Chapter 3
Anaplerosis for Glutamate Synthesis in the Neonate and in Adulthood

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Abstract A central task of the tricarboxylic acid (TCA, Krebs, citric acid) cycle in brain is to provide precursors for biosynthesis of glutamate, GABA, aspartate and glutamine. Three of these amino acids are the partners in the intricate interaction between astrocytes and neurons and form the so-called glutamine–glutamate (GABA) cycle. The ketoacids α-ketoglutarate and oxaloacetate are removed from the cycle for this process. When something is removed from the TCA cycle it must be replaced to permit the continued function of this essential pathway, a process termed anaplerosis. This anaplerotic process in the brain is mainly carried out by pyruvate carboxylation performed by pyruvate carboxylase. The present book chapter gives an introduction and overview into this carboxylation and additionally anaplerosis mediated by propionyl-CoA carboxylase under physiological conditions in the adult and in the developing rodent brain. Furthermore, examples are given about pathological conditions in which anaplerosis is disturbed.

Keywords Pyruvate carboxylation • Neonate • Adult • Anaplerosis • Pyruvate • Glutamate

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Abbreviations

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AD	Alzheimer's disease
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	AMP activated protein kinase
CBZ	Carbamazepine
GS	Glutamine synthetase
ME	Malic enzyme
MRS	Magnetic resonance spectroscopy
P	Postnatal day
PAG	Phosphate activated glutaminase
PC	Pyruvate carboxylase
PCC	Propionyl-CoA carboxylase
PDH	Pyruvate dehydrogenase
PEPCK	Phosphoenolpyruvate carboxykinase
PPP	Pentose phosphate pathway
PTZ	Pentylenetetrazole
TCA	Tricarboxylic acid

3.1   Introduction

The major fuel for the brain is glucose and relevant metabolic pathways are shown in Fig. 3.1. Glucose metabolism is necessary for glutamate synthesis which is essential for brain function since the blood–brain barrier prevents glutamate from entering the brain. Synthesis of this excitatory amino acid neurotransmitter in neurons is particularly important since 90 % of synapses in the forebrain are glutamatergic (Attwell and Laughlin 2001) and glutamate is the precursor of the other major amino acid neurotransmitter, GABA. The synthesis of the carbon skeleton of these neurotransmitters is dependent on the production of the anaplerotic substrate oxaloacetate from glucose (Fig. 3.2). Sir Hans Kornberg coined the expression: *anaplerotic sequences* to describe a series of enzymatic reactions or pathways that replenish the pools of metabolic intermediates in the tricarboxylic acid (TCA) cycle. Since both glutamate and GABA are derived from the TCA cycle intermediate α-ketoglutarate the anaplerotic reactions securing the synthesis of α-ketoglutarate in brain are important. However, because of the coupling of this process with cataplerosis, i.e. the exit of intermediates from the cycle, both pathways are equally important for the regulation of amino acid, glucose and fatty acid homeostasis (Sonnewald 2014).

