Among the three OCTN, OCTN3 has the highest specificity for carnitine, OCTN1 has the lowest one (Tamai et al., 2000). OCTN operate on the intestinal absorption and renal reabsorption of carnitine and play a major role in tissue distribution by catalysing the uptake of carnitine into body cells. Due to its high binding affinity for carnitine and its wide expression, OCTN2 is the physiologically most important carnitine transporter, operating for the reabsorption of carnitine from the urine as well as playing a major role in tissue distribution. OCTN1 contributes less to carnitine transport than OCTN2 due to its low carnitine transport activity. OCTN3 may be important for carnitine uptake into testis, and may contribute to reabsorption of carnitine in kidney (Tamai et al., 2000).

Many years ago it has been shown that starvation or treatment of rats with clofibrate increases the concentration of carnitine in the liver (McGarry et al., 1975; Brass and Hoppel, 1978; Paul and Adibi, 1979). As both starvation and clofibrate treatment lead to an activation of peroxisome proliferator-activated receptor (PPAR)-α, a transcription factor belonging to the nuclear hormone receptor superfamily (Schoonjans et al., 1996), we have recently tested the hypothesis that activation of this nuclear receptor is responsible for the increased liver carnitine concentrations observed in those studies. We found that activation of PPARα by clofibrate increases mRNA concentrations of OCTN2 in liver and small intestine of rats (Luci et al., 2006; Ringseis et al., 2007). These data suggest that PPARα activation stimulates intestinal absorption of carnitine and the delivery of carnitine from blood into the liver which provides an explanation for the increased carnitine concentration in the liver of rats starved or treated with clofibrate. More recently, Van Vlies et al. (2007) have shown that treatment with WY-14,643, another synthetic PPARα agonist, increases gene expression of OCTN2 and the activity of γ-butyrobetaine dioxygenase in the liver in wild-type mice but not in PPARα-deficient mice. These findings clearly show that up-regulation of OCTN2 and hepatic carnitine synthesis are directly mediated by PPARα activation.

Regarding the expression of PPARα in tissues and the effects of PPARα activation on transcription of its target genes, there are great differences between various species. In rodents, PPARα is highly expressed, and activation of PPARα not only induces many genes involved in various metabolic pathways but also causes severe peroxisome proliferation in the liver (Peters et al., 2005). In contrast to rodents, PPARα agonists do not induce peroxisome proliferation in the liver of many other species, such as guinea pigs, swine, monkeys and humans. These non-proliferating species have a lower expression of PPARα in the liver and the response of many genes to PPARα activation is much weaker than in proliferating species (Holden and Tugwood, 1999). For that reason, effects related to PPARα activation observed in rodents cannot be directly applied for non-proliferating species such as humans.

We have recently shown that pigs have a similar mRNA concentration of PPARα in the liver as humans, which is approximately ten-fold lower than in rats. Therefore, we proposed that the pig may be a useful model to study biochemical effects induced by treatment with PPARα agonists (Luci et al., 2007). The aim of the present study was to find out whether treatment with PPARα activators influences carnitine homeostasis in the pig as a non-proliferating species. Therefore, we determined gene expression of OCTN2 in enterocytes of small intestine, liver and muscle and carnitine concentrations in plasma, liver and muscle of these pigs treated with clofibrate. We also determined concentrations of γ-butyrobetaine in these tissues and examined mRNA concentration and activity of γ-butyrobetaine dioxygenase in the liver in order to explore whether clofibrate treatment enhances carnitine biosynthesis in the liver.

2. Materials and methods

2.1. Animals and treatments

Eighteen male 8 weeks old crossbred pigs [(German Landrace × Large White) × Pietrain] were kept in a room under controlled temperature at 23 ± 2 °C and 55 ± 5% relative humidity with light from 0600 to 1800 h. One day before the beginning of the experimental feeding period, the pigs were weighted and randomly allocated to two groups with body weights of 12.0 ± 1.1 kg in control group and 11.9 ± 0.6 kg in treatment group. Both groups of pigs received a nutritionally adequate diet (National Research Council, 1998) for growing pigs containing (in g/kg) wheat (400), soybean meal (230), wheat bran (150), barley (100), sunflower oil (90) and mineral premix including L-lysine, DL-methionine and L-threonine (30). This diet contained 14.4 MJ metabolisable energy and 185 g crude protein per kg. The native carnitine concentration of the diet was low (<5 mg/kg as determined by tandem mass spectrometry, see Section 2.3). The diet of the treatment group was supplemented with 5 g clofibrate per kg. Diet intake was controlled, and each animal in the experiment was offered an identical amount of diet per day. The amount of diet administered was about 15% below that consumed ad-libitum by pigs of a similar weight (as assessed in a previous study). Therefore, the diet offered was completely taken in by all pigs in the experiment. During the feeding period, the amount of diet offered each day was increased continuously from 400 to 1200 g. The intake of metabolisable energy was in clear excess of the requirement for maintenance (as given by National Research Council, 1998). Therefore, all the pigs had a normal growth rate. The pigs had free access to water via nipple drinking systems. The experimental diets were administered for 28 days. All experimental procedures described followed established guidelines for the care and use of laboratory animals and were approved by the local veterinary office.

2.2. Sample collection

After completion of the feeding period the animals were killed under light anaesthesia. Four hours before euthanasia each pig was fed its respective diet. After killing, blood was