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Interplay between NAD+ and acetyl-CoA metabolism in ischemia-induced mitochondrial pathophysiology☆

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Abstract

Brain injury caused by ischemic insult due to significant reduction or interruption in cerebral blood flow leads to disruption of practically all cellular metabolic pathways. This triggers a complex stress response followed by overstimulation of downstream enzymatic pathways due to massive activation of post-translational modifications (PTM). Mitochondria are one of the most sensitive organelle to ischemic conditions. They become dysfunctional due to extensive fragmentation, inhibition of acetyl-CoA production, and increased activity of NAD^+ consuming enzymes. These pathologic conditions ultimately lead to inhibition of oxidative phosphorylation and mitochondrial ATP production. Both acetyl-CoA and NAD^+ are essential intermediates in cellular bioenergetics metabolism and also serve as substrates for post-translational modifications such as acetylation and ADP-ribosylation. In this review we discuss ischemia/reperfusion-induced changes in NAD^+ and acetyl-CoA metabolism, how these affect relevant PTMs, and therapeutic approaches that restore the physiological levels of these metabolites leading to promising neuroprotection.

Keywords

Ischemia; Brain; Mitochondria; NAD^+; Acetyl-CoA

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1. Introduction

At the onset, brain ischemia triggered by severely limited or absent blood flow, can be considered an acute mitochondrial disease. This is because the essential mitochondrial function, oxidative phosphorylation that generates ATP, is the first one ceased due to the depletion of tissue oxygen. Although the brain represents only 2% of body weight it is particularly sensitive to ischemic conditions since it utilizes about 20% of the total body oxygen consumption under resting conditions. Due to the ATP requirement for the majority of cellular functions, ischemia impacts practically every aspect of cellular metabolism in all cell types. Therefore, ischemia-induced brain damage is one of the most complex and devastating neurological condition.

Mitochondria are one of the major targets for development of effective treatment strategies in both acute brain injury and neurodegenerative diseases. They are essential in the maintenance of normal cellular functions, mainly through regulation of energy production [1–5]. The cells' energy demand is affecting not only the activity of mitochondrial oxidative phosphorylation but also the mitochondrial structure and movement. Mitochondria respond to energetic stress by re-organizing their sub-cellular distribution and also by structural and morphological alterations [4,6–8]. This unique ability of mitochondria to spatially and morphologically adapt to changing intracellular environments is termed "mitochondrial dynamics".

Bioenergetic stress and downstream effects caused by ischemic insult depletes NAD^+ levels and compromises several metabolic pathways and mitochondrial functions [9–13]. Mitochondrial protein acetylation is dramatically reduced suggesting a deficiency in production of acetyl-CoA (AcCoA), a substrate for acetyltransferases. Additionally, mitochondria are extensively fragmented leading to failure in oxidative phosphorylation [14–16]. All these events lead to bioenergetics failure and ultimately cell death. In this review we will address the metabolic links between NAD^+, AcCoA, and mitochondrial dynamics that are altered by ischemia/reperfusion triggered conditions.

2. Role of mitochondrial dynamics in mechanisms of pathophysiology

The state of mitochondrial dynamics is determined by the balance between activities of fission and fusion processes. There are several physiological functions of mitochondrial fission. Fragmented mitochondria can move more efficiently within the cell to reach areas where there is a higher demand for local ATP generation. Furthermore, by fragmentation the damaged and healthy mitochondrial proteins and DNA can be segregated into separate smaller organelles allowing the damaged, dysfunctional subpopulation to be eliminated by mitophagy [17,18]. Finally, during cell division mitochondrial fragmentation facilitates proper redistribution of mitochondrial mass into daughter cells [19]. However, an extensive and prolonged fission due to pathologic stress can lead to transformation of the whole mitochondrial population into submicron size organelles [14]. These individual organelles are too small to harbor the required amount of all essential metabolites and proteins for proper and effective function. Thus, the cellular demand for ATP generation cannot be met and ultimately will lead to bioenergetics failure and cell death. To reverse this process, the

fragmented mitochondria need to fuse back so the contents of the small organelles can combine and stabilize protein and DNA levels for normal mitochondrial function. Therefore, fusion, by combining the contents of functionally compromised small organelles into functioning mitochondria, mitigates the effects of cellular stress. For example, CA1 neurons in the hippocampus are the most vulnerable to ischemic attack and mitochondria in these neurons are extensively fragmented following ischemic insult [14,15]. This highly fragmented state lasts for several days and CA1 neurons ultimately die. On the other hand the CA3 and dentate gyrus neurons of the hippocampus are resistant to ischemic conditions [20–22]. Although, mitochondria in these cells are fragmented directly after the ischemic insult, later at 24 h of reperfusion the highly fragmented population is significantly reduced, and the number of longer mitochondria increases when compared to immediate post-insult state [14]. This suggests that at later recovery time, factors stimulating the fission process are diminished or the fusion activity is sufficiently increased to reverse the fission process. Interestingly, similar temporal profile of mitochondrial fragmentation is observed also in astrocytes following acute brain injury [14,23].