One role of this cycle in all mammalian cells is the oxidation of acetyl-CoA to carbon dioxide and energy production. However, in brain synthesis of precursors for the neurotransmitters glutamate, GABA and aspartate are also an essential part of its function. Pyruvate carboxylase (PC) is the enzyme with major responsibility for oxaloacetate production from glucose and thus anaplerosis in
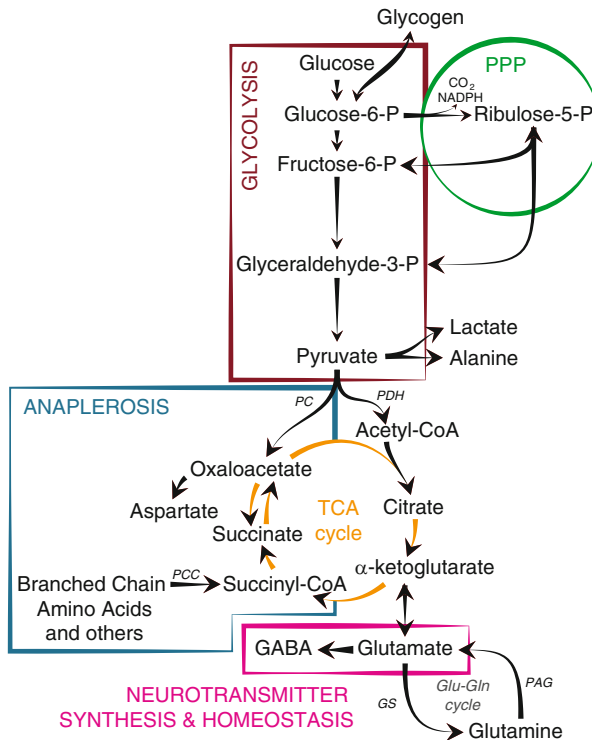
Fig. 3.1 Pathways for glucose metabolism. *Gln* glutamine, *Glu* glutamate, *KG* ketoglutarate, *ME* malic enzyme, *GS* glutamine synthetase, *NADPH* nicotinamide adenine dinucleotide phosphate, *PAG* phosphate-activated glutaminase, *PC* pyruvate carboxylase, *PCC* propionyl-CoA carboxylase, *PDH* pyruvate dehydrogenase, *PPP* pentose phosphate pathway, *TCA* tricarboxylic acid

brain (Mohler et al. 1974; Patel 1974b; Kanamatsu and Tsukada 1999). However, pyruvate carboxylation and neurotransmitter synthesis and release do not occur in the same compartment (Fig. 3.3). In the brain only astrocytes express PC (Yu et al. 1983; Shank et al. 1985; Cesar and Hamprecht 1995) but the two amino acid neurotransmitters are produced in the neurons. This dependence of neurons on astrocytes has been investigated extensively in the adult brain of animals and humans (McKenna et al. 2012) and is defined by the details of the glutamate–glutamine cycle (Fig. 3.3) which can also encompass GABA (Berl and Clarke 1983). Using ^{13}C magnetic resonance spectroscopy (MRS) and ^{13}C-labelled glucose it has been shown that pyruvate carboxylation occurs in humans, rats and mice in vivo as well as in vitro in neural tissue and cell preparations (Badar-Goffer et al. 1990; Shank et al. 1993; Hassel and Sonnewald 1995; Griffin et al. 1999; Waagepetersen et al. 2001a; Rae et al. 2005; Mason et al. 2007). An excellent review article gives an overview over the different MRS studies performed to evaluate the relationship between pyruvate carboxylation and dehydrogenation (Hertz 2011). In order to perform de novo synthesis of glutamate, an
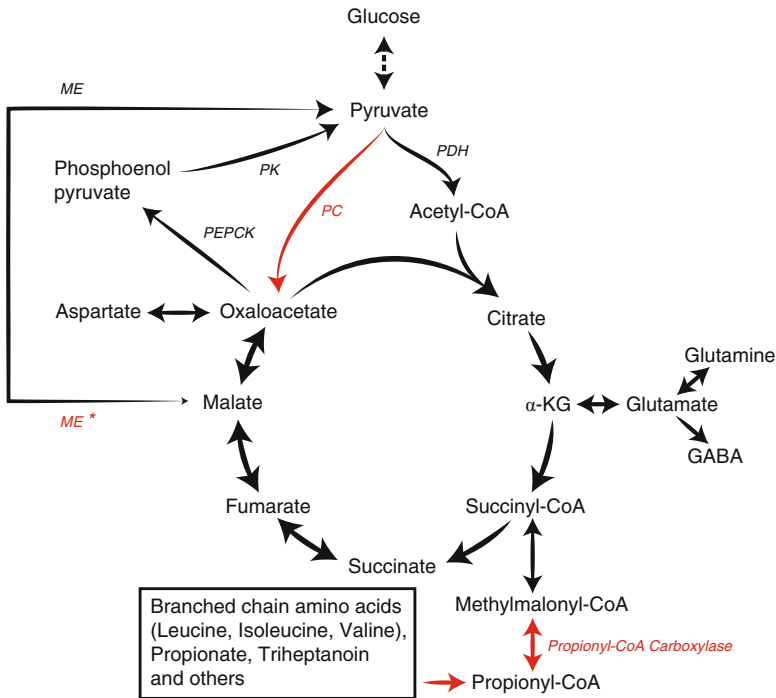
Fig. 3.2 Schematic presentation of reactions pertinent to pyruvate carboxylation. *In the brain ME appear to operate primarily in the decarboxylation direction, see Section 3.2. *KG* ketoglutarate, *ME* malic enzyme, *PC* pyruvate carboxylase, *PDH* pyruvate dehydrogenase, *PEPCK* phosphoenolpyruvate carboxykinase, *PK* pyruvate kinase

