Intracellular redistribution of acetyl-CoA, the pivotal point in differential susceptibility of cholinergic neurons and glial cells to neurodegenerative signals

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Abstract

Intramitochondrial decarboxylation of glucose-derived pyruvate by PDHC (pyruvate dehydrogenase complex) is a principal source of acetyl-CoA, for mitochondrial energy production and cytoplasmic synthetic pathways in all types of brain cells. The inhibition of PDHC, ACO (aconitase) and KDHC (ketoglutarate dehydrogenase complex) activities by neurodegenerative signals such as aluminium, zinc, amyloid β-peptide, excess nitric oxide (NO) or thiamine pyrophosphate deficits resulted in much deeper losses of viability, acetyl-CoA and ATP in differentiated cholinergic neuronal cells than in non-differentiated cholinergic, and cultured microglial or astroglial cell lines. In addition, in cholinergic cells, such conditions caused inhibition of ACh (acetylcholine) synthesis and its quantal release. Furthermore, cholinergic neuronal cells appeared to be resistant to high concentrations of LPS (lipopolysaccharide). In contrast, in microglial cells, low levels of LPS caused severalfold activation of NO, IL-6 (interleukin 6) and TNFα (tumour necrosis factor α) synthesis/release, accompanied by inhibition of PDHC, KDHC and ACO activities, and suppression of acetyl-CoA, but relatively small losses in their ATP contents and viability parameters. Compounds that protected these enzymes against inhibitory effects of neurotoxins alleviated acetyl-CoA and ATP deficits, thereby maintaining neuronal cell viability. These data indicate that preferential susceptibility of cholinergic neurons to neurodegenerative insults may result from competition for acetyl-CoA between mitochondrial energy-producing and cytoplasmic ACh-synthesizing pathways. Such a hypothesis is supported by the existence of highly significant correlations between mitochondrial/cytoplasmic acetyl-CoA levels and cell viability/transmitter functions respectively.

Introduction

Brain neurons require large amounts of acetyl-CoA for energy production to support their neurotransmitter functions and synthetic acetylation reactions. Glial cells use less acetyl-CoA due to severalfold slower energy metabolism. Pyruvate derived from glucose is an almost exclusive source of this key energy metabolite feeding the TCA (tricarboxylic acid) cycle. Rates of glucose and oxidative metabolism in brain are on average 10-fold higher than in other body tissues, utilizing 20% of whole-body glucose and oxygen consumption under resting conditions (Figure 1). Accordingly, activities of PDHC (pyruvate dehydrogenase complex) and KDHC (ketoglutarate dehydrogenase complex) in whole brain and its mitochondrial fraction were found to be 4–10-fold higher than in respective preparations from non-excitable tissues. Glucose is transported into brain's interstitial compartment through the low-affinity high-capacity GLUT1 (glucose transporter 1) on the BBB (blood–brain barrier). The GLUT1 level is up-regulated by chronic hypoglycaemia and down-regulated by chronic hyperglycaemia, thereby assuring relatively stable long-term provision of glucose into the brain. Neurons avidly take up glucose thanks to the presence of insulin-independent high-affinity GLUT3 [1]. Under specific conditions such as starvation or pathological ketoacidosis, acetoacetate and β-hydroxybutyrate can partially substitute for glucose, providing acetyl-CoA through the β-ketothiolase pathway [2]. In addition, lactate released from astroglia can be taken up avidly by neuronal MCT2 (monocarboxylate transporter 2) to be converted into pyruvate and subsequently into acetyl-CoA. The lactate consumption by neurons increased proportionally to its systemic increase under conditions such as heavy exercises or hypoxia. In fact, *in vitro* experiments demonstrated that lactate/pyruvate may be better energy substrates for cultured neurons than glucose [3]. On the other hand, *in vivo* data revealed that lactate, even at high concentrations in blood could not replace glucose in full as an energy substrate for the brain [4]. Sources of this

Key words: acetyl-astragalin, cholinergic neurons, CoA, microglia, neurotoxicity.

Abbreviations: ACh, acetylcholine; ACL, ATP-citrate lyase; ACO, aconitase; CAT, carnitine acetyltransferase; ChAT, choline acetyltransferase; GLUT, glucose transporter; ICDH, isocitrate dehydrogenase; KDHC, ketoglutarate dehydrogenase complex; LPS, lipopolysaccharide; NAA, *N*-acetyl-L-aspartate; PDHC, pyruvate dehydrogenase complex; TCA, tricarboxylic acid.