Mitochondrial fission and fusion is a highly controlled process by several cytosolic and mitochondrial proteins belonging to the GTPase family (for review see [24–27]). There are a separate set of fusion proteins that control the outer and the inner membrane fusion. Mitofusin1 and mitofusin2 (Mfn1 and Mfn2) mediate the mitochondrial outer membrane fusion, while the inner membrane fusion is regulated by dynamin-like GTPase encoded by optic atrophy 1 gene (Opa1) [18,28]. Fission is facilitated by the dynamin-related protein1 (Drp1) which needs to be recruited from the cytosol to the outer mitochondrial membrane. Several proteins on the outer membrane serve as recruitment factors for Drp1, mitochondrial fission factor (MFF), Fis1 protein, and mitochondrial dynamic proteins 49/51 (MiD49/51) [29,30]. Overall modulation of the fission and fusion process is rather complex, involving several post-translational modifications [4,24,26,27]. Thus, the activity of these proteins is tightly regulated by phosphorylation, acetylation, ADP-ribosylation, S-nitrosylation, SUMOylation, ubiquitination, *o*-linked-*N*-acetyl-glucosamine glycosylation, and proteolytic cleavage [27,31–33]. Maintenance of the proper balance between mitochondrial fission and fusion by post-translational modifications is essential not only for facilitating normal mitochondrial bioenergetic function but also for dynamic cellular stress response to pathological conditions. In next paragraphs we discuss the impact of ischemia on NAD^+ and AcCoA metabolism that modulates acetylation of cellular and mitochondrial proteins.

3. NAD^+ metabolism and the cellular and mitochondrial acetylome

3.1. NAD^+ metabolism and pathophysiology of brain injury

NAD^+ is one of the most abundant metabolic intermediate that is required for about 500 enzymatic reactions. As a cofactor it is essential for activity of pyruvate dehydrogenase complex (PDHC) and several mitochondrial enzymes in the TCA cycle that reduce NAD^+ to NADH. The matrix localized NADH serves as an electron donor to complex I of the respiratory chain where it is reoxidized back to NAD^+. Similarly, NADH (and its phosphorylated form NADPH) participates as a cofactor in the glycolytic pathway, pentophosphate pathway, ketone body, fatty acid, and amino acid metabolism.

NAD^+, apart being a cofactor, also serves as a substrate for NAD^+-consuming enzymes that catalyze NAD^+-dependent protein modifications including poly- and mono-ADP-ribosylation [34]. The CD38 enzyme that generates the second messenger cyclic-ADP-ribose (cADPR) also utilizes NAD^+ as a substrate [35]. Finally, sirtuins, a class III NAD^+ dependent de-acetylases remove the acetyl group from a target protein and transfer it on the ADP-ribose moiety after release of nicotinamide (Nam) [36–38] (Fig. 1).

The NAD^+ pools have been shown to decrease due to pathologic conditions including ischemic insult or traumatic brain injury (TBI) [39–41]. This is caused by the generation of free radicals during reperfusion leading to DNA damage and activation of NAD^+ consuming poly-ADP-ribose polymerase 1 (PARP1) [9,42]. PARP1 utilizes NAD^+ as a substrate for poly-ADP-ribosylation of specific nuclear proteins, including histones, to facilitate DNA repair [43,44]. Uncontrolled activation of PARP1 can deplete cellular NAD^+ leading to inhibition of ATP production and cell death [45,46]. The increase in poly-ADP-ribose (PAR) levels was reported already after the first 2 h of recovery following ischemic insult and was associated with NAD^+ depletion [9,40,42,47]. An additional depletion of tissue NAD^+ pools was observed at 24 h of recovery, which is linked to an increased activity of CD38 [47]. The NAD^+ catabolism after acute brain injury was reduced by treating the animals with PARP1 inhibitors [9,39,42] or in PARP1 null animals [48].

All enzymes that utilize NAD^+ as a substrate cleave the nicotinamide (Nam) moiety and generate ADP-ribose (for review see [13]). The released Nam can then be recycled via the NAD^+ salvage pathway by nicotinamide phosphotransferase (Nampt). Nampt generates nicotinamide mononucleotide (NMN) from Nam and phosphoribose pyrophosphate (PRPP). The NMN is then used by nicotinamide mononucleotide adenylyl transferase (NMNAT) to synthetize NAD^+ in the presence of ATP [13,49–51] (Fig. 1).