anaplerotic substrate must be transferred from astrocytes to neurons, since the latter cell type does not contain PC. In contrast to glutamate, glutamine can safely be released to the extracellular milieu without a disturbing interaction with receptors for the two mentioned amino acid neurotransmitters. When glutamine is released from astrocytes into the synapse it is taken up by specific transporters into neurons (Varoqui et al. 2000) where it can be converted to glutamate by phosphate activated glutaminase (PAG) (Hogstad et al. 1988). As can be seen in Fig. 3.3, after release in the neurotransmission process, glutamate is taken up via specialized, high affinity transporters located primarily in astrocytes (Danbolt et al. 1992; Danbolt 2001). In astrocytes, glutamate is either rapidly converted to glutamine via the enzyme glutamine synthetase (GS), localized only in astrocytes (Norenberg and Martinez-Hernandez 1979), or converted to α-ketoglutarate, thereby entering the TCA cycle (McKenna et al. 1996; Schousboe et al. 2014). This sequence of processes is termed the glutamate–glutamine cycle (Fig. 3.3) (references in (McKenna et al. 2012)), which is essential in order to ensure precise neural signalling, preserve carbon atoms for neuronal glutamate synthesis and limit excitotoxicity which may result from excessive glutamate receptor stimulation (Danbolt 2001). Only limited information is
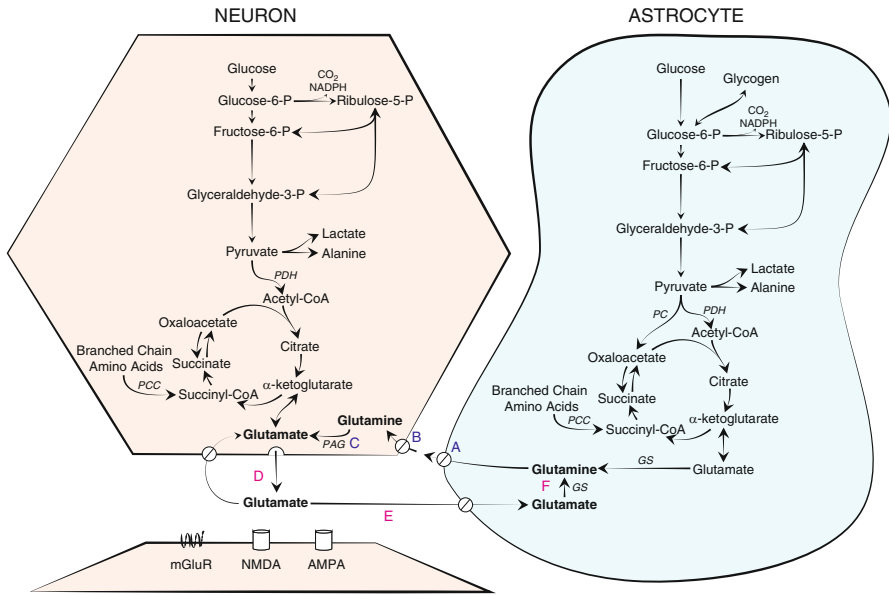
Fig. 3.3 The glutamine–glutamate cycle. *AMPA* α-amino-3-hydroxy-5-methyl-4-isoxazolepropi onic acid receptor, *A,B* glutamine transport, *C,D* glutamate transport, *E* conversion of glutamate to glutamine, *GS* glutamine synthetase, *NADPH* nicotinamide adenine dinucleotide phosphate, *NMDA* N-methyl-D-aspartate receptor, *PAG* phosphate activated glutaminase, *PC* pyruvate car- boxylase, *PCC* propionyl-CoA carboxylase, *PDH* pyruvate dehydrogenase, *mGluR* metabotropic glutamate receptors

