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Anaplerotic molecules: Current and future

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Summary This review presents the concepts of anaplerosis and cataplerosis in relation to the regulation of citric acid cycle operation. Anaplerosis is the re-filling of the catalytic intermediates of the cycle that carry acetyl-CoA as it is oxidized. The main anaplerotic substrates are pyruvate, glutamine/glutamate and precursors of propionyl-CoA (odd-chain fatty acids, specific amino acids, C_5-ketone bodies). Cataplerosis balances anaplerosis by removing excess intermediates from the citric acid cycle. The properties of the main anaplerotic substrates are reviewed from the point of view of potential clinical applications to the treatment of some inherited and acquired conditions.

The concept of anaplerosis

The oxidation of acetyl groups in the citric acid cycle (CAC) involves eight reactions, which (i) convert the two carbons of acetyl to CO_2, and (ii) regenerate the acceptor of the acetyl group, i.e., oxaloacetate. When the only source of carbon entering the CAC is acetyl-CoA, the net fluxes through the eight reactions of the cycle are identical, although most of the reactions are reversible in intact cells (except citrate synthase and α-ketoglutarate dehydrogenase). The size of the total pool of the eight CAC intermediates (1–2 μmol/g) is small compared to the throughput of the cycle (1–2 μmol acetyl/g per min). This is why the eight intermediates are referred to as 'catalytic intermediates' of the CAC. Figure 1 illustrates the large differences between the sizes of the pools of individual intermediates. As a consequence, the turnover of these pools varies greatly: 5–10 times/min for citrate, and 100–200 times/min for oxaloacetate.

Although the reactions of the CAC provide 100% recovery of the catalytic intermediates, there is a physiological 'leakage' of intermediates through the mitochondrial membranes and the cell membranes, often referred to as 'cataplerosis'. Although the word 'cataplerosis' is, *sensu stricto*, a misnomer, it is used extensively in the recent literature. The rate of physiological cataplerosis in the normal heart is estimated at 1–2% of the total pool per min. If the leakage of catalytic intermediates were not balanced by the re-filling reactions of anaplerosis, the flux through the CAC and the regeneration of ATP could not be sustained. Therefore, the maintenance of adequate pools of CAC intermediates is a *conditio sine qua non* of cell survival and homeostasis. Indeed, the mechanical performance of isolated rat hearts decreases rapidly when the perfusate contains only precursors of acetyl-CoA, i.e., acetate or acetoacetate. Recovery of cardiac mechanical performance follows the addition of an anaplerotic substrate (pyruvate, propionylcarnitine) to the perfusate (Russell and Taegtmeyer 1991; Russell et al 1995).

Figure 2 summarizes the main anaplerotic reactions in mammalian cells. Pyruvate is anaplerotic via pyruvate carboxylase and/or malic enzyme. Glutamate, and its abundant precursor glutamine, are converted to α-ketoglutarate by reactions catalysed by glutamate dehydrogenase and/or aminotransferases. Numerous precursors of propionyl-CoA

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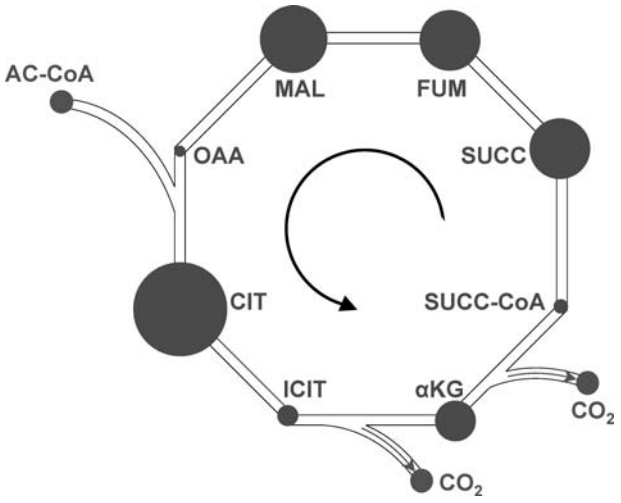