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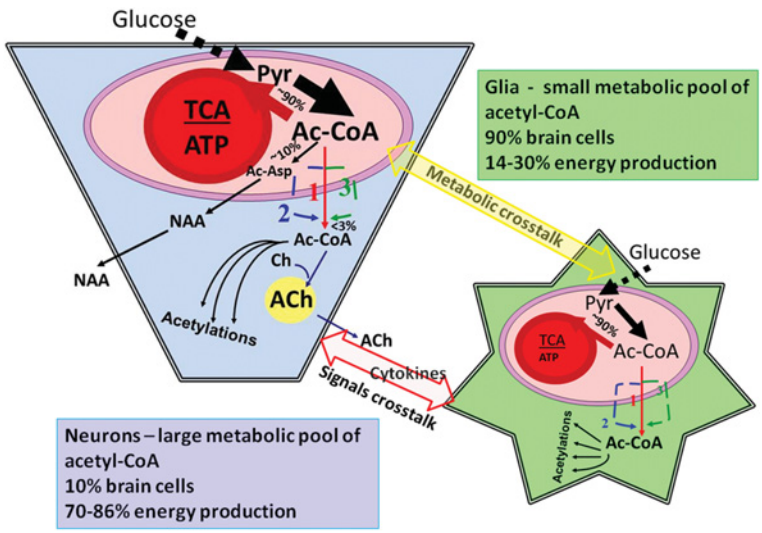
Figure 1 | Differential compartmentalization of energy and acetyl-CoA metabolism in cholinergic neuronal and glial cells in the brain

Cholinergic neurons represent a relatively small fraction (1–10 %) of diverse neuronal cells population constituting a 10 % fraction of whole brain cells. However, neurons consume approximately 80 % of oxygen supplied to the brain and produce an equivalent fraction of acetyl-CoA in mitochondria. Approximately 90 % of this metabolite is utilized for energy production to restore/maintain membrane potentials during their neurotransmitter functions. The remaining part of neuronal acetyl-CoA is utilized for NAA synthesis and hundreds of different acetylation reactions. In cholinergic neurons, an additional fraction of acetyl-CoA must be transported to the cytoplasmic compartment to maintain appropriate levels and rates of ACh release. Acetyl-CoA transport through the mitochondrial membrane takes place through: direct (1), ACL-dependent (2) and CAT-dependent (3) transport pathways [6,17]. Targets for neurotoxic factors have been described in [9]. Pathways of acetyl-CoA metabolism in the glial compartment are similar, although their rates are apparently lower than in neurons and they synthesize neither NAA nor ACh. Ac-Asp, acetylaspartate; Ac-CoA, acetyl-CoA; Ch, choline; Pyr, pyruvate.



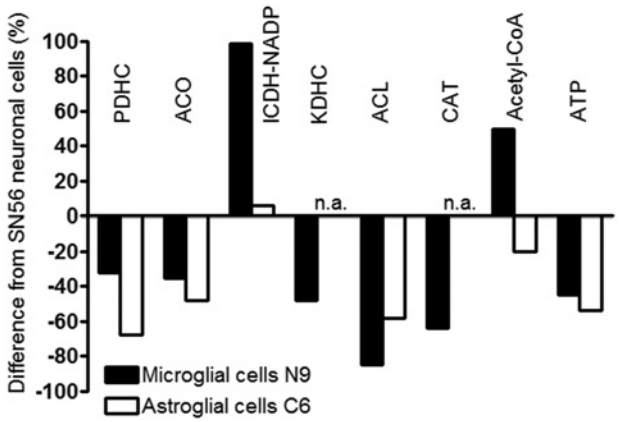
inconsistency have not been found. Nevertheless, there is evidence that acetyl-CoA plays an exclusive role as a merging point of metabolism of multiple precursors and intermediates. It is also a starting point for many hundreds of acetylation reactions in each subcellular compartment in a specific pattern for each cell type. For instance, L-aspartate was found to be acetylated exclusively in neuronal mitochondria, yielding the most abundant brain metabolite NAA (*N*-acetyl-L-aspartate). Owing to its high level (~10 mmol/kg), NAA serves as an indirect marker of energy equilibrium and acetyl-CoA availability in the brain. There are suggestions that NAA synthesis may consume up to 10 % of the neuronal acetyl-CoA pool [5] (Figure 1). On the other hand, ACh (acetylcholine) is synthesized from acetyl-CoA exclusively in the cytoplasmic compartment of the cholinergic nerve terminals. This transmitter function may utilize up to 3 % of the metabolic output of PDHC of cholinergic neurons [6]. In addition, acetyl-CoA, after its conversion into citrate, may be transported from mitochondria to the cytoplasm. In this compatment, acetyl-CoA is resynthesized from citrate through the ACL (ATP-citrate lyase) pathway, to feed the ChAT (choline acetyltransferase) reaction. This pathway is linked with consumption of ATP. Glial cells do not utilize their own acetyl-CoA for those two synthetic pathways, which might contribute to their greater resistance to degenerative conditions, frequently accompanied by inhibition of acetyl-CoA synthesis in the PDHC reaction [7–9] (Figure 1). Such a diversity of specific acetyl-CoA-consuming pathways in different brain cell classes suggests the existence of significant differences in activities of enzymes involved in its metabolism, that in turn may variably modify reactions of different cell types to similar neurotoxic conditions.