available on the above-mentioned processes in the neonatal brain. However, there are indications that the glutamate–glutamine cycle at this stage does not operate as a cycle, but rather as a delivery system of glutamine from astrocytes to neurons, since transport of glutamate from neurons to astrocytes is negligible while that of glutamine from astrocytes to neurons is higher than in the adult brain (Morken et al. 2013; Brekke et al. 2014).

3.2 Carboxylation in the Brain

There are several enzymes that theoretically would have the potential for catalys- ing carboxylation processes in the brain (Fig. 3.2) such as pyruvate carboxylase (PC), propionyl-CoA carboxylase, phosphoenolpyruvate carboxykinase (PEPCK) and malic enzyme (ME). The latter three enzymes are present both in astrocytes and neurons (Kurz et al. 1993; Alves et al. 1995; McKenna et al. 1995, 2000; Vogel et al. 1998a, b). However, in the brain PEPCK and ME appear to operate primarily if not exclusively in the decarboxylation direction (Kurz et al. 1993; Alves et al. 1995; McKenna et al. 1995, 2000; Vogel et al. 1998a, b).

3.3   Propionyl CoA Carboxylation

The anaplerotic pathway from propionyl-CoA via methylmalonyl-CoA to succinyl-CoA (Fig. 3.2) is known to operate in peripheral tissues. Anaplerotic molecules metabolized to propionyl-CoA and the propionyl-CoA carboxylase pathway include the branched chain amino acids, isoleucine and valine (Bak et al. 2009), propionate and molecules containing fatty acids with an uneven number of carbon atoms, such as triheptanoin (Borges and Sonnewald 2012). This pathway has been shown to be active in brain and supplementation of diet with triheptanoin has been attempted as a treatment against epilepsy (Willis et al. 2010; Borges and Sonnewald 2012; Kim et al. 2013; Marin-Valencia et al. 2013; Smeland et al. 2013).

3.4   Pyruvate Carboxylation

3.4.1   *Pyruvate Carboxylation in the Adult*

Pyruvate carboxylase is an ATP-dependent mitochondrial enzyme catalysing the carboxylation of pyruvate to form oxaloacetate. It is a nuclear encoded homotetramer found in most tissue from eukaryotes and in many prokaryotes and is a member of the family of biotin-dependent carboxylases (Wallace et al. 1998). In the brain it is involved in various anabolic pathways such as lipogenesis and the synthesis of neurotransmitter substances (Wallace et al. 1998; Schousboe et al. 2015). A defect in the expression or biotinylation of pyruvate is a rare autosomal recessively inherited disorder in humans resulting in developmental delays and failure to thrive starting in the neonatal or early infantile period, early death, or severe debilitating psychomotor retardation (Wallace et al. 1998; Schiff et al. 2006).