Fig. 1 Scheme of the citric acid cycle emphasizing the relative sizes of the pools of intermediates (dark circles)

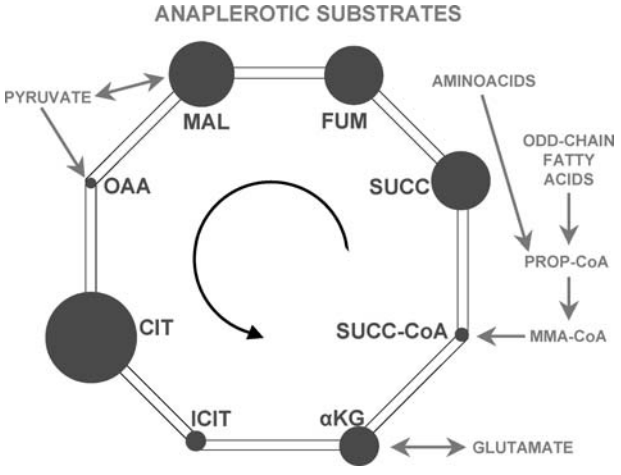

Fig. 2 Main anaplerotic processes feeding into the citric acid cycle

(odd-chain fatty acids, propionylcarnitine, C_5-ketone bodies) form succinyl-CoA via methylmalonyl-CoA. Lastly, aspartate derived from protein degradation forms oxaloacetate by transamination reaction, or fumarate via the reactions of the purine nucleotide cycle (Aragon et al 1981) and of the urea cycle.

There is good evidence that the total concentration of CAC intermediates can vary by up to a few fold during transitions between metabolic situations. This is observed (i) in muscle during the transition from rest to exercise, (ii) in heart and liver upon supply of anaplerotic substrates (Williamson et al 1969), and (iii) in liver during the transition from fasting to feeding (Brunengraber et al 1973). However, in a given metabolic situation, anaplerotic flux in excess of physiological leakage must be balanced by a corresponding cataplerotic flux. For example, in the liver, gluconeogenic carbons of pyruvate and propionyl-CoA, which enter the CAC via pyruvate carboxylase and methylmalonyl-CoA mutase, leave the cycle via phosphoenolpyruvate (PEP) carboxykinase. In the perfused rat heart, anaplerosis from high concentrations of propionate is balanced by an efflux of malate. As anaplerotic molecules pass through reactions of the CAC, there is no net CO_2 production, except from C1 of glutamine/glutamate. Thus, except for the latter, the production of labelled CO_2 from a labelled anaplerotic substrate reflects not net oxidation of the substrate but isotopic exchanges in the CAC. This is true unless additional reactions form labelled mitochondrial acetyl-CoA from the anaplerotic substrate, for example via malic enzyme+ pyruvate dehydrogenase, or PEP carboxykinase and pyruvate dehydrogenase.

In addition to maintaining the pool of CAC intermediates, anaplerotic and cataplerotic reactions play a central role in gluconeogenesis from amino acids, lactate and pyruvate, as well as in the urea cycle. In the brain, anaplerotic reactions contribute to the regulation of the metabolism of neurotransmitters. Also, anaplerotic glutamine synthesis is coupled to removal of nitrogen from the brain in hyperammonaemia (Sibson et al 2001). Lastly, anaplerosis appears to play an important role in the secretion of insulin by pancreatic β-cells (MacDonald et al 2005). The stimulation of anaplerosis by physiological fuel secretagogues is accompanied by a corresponding cataplerosis that transfers CAC intermediates to the extramitochondrial space. The transferred molecules could act as secretagogues or as exporters of equivalents of NADPH, acetyl-CoA or malonyl-CoA.