Energy and acetyl-CoA metabolism in encephalopatic brain

The predominant losses of cholinergic innervation have been documented in autopsied brain cortices of AD (Alzheimer's disease), dialysis encephalopathy or thiamine-deficient patients by decreases in levels of cholinergic markers: ChAT, muscarinic presynaptic M_2 receptors or vesicular ACh transporter or high-affinity choline uptake system [9–11]. Also losses of PDHC, KDHC, respiratory chain complexes and other enzymes of energy and acetyl-CoA metabolism were found to take place in these pathologies [12]. The depth of those deficits demonstrated strong correlations with deterioration of cognitive functions according to the

Figure 2 | Relative differences between enzymological and metabolic parameters of energy and acetyl-CoA metabolism in cholinergic neuronal SN56 (baseline) and microglial N9 and astroglial C6 cells

Baseline corresponds to activities of: PDHC, 7.60 ± 0.25; ACO, 33.5 ± 3.5; ICDH–NADP, 20.8 ± 0.5; KDHC, 4.1 ± 0.3; ACL, 38.4 ± 1.4; CAT, 14.7 ± 0.7 nmol/min per mg of protein, and levels of acetyl-CoA of 27.0 ± 2.2 pmol/mg of protein and ATP of 20.3 ± 1.3 nmol/mg of protein ([18,23,26,30,33], A. Dyś and J. Klimaszewska-Łata, unpublished work).



Mini Mental State of Examination/Mattis Dementia Rating Scale, diagnosed shortly before the patient's death [13]. These findings indicate that inhibition/inactivation of PDHC may both trigger depression in cholinergic neurotransmission and compromise cholinergic neuron viability due to deficits of acetyl-CoA [6,12,14–16] (Figure 1).

However, those studies were performed in samples of whole brain cortex. Thereby, they reflected neither compartmentalization nor possible differences in enzyme alterations taking place in each of particular neuronal and glial cell compartments of the diseased brain. The same reservation applies to putative differences in acetyl-CoA and energy metabolism, which may contribute to variable susceptibility of specific groups of brain cells to the same pathological conditions (Figure 2). They also did not identify individual neurodegenerative signals that could inhibit/inactivate one or more of the enzymes, specified above, in either all or selected brain cell types.

Compartmentalization of acetyl-CoA and energy metabolism in brain cells

The partial solution for this problem may be cytotoxicity/cytoprotection studies performed on isolated primary neuronal and glial or specific clonal respective cell lines [7,9]. Those studies revealed that activities of energy metabolism enzymes such as PDHC, ACO (aconitase), KDHC and enzymes of acetyl-CoA metabolism in cholinergic neuronal, microglial or astroglial cells lines may vary markedly, yielding differences in their acetyl-CoA and ATP levels (Figure 2). In fact, activities of PDHC, ACO and KDHC in neuronal SN56 cells were from 50 to 200 % higher than in microglial N9 or astroglial C6 cells, which is compatible with greater energy demand to support a high rate of neurotransmission-linked depolarization/repolarization cycles in neuronal SN56 cells (Figure 2). Also, activities of CAT (carnitine acetyltransferase) and ACL, involved in transport of acetyl-CoA to the cytoplasmic compartment, appeared to be from 3- to 7-fold higher respectively than in microglial cells (Figure 2). Such a pattern would be compatible with the demand of cholinergic cells for acetyl-CoA necessary for cytoplasmic ACh synthesis [6,17]. These data preclude significance studies performed in whole brain tissue for explaining acetyl-CoA-dependent mechanisms in individual types of brain cells. For instance, whole microglial cell acetyl-CoA levels were found to be 50 and 90 % higher than in cholinergic neuronal and astrocytic cells respectively (Figure 2).