While the cellular location of PC in astrocytes but not neurons (Yu et al. 1983) is not disputed, pyruvate carboxylation has been suggested to take place also in neurons (Hassel 2001; Merle et al. 2002). However, a whole range of experiments, using different preparations, in different laboratories have essentially unequivocally confined this carboxylation to glia. Primary mono-cultures of neurons and astrocytes were used to obtain information about the cellular location and magnitude of pyruvate carboxylation using ^{13}C labelled precursors. However, the interpretation of results obtained in these studies may be ambiguous since oxaloacetate can be converted not only to citrate but also via several steps to the symmetrical molecule fumarate (back cycling) and subsequently from oxaloacetate and citrate (cycling). The latter pathway will lead to scrambling of label resulting in misinterpretation of some labelling results. Merle et al. (2002) suggested incomplete back cycling for astrocytes and complete back cycling for cerebellar neurons. If back cycling in neurons is indeed complete, the study mentioned in the previous sentence, utilizing [1-^{13}C]glucose, was not capable of showing carboxylation in neurons. In order to

clarify this, Waagepetersen et al. (2001a) have performed a study using [U-^{13}C]glucose or [U-^{13}C]lactate and 3-nitropropionic acid to specifically block the TCA cycle at the succinate dehydrogenase step (Alston et al. 1977). In this way multiple cycling of TCA cycle constituents was avoided and carboxylation could be clearly detected in labelling of the C-3 position, regardless of back cycling. Analysing the ^{13}C labelling patterns in amino acids, pyruvate carboxylation was detected in astrocytes but not in neurons (Waagepetersen et al. 2001a). Furthermore, also in studies of brain tissue slices and cell cultures carboxylation was detected in astrocytes (Griffin et al. 1998, 2003; Waagepetersen et al. 2001a). In a very elegant study using unrestrained awake rats which were continuously infused with a combination of H^{14}CO$_3^-$ and [1-^{13}C] glucose in over 50 infusions ranging from 5 to 60 min it was shown that synthesis of glutamine from H^{14}CO$_3^-$ was substantial, amounting to 32 % of the glutamate/ glutamine cycle (Xu et al. 2004).

3.4.2 Pyruvate Carboxylation in the Neonate

Also in the neonatal brain pyruvate carboxylation is predominantly localized in glia since the level of [2,3-^{13}C]glutamine derived from [1,2-^{13}C]glucose is higher than that of [2,3-^{13}C]glutamate (Morken et al. 2013; Brekke et al. 2014). As pointed out earlier, synthesis of the neurotransmitters glutamate and GABA is coupled to pyruvate carboxylation and this is particularly important in development when neurotransmitter levels increase. GABA levels are only slightly lower at postnatal day 7 (P7) compared to later in life, but glutamate has a sharp increase early in brain development (Chowdhury et al. 2007; Morken et al. 2013). In humans, glutamate levels double from gestational week 32 until term and continue to increase during the first year of life (Kreis et al. 2002). In rats, glutamate levels double between P7 and adulthood. This increase in de novo synthesis of glutamate must happen via anaplerosis catalysed by PC.

PC activity in the rat brain is very low at birth, but increases sharply during the first weeks of life (Larsson et al. 1985), coinciding with the major period of gliogenesis (Schousboe 1972; Bandeira et al. 2009). During the first days of life, the activity of the TCA cycle-related enzymes is also low (Wilbur and Patel 1974; Larsson et al. 1985). Thus, at the time of birth, astrocytes may not have the same capacity as in the adult brain for metabolic support of neurons but the relative contribution of PC to pyruvate metabolism might still be significant. Indeed, a larger part of the available glucose is channelled into this pathway in the P7 rat than in the adult (Morken et al. 2013; Brekke et al. 2014). In fact, for every glucose molecule metabolized through glycolysis to become pyruvate, half is metabolized via pyruvate dehydrogenase, and the other half is used for anaplerosis in the P7 rat (Morken et al. 2013; Brekke et al. 2014).