There is good evidence that a number of pathological conditions could benefit from anaplerotic therapy. Conditions associated with reperfusion injury (myocardial infarction, stroke, organ transplantation) are associated with damage to cell membranes and possible decrease in the pools of some CAC intermediates. Applications of anaplerotic therapy to the dietary treatment of some inherited metabolic diseases are extensively discussed in a paper published in this issue (Roe and Mochel 2006). Note that, when considering anaplerotic therapy, it is impossible in most cases to demonstrate either a decrease in the concentration of CAC intermediates in tissues or an excessive leakage of intermediates from a tissue. The latter might be inferred from evidence of the leakage of large molecules such as creatine kinase during myocardial infarction or rhabdomyolysis (Roe et al 2002). Still, one cannot assess whether the concentration of oxaloacetate, the acetyl-CoA acceptor and least abundant CAC intermediate, is sufficient to allow proper CAC flux and energy production. In most cases, evidence of the need for anaplerotic therapy can only be inferred *a posteriori* from the improvement of cardiac function (Hermann et al 2004; Roe et al 2002; Russell and Taegtmeyer 1991; Russell et al 1995), muscle strength (Roe et al 2002), or neurological status (Mochel et al 2005).

Properties of anaplerotic substrates

The following subsections discuss and compare the properties of anaplerotic compounds that might be used for the chronic or acute therapy of some inherited or acquired pathological conditions.

Pyruvate

There is good evidence from animal experiments that supraphysiological concentrations of pyruvate improve the mechanical function of myocardium damaged by ischaemia–reperfusion injury (Bunger et al 1986; Kristo et al 2004; Mentzer et al 1989). The beneficial effects of pyruvate probably result from the combination of anaplerosis, trapping of reactive oxygen species (Bassenge et al 2000), and trapping of reducing equivalents that accumulate during ischaemia. There are, however, a number of problems associated with the use of pyruvate for anaplerotic therapy. First, since the K_m of pyruvate carboxylase for pyruvate is high (0.4 mmol/L), acute anaplerotic therapy requires achieving and maintaining arterial pyruvate concentrations of about 1 mmol/L. Second pyruvate, whether administered orally or parenterally, equilibrates rapidly with lactate. This necessitates infusing pyruvate in amounts corresponding to 50–100% of the caloric requirement (Stanley et al 2003). Third, pyruvic acid is an unstable strong acid, which cannot be administered as such. Fourth, sodium pyruvate and calcium pyruvate, albeit stable as dry powders, cannot in most cases be administered in therapeutic amounts because of the accompanying sodium or calcium load. When acute anaplerotic therapy is targeted at the heart, systemic sodium overload can be avoided by infusing sodium pyruvate via an intracoronary catheter. This has resulted in improved myocardial function in patients with congestive heart failure (Hermann et al 2004). One strategy for systemic pyruvate infusion without sodium load is to administer a glycerol pyruvate ester, which is instantly hydrolysed by plasma esterases. Following a one-hour coronary occlusion in anaesthetized pigs, systemic infusion of dipyruvylacetylglycerol for 2 h at 95% of caloric requirement resulted in average plasma pyruvate concentration of 0.8 mmol/L, and a decrease in infarct size from 31 to 20% of the area at risk (Stanley et al 2003). In view of the harsh taste of glycerol pyruvate esters, their potential use is limited to intravenous administration for acute decompensation conditions.

Glutamine/glutamate

Glutamate and the CAC intermediate α-ketoglutarate are interconverted by reversible reactions catalysed by glutamate dehydrogenase and aminotransferases. Glutamate can also be formed from glutamine, the most abundant circulating amino acid, via glutaminase. Thus, theoretically, the glutamine/glutamate couple could be an effective anaplerotic system. The evaluation of anaplerosis from ^{13}C-labelled glutamine or glutamate is difficult because of the reversibility of the reactions linking glutamine, glutamate and α-ketoglutarate. Isotopic exchanges through these reactions can label CAC intermediates without net anaplerosis. As H.A. Krebs wrote in 1966: 'The fate of the label does not allow predictions to be made about the net fate of the labelled metabolites' (Krebs et al 1966). Evidence of net anaplerosis from glutamine/glutamate would require the demonstration of simultaneous cataplerosis evidenced by (i) stimulation of gluconeogenesis in liver and kidney, or (ii) release of CAC intermediates and aspartate from non-gluconeogenic tissues. The effect of anaplerosis from glutamine/glutamate on cardiac function has been controversial, with positive and negative reports of improvement of cardiac function in animal models (Jessen et al 1993; Khogali et al 1998).