The increase in expression of cholinergic phenotype in SN56 neuronal cells through *TrkA* (tropomyosin receptor kinase A) or *ChAT* gene inserts caused a decline in whole-cell acetyl-CoA levels [11,18]. This indicates that up-regulation of cholinergic neurotransmission may limit the availability of acetyl-CoA for energy production. Moreover, phenotypic modifications caused by physiological differentiating signals markedly altered acetyl-CoA distribution between mitochondrial and cytoplasmic compartments of those cholinergic cells. Thus the increase in expression of the cholinergic phenotype in septal neuroblastoma cells by cAMP/ATRA (all-*trans*-retinoic acid) was linked with a 50 % decline in acetyl-CoA in the mitochondrial compatment and its 100 % elevation in the cytoplasmic compartment [19]. Such redistribution of acetyl-CoA from the mitochondrial to the cytoplasmic compartment would be compatible with the high demand of mature cholinergic neurons for cytoplasmic acetyl-CoA for ACh synthesis [20]. These data indicate that this redistribution may take place through PTP (protein tyrosine phosphatase)–Ca^{2+}-dependent mitochondrial channels in depolarization-activated cholinergic neurons [18,20,21]. On the other hand, such conditions might augment the vulnerability of cholinergic neurons to neurotoxic influences due to a transient shortage of acetyl-CoA in mitochondria [9,19].

Compartmentalization of acetyl-CoA metabolism under neurotoxic conditions

Exposure of different cell types to diverse neurodegenerative signals such as aluminium, zinc, amyloid β-peptide, NO excess or thiamine pyrophosphate deficits differentially suppressed activities of PDHC, ACO and KDHC in cholinergic and non-cholinergic cells. Zinc, ROS (reactive oxygen species) and NO-derived peroxynitrite radicals, are known components of excitotoxicity caused by an excessive activity of glutamatergic neurons under a vast range of neurodegenerative conditions [22–27]. Exposure of

Table 1 | Differential effects of common neurotoxic signals on acetyl-CoA metabolism and viability of cholinergic SN56 neuronal, N9 microglial and C6 astroglial cells in culture

NC, non-differentiated cells; DC, cAMP/retinoic acid-differentiated cells.

Cytotoxic compound concentration (mmol/l)/exposure time (h)	Cell type	PDHC (% of change)	Acetyl-CoA (% of change)	Non-viable cells (% of total)	Reference(s)
Zinc 0.10/30 min	SN56NC	−31	−23	22	[27], A. Dyś, unpublished work
	SN56DC C6	−38	−53	42	
		+24	0	3	
SNP 1.0/10 min SNP 0.4‡ 24 h	SN56NC	−8	0	15	[8,22,23], J. Klimaszewska-Łata, unpublished work
	SN56DC	−28	−42	27	
	SN56DC N9	−31	−39	26	
		−53	−2	4	
LPS 1.0*/24 h LPS 0.01*†/24 h LPS 1.0*†/24 h	SN56 DC N9	+1	+2	3	J. Klimaszewska-Łata, unpublished work
		−22	−20	4	
		−48	−35	7	
Amyloid-β_{25-35} 0.001/24 h	SN56NC	−7	−8	11	[22,23,31]
	SN56DC	−20	−40	31	
Aluminium 1.0/72 h	SN56NC	−2	−29	9	[8,41]
	SN56DC	−39	−36	14	
Amprolium 2.0/48 h	SN56NC	−27	−37	5	[42]
	SN56DC	−44	−46	13	

*LPS additions in μg/ml medium.

†Addition of 0.01 and 1.0 μg/ml LPS to N9 culture caused synthesis/release of endogenous NO at rates of 620 and 820 nmol/h per litre of medium, respectively.