Since pyruvate carboxylation occurs in astrocytes, it is possible to calculate the relative glucose utilization in astrocytes compared to neurons at the level of pyruvate using results from experiments with [1,2-^{13}C]glucose injection. This was calculated to be 34 ± 3 % of glucose metabolism at the level of pyruvate in the P7 rat

brain (Morken et al. 2013; Brekke et al. 2014). This is in surprisingly good accordance with results from the adult brain based on a corresponding equation using [U-^{13}C]glucose. Qu et al. (2000) found that astrocytes metabolize maximally 34 % of glucose at the level of pyruvate in the adult brain, and similar results have been reported by Hassel et al. (1995). This could indicate that in rats both astrocytes and neurons have lower glucose metabolism at P7 compared to adult animals. However, the distribution between nonneuronal cells and neurons changes considerably from P7 until adult age, since the number of astrocytes increases dramatically in this period to outnumber neuronal cells at adult age (Baburamani et al. 2013; Bandeira et al. 2009). This may suggest that each astrocyte has a higher metabolic rate relative to each neuron in the neonatal brain compared to later in life.

The exchange of glutamate and glutamine between neurons and astrocytes in rats is radically different in the neonatal brain compared to the adult brain (Morken et al. 2013; Brekke et al. 2014). The necessary transporters for glutamine to enter neurons are present from late gestation (Weiss et al. 2003) (Fig. 3.3B) and the number of glutamine transporters on astrocytes actually reaches higher levels than the adult brain on postnatal day 14 (Boulland et al. 2003) (Fig. 3.3A). However, the expression of astrocytic glutamate transporters (GLT-1 and GLAST) is low early in brain development (Danbolt 2001) (Fig. 3.3E), indicating that the ability of astrocytes to take up glutamate from the synapse may be limited in the neonatal brain. Indeed, the transport of newly synthesized glutamine from astrocytes to glutamatergic neurons is higher in the P7 rat brain than the adult rat brain (Morken et al. 2013, 2014) while the reciprocal transfer of glutamate from neurons to astrocytes was very low at P7 compared to adults. The latter may also be due to low release of glutamate from neurons (Fig. 3.3D) at this stage and/or low rate of conversion into glutamine in the astrocytes (Fig. 3.3F). In the neonatal rat brain the action potentials are smaller in amplitude and the firing rate is lower (McCormick and Prince 1987) and the EEG does not develop an adult pattern until P12 (Snead and Stephens 1983). Moreover, glutamine synthetase activity, just like PC activity, is very low at birth, but increases sharply during the first weeks of life (Wilbur and Patel 1974; Hertz et al. 1978; Juurlink et al. 1981; Larsson et al. 1985). Thus, several factors may lead to this difference regarding astrocyte–neuron interactions in the neonatal and the adult brain.

3.5   What Factors Affect Pyruvate Carboxylation?

3.5.1   Increased Pyruvate Carboxylation

Cerebral hyperammonemia is believed to play a pivotal role in the development of hepatic encephalopathy (HE), a debilitating condition arising due to acute or chronic liver disease (Butterworth 2003). In the brain, ammonia is thought to be detoxified via the activity of the astrocytic enzyme glutamine synthetase. However, glutamine cannot be synthesized without increased activity of PC as pointed out earlier. Zwingmann

(2007) showed that an elevated ammonia concentration increased pyruvate carboxylation both in astrocytes in culture and in animal models. This carboxylation might be coupled to the increased glutamine formation which is a hallmark of ammonia detoxification (Zwingmann 2007). Glutamine formation is the only efficient method of ammonia fixation in brain and also in other cell culture studies increased production of glutamine was detected (Leke et al. 2011). It may be of interest that upon inhibition of the glutamine synthesizing enzyme glutamine synthetase ammonia fixation is brought about by the concerted action of glutamate dehydrogenase and alanine aminotransferase leading to net synthesis of alanine, a process stimulating glycolysis to provide pyruvate for alanine production (Dadsetan et al. 2011, 2013).