Precursors of propionyl-CoA

Anaplerosis from propionyl-CoA

Propionyl-CoA is anaplerotic via the reactions catalysed by propionyl-CoA carboxylase, methylmalonyl-CoA racemase and methylmalonyl-CoA mutase. The reactions of the propionyl-CoA pathway are kept far from thermodynamic equilibrium, presumably by the pull of the CAC reactions converting succinyl-CoA to succinate. This is evidenced by the mass action ratio [succinyl-CoA]/[propionyl-CoA] which, in liver and heart, differs by 2–3 orders of magnitude from the thermodynamic equilibrium ratio calculated from the combined equilibrium constants of the three reactions (Reszko et al 2003). Thus, propionyl-CoA is a very effective anaplerotic precursor, even at low concentration. We now discuss various precursors of propionyl-CoA.

Propionate

Propionate is physiologically derived from intestinal fermentation. The portal vein concentration of propionate is about 0.1–0.2 mmol/L, but the liver takes up 99% of portal vein propionate in a single pass (Puchowicz et al 1999). This explains the very low systemic concentration of propionate (about 0.05 mmol/L). One can thus infer that enteral administration of sodium propionate or of a glycerol propionate ester would not result in a substantial increase in systemic propionate concentration. Therefore, to achieve an elevated plasma propionate concentration, one could infuse an isotonic solution of sodium propionate. However, such infusion might result in sodium overload. An alternative would be to infuse a solution of the fairly soluble glycerol monopropionate.

In isolated rat hearts perfused with 0.1–1.0 mmol/L [U-$^{13}C_3$]propionate, we found that the production of unlabelled propionyl-CoA from endogenous sources is markedly inhibited by exogenous [U-$^{13}C_3$]propionate (Kasumov et al 2006). This strongly suggests that exogenous propionate inhibits protein degradation in the heart. Note that, although propionate is an excellent anaplerotic substrate, it does not provide a direct energy source, i.e. acetyl-CoA, in most organs. For example, in the heart, which has no PEP carboxykinase, carbons from propionyl-CoA leave the heart as malate, which can be converted to glucose in the liver or be excreted in urine. In contrast, in the liver, part of the propionyl-CoA carbon can be converted to acetyl-CoA by the sequence of reactions: propionyl-CoA →→ succinyl-CoA → (CAC reactions) → oxaloacetate → PEP → pyruvate → acetyl-CoA.

Triheptanoin

The enteral use of triheptanoin is extensively discussed in the accompanying paper in this issue (Roe and Mochel 2006). After oral administration of a triheptanoin emulsion and enteral hydrolysis, glycerol and heptanoate are absorbed into the portal drainage system. Most of the heptanoate reaching the liver is β-oxidized to 1 anaplerotic propionyl-CoA + 2 acetyl-CoA. Propionyl-CoA derived from heptanoate has two main fates in the liver: (i) conversion to glucose via CAC reactions, PEP carboxykinase and the gluconeogenic pathway, and (ii) incorporation into C_5-ketone bodies β-ketopentanoate (3-ketovalerate) and (*R*)-β-hydroxypentanoate (3-hydroxyvalerate) via the reactions of the hydroxymethylglutaryl-CoA cycle. Note that the ingestion of meals containing triheptanoin also increases the production of C_4-ketone bodies, acetoacetate and (*R*)-β-hydroxybutyrate (Mochel et al 2005; Roe et al 2002). The production of C_4- and C_5-ketone bodies from dietary triheptanoin occurs even when the meal contains carbohydrates. This is because the oxidation of heptanoate, a medium-chain fatty acid, in liver mitochondria is not regulated by the carnitine palmitoyltransferase system, the activity of which is inhibited by dietary carbohydrates (McGarry and Foster 1980). The rapid oxidation of heptanoate to propionyl-CoA + acetyl-CoA exceeds the capacity of the CAC to oxidize acetyl-CoA. The excess acetyl-CoA and some propionyl-CoA are channelled to C_4- and C_5-ketone bodies, which are exported from the liver. In peripheral tissues, the C_4- and C_5-ketone bodies are metabolized by the same enzymes, i.e., (*R*)-β-hydroxybutyrate dehydrogenase, 3-oxoacid-CoA transferase and acetoacyl-CoA thiolase. Through these reactions, the C_5-ketone bodies are converted back to anaplerotic propionyl-CoA and to acetyl-CoA.