‡Addition of 0.4 mmol/l SNP caused generation of exogenous NO at rate of 690 nmol h^{-1} l^{-1} medium (J. Klimaszewska-Łata, unpublished work).

differentiated SN56 cholinergic cells to pathophysiologically relevant concentrations of zinc or NO strongly inhibited activities of PDHC, ACO or KDHC *in situ*. On the other hand, in non-differentiated SN56, microglial or astroglial cells, these cytotoxins caused less or no inhibition of their activities (Table 1). That may be because such conditions resulted in much greater loses of viability in differentiated cholinergic neuronal cells than in the non-differentiated cholinergic or microglial/astroglial cells, due to a greater reduction in acetyl-CoA and ATP levels in the former [9,28] (Table 1). Lipoamide/lipoic acid which protected PDHC and ACO, in neuronal cell homogenates, against direct inhibition by zinc/NO, was also found to overcome depletion of acetyl-CoA and rescued cultured cells [23,26].

These data support the hypothesis that enzyme-inhibition induced depletion of acetyl-CoA is an important element in mechanism(s) promoting preferential loss of cholinergic neurons in encephalopatic brains [9]. That does not preclude the appearance of other pathogenic alterations in neurotoxin-challenged neurons. It has been shown that the exposure of cholinergic neuronal cells to zinc, aluminium or NO brought about severalfold increases in their Ca^{2+} contents, triggering a vast range of cell-damaging reactions including release/activation of several pro-apoptotic peptides from mitochondria [8,22,27,29,30]. Increased expression of voltage-gated calcium channels in cAMP/NGF (nerve growth factor)-differentiated SN56 cells, accompanied by 2–4-fold elevations in Ca^{2+} levels, could also make them more susceptible to neurodegeneration [19,29,31].

Astroglial and microglial cells are known to be relatively resistant to neurodegenerative conditions despite multiple aberrations in their functions [7,24]. Moreover, microglial cells retain proliferative potential, which is a key element of their inflammatory response under various pathogenic conditions. Activation of both primary and clonal cultured microglial cells by LPS (lipopolysaccharide) caused TLR-4 (Toll-like receptor 4)-mediated severalfold increases in the release/synthesis of NO, IL-6 (interleukin 6), TNFα (tumour necrosis factor α) and other pro-inflammatory cytokines [24,32]. Exposure of microglial cells either to endogenous LPS-induced or exogenous SNP (sodium nitroprusside)-derived NO excess, was found to exert weak inhibition of PDHC and stronger inhibition of ACO and KDHC activities (Table 1). As a result, only small or no decreases in microglial acetyl-CoA, ATP and viability were observed (Table 1). Such an enzyme pattern would be compatible with paradoxically high levels of acetyl-CoA and low levels of ATP in the microglial cells, and low contents of both energy intermediates in the astroglia ([33], and A. Dyś and J. Klimaszewska-Łata, unpublished work) (Figure 2).

In addition, relatively high activity of ICDH (isocitrate dehydrogenase)–NADP, which is insensitive to NOO^-,

could support self-protection of microglia against endogenous and exogenous NOO^- through provision of NADPH for glutathione synthesis [34,35]. Also, the glutathione pool in astroglial cells was well preserved upon exposition to NO excess [36].

Astroglia are known to exert several neuroprotective functions through the supply of lactate, glutamine, glutathione and other metabolites into the neurons, as well as by the uptake of glutamate, zinc and various cytotoxic compounds from the synaptic cleft [37,38]. Oxidative metabolism in microglia is relatively slow, apparently due to lower activities of PDHC and KDHC than in neurons (Figure 2). That would result in a rate of acetyl-CoA production lower than in neurons and its utilization in the TCA cycle. Astrocytes are capable of accumulating zinc co-released as a complex with glutamate during excitation of glutamatergic terminals [27,39]. Thereby, they may alleviate possible cytotoxic effects of glutamatergic neuron overstimulation. In addition, PDHC and ACO *in situ* in astrocytes were resistant to their exposure to high, but pathophysiologically relevant, concentrations of zinc in the extracellular space (Table 1). Finally, these conditions altered neither acetyl-CoA levels nor viability of astrocytic cells. On the other hand, astrocytes were reported to be sensitive to nitrosative stress in ammonia neurotoxicity [40]. However, their acetyl-CoA status under those conditions remains unknown.

Previous findings indicate that different populations of brain cells display variable parameters of acetyl-CoA metabolism. Such specific metabolic diversity may determine either susceptibility or resistance of neurons or glia respectively to various neurodegenerative signals. Preservation of key pathways of acetyl-CoA seems to be the prerequisite condition for sustaining the viability and functional competency of any type of brain cell under diverse pathological conditions.

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