The ketogenic diet is a high-fat, adequate-protein, low-carbohydrate diet that is used primarily to treat refractory epilepsy in children (Yudkoff et al. 2004). The diet causes an increase in the metabolism of fatty acids rather than carbohydrates. The effect of ketone bodies on pyruvate carboxylation by rat brain mitochondria has been investigated (Patel 1974a) and it was shown that pyruvate carboxylation increased subsequent to feeding rats a ketogenic diet (Melø et al. 2006). This increase in pyruvate carboxylation was coupled to an elevated pyruvate recycling (Melø et al. 2006), i.e. conversion of oxaloacetate to pyruvate which re-enters the TCA cycle via pyruvate dehydrogenase, as expected since synthesis of a metabolite has to be equalled by its degradation.

3.5.1.1 Neurotransmission

Activation of the glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor induces large post-synaptic action potentials (Rao and Finkbeiner 2007) and should therefore stimulate release of neurotransmitter glutamate. In experiments with brain tissue slices metabolizing [1-^{13}C]glucose it was possible to show evidence of pyruvate carboxylation in aspartate which is obtained by transamination of oxaloacetate, the product of pyruvate carboxylation by pyruvate carboxylase (Rae et al. 2009). Increased pyruvate carboxylation was detected when the slices were incubated with AMPA (Rae et al. 2009) suggesting that increased neuronal activity increases carboxylation of pyruvate, a finding compatible with the notion that glutamate biosynthesis requires anaplerosis coupled to pyruvate carboxylation. Furthermore, it was shown that pyruvate carboxylation in the awake rat brain was several fold higher than under deep pentobarbital anaesthesia (Oz et al. 2004).

3.5.2 Decreased Pyruvate Carboxylation

3.5.2.1 Anxiety and Epilepsy

Decreased pyruvate carboxylation has been reported after pentylenetetrazole (PTZ) injection in rats (Eloqayli et al. 2004). PTZ administration is a generally accepted model for epileptic seizures but when given in low doses PTZ can cause anxiety

(Eloqayli et al. 2004). Interestingly, the antiepileptic drug carbamazepine (CBZ) decreased the biotin concentration in both humans and rats and decreased pyruvate carboxylase activity in rat brain (Rathman et al. 2003). These results support the use of biotin supplementation as a concurrent strategy during CBZ administration to help maintain pyruvate carboxylation.

3.5.2.2 Ischaemia

Not surprisingly, ischaemia has a profound effect on pyruvate carboxylation, a pathway dependent on adenosine triphosphate availability. In adult rats subjected to 120 min of middle cerebral artery occlusion followed by 120 min of reperfusion it could be shown that the utilization of precursors in astrocytic metabolism originating from the pyruvate carboxylase pathway was markedly reduced compared to that of precursors originating from the pyruvate dehydrogenase pathway. Hence, glutamate synthesis was reduced and that of GABA completely stopped in the ischaemic core area (Håberg et al. 2006). Furthermore, in the re-perfused penumbra, glutamatergic and GABAergic neurons used relatively more astrocytic metabolites derived from the pyruvate carboxylase pathway than in the ischaemic core (Håberg et al. 2006). In the canine cardiac arrest model of global ischaemia, pyruvate carboxylation was decreased in the hippocampus in animals that had undergone hyperoxic resuscitation (Scafidi et al. 2009).

Interestingly, in the Rice–Vannucci model of neonatal hypoxia–ischaemia in postnatal day 7 rats, PC activity is relatively preserved compared to that of the pyruvate dehydrogenase pathway. Labelling via pyruvate dehydrogenase was reduced by 44 % while labelling via pyruvate carboxylase was reduced by 30 % in the hypoxic–ischaemic hemisphere in the Rice–Vannucci model (Brekke et al. 2014) while, as pointed out, in adult models of middle cerebral artery occlusion, PC activity is more profoundly reduced than pyruvate dehydrogenase activity (Haberg, Qu et al. 1998, 2006). This illustrates how the neonatal and adult brain can respond differently to similar insults and may need different treatments.