Another approach applied to prevent the depletion of post-ischemic NAD^+ pools was to stimulate the NAD^+ salvage pathway by administering precursors, Nam or NMN [40,52,53]. By feeding into the NAD^+ salvage pathway one can facilitate NAD^+ synthesis. However, Nam and NMN also inhibit PARP1 and CD38 [40,47,52,54,55] and therefore also inhibit the NAD^+ depletion by reducing its degradation.

3.2. Brain injury and mitochondrial NAD^+ metabolism

Although, it was established that acute brain injury is associated with brain tissue NAD^+ depletion it is still elusive in which subcellular compartment the NAD^+ levels are the most affected. In brain cells, depending on the cell type, 25% (in astrocytes) to 50% (in neurons) of NAD^+ is localized to mitochondria [56]. The mechanisms that maintain mitochondrial NAD^+ pools remain unclear.

Nampt, the rate-limiting enzyme of the NAD^+ salvage pathway, has been detected in the nucleus and cytoplasm [57]. In the brain it is expressed mainly in the cytoplasmic fraction of neurons [58]. Therefore, to generate NAD^+ in the mitochondria the product of Nampt, NMN, needs to be transported into the mitochondrial matrix. Mitochondrial NAD^+ is then synthetized by mitochondrial isoform of NMNAT (NMNAT3) [59,60]. However, the mechanisms of NMN transport across the mitochondrial membrane are not known.

One could also replenish mitochondrial NAD^+ via its translocation from the cytosolic compartment. In yeast and plants, a membrane carrier protein transporting NAD^+ across the inner mitochondrial membrane from the cytoplasm into mitochondria has been identified [61,62]. Surprisingly, overexpression of plant and yeast mitochondrial NAD^+ carrier in human cells caused a switch from oxidative phosphorylation to glycolytic metabolism, reduction of cellular ATP levels, and resulted in dramatic growth retardation [63]. Thus, these data suggest that direct NAD^+ import is likely to be absent from mammalian mitochondria and the major mechanism that replenishes intra-mitochondrial NAD^+ is the NMNAT3 enzyme driven synthesis [60,63]. However, recent findings challenge this conclusion [64] and also the presence of active NMNAT3 enzyme in human mitochondria was questioned [65]. Thus, until the NMN or NAD^+ transporter in mammalian mitochondria will be unequivocally identified and characterized, the intra-mitochondrial NAD^+ metabolism will remain a matter of debate.

There are two possible mechanisms that can lead to reduction of mitochondrial NAD^+ levels. First, under high oxidative stress or mitochondrial calcium overload a large, high conductance pore can be formed in the inner mitochondria membrane, called the mitochondrial permeability transition (MPT) pore [66]. Since it allows solutes of molecular weight up to 1500 Da to diffuse across the inner membrane, the activation of MPT leads to mitochondrial depolarization, leakage of mitochondrial NAD^+ into the cytosol, and osmotic swelling [67–69]. Even if pore opening is transient and does not lead to an extensive mitochondrial swelling, a potentially significant loss of matrix NAD^+ will result in inhibition of all NAD^+ dependent metabolic processes including oxidative phosphorylation [70].

Second, mitochondrial NAD^+ pools can also be reduced by activation of intra-mitochondrial enzymes that utilize NAD^+ as substrate. The major NAD^+ consuming enzyme following TBI or ischemic brain injury is PARP1. Although there are reports suggesting a presence of intra-mitochondrial PARP1 activity causing increase in poly-ADP-ribosylation of mitochondrial proteins following TBI, so far there is no consensus whether it can lead to pathologic depletion of intra-mitochondrial NAD^+ pools [13]. Furthermore, to our knowledge there are no systematic studies examining the effect of acute brain injury on brain mitochondrial NAD^+ pools.

3.3. NAD^+ and mitochondrial protein acetylation

Sirtuins (Sirts) serve as metabolic sensors due to their dependence on NAD^+ as a substrate [36]. The Sirt family of proteins is comprised from seven members that show a discrete pattern of subcellular localization. Sirt1, Sirt6, and Sirt7 are localized in the nucleus but also reports show a presence of Sirt1 in the cytosol, suggesting that Sirt1 can shuttle to the cytosol under specific circumstances [71]. Sirt2 is localized in the cytosol, and Sirt3, Sirt4, and Sirt5 were identified as mitochondrial proteins [72]. However, only Sirt3 is considered the major mitochondrial deacetylase [73,74]. Stimulation of Sirt3 by caloric restriction or following administration of NAD^+ precursors leads to activation of TCA cycle enzyme glutamate dehydrogenase (GDH) [73] and isocitrate dehydrogenase 2 (IDH2) [75]. Furthermore, Sirt3 deacetylates components of the mitochondrial respiratory complexes,