After intravenous infusion of triheptanoin emulsion, and hydrolysis by plasma esterases, most heptanoate is immediately available to peripheral tissues. A fraction of plasma heptanoate is metabolized in liver to glucose and C_5-ketone bodies. This explains why the plasma concentration ratio [C_5-ketone bodies]/[heptanoate] is high after enteral administration of triheptanoin, but low during parenteral infusion.

To illustrate in quantitative terms the anaplerotic potential of dietary triheptanoin, let us consider the propionyl content of two adult dietary regimens. Both regimens provide 8790 kJ/day, including 2930 kJ/day from fat, and 100 g/day of proteins. In the control diet, in which the fat component comprises only even-chain triglycerides, the propionyl groups derive only from proteins (63 mmol propionyl equivalents per 100 g mixed proteins). If one replaces one-third of the energy from fat by triheptanoin, the latter provides an additional 700 mmol propionyl equivalents. This calculation shows that it would not be possible to substantially boost anaplerosis from propionyl-CoA by increasing dietary proteins.

Tripentanoin

Pentanoate is also an anaplerotic substrate since its initial degradation forms propionyl-CoA and acetyl-CoA. Unlike heptanoate, it can be metabolized by patients with medium-chain fatty acid oxidation disorders. This is because pentanoyl-CoA can be converted to pentenoyl-CoA by isovaleryl-CoA dehydrogenase, an enzyme of branched-chain amino acid catabolism.

In anaesthetized pigs receiving an intracoronary infusion of [3,4,5-$^{13}C_3$]pentanoate, we observed a strong decrease in anaplerosis from endogenous sources in the infused myocardium (Yu et al 2006). This again reflects a decrease in the degradation of myocardial proteins.

C_5-ketone bodies

In nondiabetic animals, C_5-ketone bodies ((*R*)-β-hydroxypentanoate and β-ketopentanoate) are very rapidly used when administered intravenously at doses corresponding to 75% of the energy requirement (Leclerc et al 1995). Since the heart, brain and kidney are the three organs with the highest activity of enzymes utilizing ketone bodies (Ardawi and Newsholme 1982), one can surmise that C_5-ketone bodies are very effective anaplerotic agents in these tissues. This is supported by the improvement in the neurological status of a newborn with pyruvate carboxylase deficiency during treatment with triheptanoin (Mochel et al 2005; Roe and Mochel 2006).

Because of their very rapid metabolism, C_5-ketone bodies cannot be infused as sodium salts without causing rapid sodium overload. A monoglycerol ester of β-ketopentanoate is a suitable sodium-free ester, which is rapidly hydrolysed by plasma esterases, like the analogous glycerol monoacetoacetate (Birkhahn and Border 1978). Glycerol mono-β-ketopentanoate, which is much easier to synthesize that the

(*R*)-β-hydroxypentanoate ester, could be used for the acute treatment of fatty acid oxidation disorders.

Propionyl overload

The administration of a large amounts of a propionyl-CoA precursor raises the theoretical possibility of saturating the capacity for disposing of a propionyl-CoA load. In patients with disorders of propionyl-CoA carboxylase or methylmalonyl-CoA mutase, propionyl-CoA combines with oxaloacetate to form methylcitrate. The formation of the latter is a cataplerotic process that depletes the CAC of catalytic intermediates. However, in animals treated with large doses of β-ketopentanoate (Leclerc et al 1995), and in humans treated with triheptanoin (Mochel et al 2005; Roe et al 2002), the increase in the urinary excretion of markers of propionyl-CoA overload (methylcitrate, 3-hydroxypropionate) was orders of magnitude lower than in propionic acidaemia patients, and thus clinically insignificant.

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