The consequences of relatively preserved PC activity in the neonatal brain following hypoxia–ischaemia may be ambiguous. As mentioned earlier there is negligible transport of glutamate from neurons to astrocytes during normal conditions in the neonatal brain (Morken et al. 2014). During hypoxia–ischaemia, there is a large release of glutamate that may originate from neurons as well as astrocytes through reversal of glutamate transporters (Puka-Sundvall, Sandberg et al. 1997; Kusaka et al. 2004). However, no increase in transfer of glutamate from neurons to astrocytes was found following neonatal hypoxia–ischaemia at postnatal day 7 in rats. Both glutamate uptake and glutamine synthesis are energy demanding processes, which may explain the lacking augmentation in transfer following hypoxia–ischaemia. Contradicting this, transfer of glutamine from astrocytes to neurons as well as glutamine synthesis and PC activity, which are ATP demanding processes were preserved at this time point following injury suggesting that astrocytes have at least partly maintained or re-established their energy balance (Morken et al. 2014). It is conceivable that preservation of anaplerosis and transfer of newly synthesized glutamine to neurons in the immediate recovery phase following hypoxia–ischaemia

may have harmful effects, but this remains to be investigated. On the other hand, following the massive release of glutamate into the extracellular space during hypoxia–ischaemia, neurons might be depleted of TCA cycle intermediates (such as α-ketoglutarate). Under these conditions anaplerosis might be beneficial since de novo synthesized glutamine transferred from astrocytes may be necessary for neurons to re-establish oxidative metabolism.

Surprisingly, at 6 h after hypoxia–ischaemia the transfer of glutamate from neurons to astrocytes was transiently decreased, even though at this point, the energy levels are almost back to normal (Yager et al. 1992). The exact mechanism of this has yet to be explored.

3.5.2.3 Glutamate Exposure

Qu et al. (2001) showed that exogenous glutamate had an inhibitory effect on pyruvate carboxylation in astrocytes, presumably by formation of oxaloacetate from α-ketoglutarate derived from glutamate metabolism. Inhibition of pyruvate carboxylation was also observed when glutamine was added to astrocytes (Hassel and Sonnewald 2002). When metabolism of ^{13}C-labelled glucose was investigated in cultured mouse cerebellar astrocytes, it could be shown that carboxylation of pyruvate was more important for biosynthesis of releasable glutamine and citrate, compared with their intracellular pools (Waagepetersen et al. 2001b). Activation of the cellular energy sensor, AMP-activated protein kinase (AMPK), has been shown to reduce the metabolism of glutamate in the TCA cycle of astrocytes. Specifically, the synthesis of citrate from glutamate involving malic enzyme and pyruvate carboxylase was reduced. Since AMPK is known to upregulate catabolic processes, this may suggest that glutamate has an anabolic function in astrocytes (Voss et al. 2015).

3.5.2.4 Alzheimer's Disease

Regional hypometabolism of glucose in the brain is a hallmark of Alzheimer's disease (AD) (Gibson et al. 1998, 1999; Huang et al. 2003; Klivenyi et al. 2004). However, little is known about the specific alterations of neuronal and astrocytic metabolism involved in homeostasis of glutamate and GABA in AD. In a recent study, the effects of amyloid β pathology on energy and neurotransmitter metabolism were investigated in the transgenic McGill-R-Thy1-APP rat model of AD, at age 15 months (Nilsen et al. 2014). Rats were injected with [1-^{13}C]glucose and [1,2-^{13}C]acetate, and brain extracts of the hippocampal formation as well as several cortical regions were analysed using ^{1}H- and ^{13}C nuclear magnetic resonance spectroscopy and high performance liquid chromatography. A decrease of pyruvate carboxylation in the hippocampal formation and retrosplenial/cingulate cortex was observed in the McGill-R-Thy1-APP rat and this decrease might have affected the level of glutamine in the hippocampal formation and levels of glutamate, glutamine, GABA and aspartate in the retrosplenial/cingulate cortex (Nilsen et al. 2014). Future treatment of AD patients might be targeted at increasing anaplerosis in the brain.

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