interacts with ATP synthase [76], and activates mitochondrial superoxide dismutase (SOD2). Deacetylation of SOD2 protects the cells against reactive oxygen species (ROS) [75,77]. Finally, Sirt3 plays an important role in protecting mitochondria against excitotoxic insult by deacetylating cyclophilin D (cypD), which leads to inhibition of its activity. CypD is a major regulator of the MPT pore [78]. As mentioned in the previous paragraph a prolonged opening of the MPT pore leads to dissipation of mitochondrial membrane potential, loss of matrix solutes including NAD^+, inhibition of oxidative phosphorylation, cessation of mitochondrial ATP production, and swelling [68,79,80]. Thus, by deacetylating and inhibiting CypD, Sirt3 prevents MPT formation, and helps to maintain mitochondrial functions under stress conditions [77,81].

In summary, loss of mitochondrial NAD^+ has multiple pathologic consequences that are associated with NAD^+ roles as a cofactor for several key metabolic enzymes and also as a substrate for post-translational modifications. Therefore, depleted mitochondrial NAD^+ pools lead to inhibition of oxidative phosphorylation and the TCA cycle enzyme activity. Furthermore, the activity of intra-mitochondrial enzymes that utilize NAD^+ as a substrate for signaling reactions, most importantly deacetylation, are compromised. As a result, due to the reduced activity of Sirt3, increased acetylation of CypD and SOD2 will increase mitochondrial sensitivity to MPT inducing stress and mitochondria will be a more active source of free radicals.

4. Metabolic interplay of NAD^+ and acetyl-CoA

4.1. Acetyl-CoA, protein acetylation, and ischemic injury

The acetylation status of proteins is determined by the dynamic interplay between deacetylases and acetyl-transferases. The level of mitochondrial protein acetylation is controlled by mitochondria specific acetyl-transferase, GCN5L1, and deacetylase, Sirt3 [82] (Fig. 2). Acetyl-transferases use AcCoA as source of acetyl groups that are transferred onto lysine residues of the target protein. In brain cells the AcCoA is a metabolite mainly derived from glucose [83]. During glycolysis, glucose is converted to pyruvate, which is transported into mitochondria where the mitochondrial pyruvate dehydrogenase complex (PDHC) catalyzes the oxidative decarboxylation of pyruvate to generate AcCoA. As mentioned above PDHC requires NAD^+ as a cofactor since during this process the NAD^+ is reduced to NADH. In the next step of the TCA cycle mitochondrial citrate synthase (CS) forms citrate from AcCoA and oxaloacetate (Fig. 2). Citrate can either be oxidized by aconitase in the TCA cycle or it can be transported to the cytosol as a substrate for the ATP citrate lyase (ACLY). This enzyme generates cytosolic AcCoA from citrate in the presence of ATP (Fig. 3) [84]. Furthermore, in cytosol AcCoA can also be generated from acetate by acetyl-CoA synthetase (ACECS1) (for review see [85]). During this reaction ATP is used and pyrophosphate is also released. Two isoforms are known in mammalian cells [86].

Interestingly, mainly neurons are immunopositive for acetylated histones [87], probably due to their higher AcCoA levels. This is most likely because although neurons constitute only about 10% of brain cells they consume 70% of glucose and oxygen supplied to this organ. Thus, high glycolytic and TCA cycle metabolic flux leads to generation of higher AcCoA levels that drive the acetyl-transferase activity. This is then reflected in increased acetylation

of neuronal histone and non-histone proteins. To our knowledge there are no reports of either cell-type specific or subcellular distribution of AcCoA pools in the brain. Furthermore, studies examining changes in cellular or mitochondrial AcCoA levels following ischemic insult would be also required to shed more lights on mechanism that lead to post-ischemic pathophysiology.

During ischemia the glucose and oxygen delivery to the brain is abolished and the production of AcCoA is discontinued after glucose pools are depleted [88,89]. As a consequence of reduced cytosolic and nuclear AcCoA levels the histone acetylation is significantly reduced following ischemia [87,90]. This substantial reduction of histone acetylation affects chromatin folding, the control of DNA accessibility and transcriptional activation [91], leading to deficiency in proper response to stress conditions. The pathologic implications of such excessively low levels of histone acetylation are supported by the neuroprotective effect of class I, II and IV histone deacetylase (HDAC) inhibitors [87,92,93]. Treatment of animals subjected to ischemia with pan HDAC inhibitors such as Trichostatin A (TSA) or suberanilohydroxamic acid (SAHA), normalized the post-ischemic histone acetylation and resulted in significant neuroprotection [92], for review see [94].

Interestingly, to achieve neuroprotective effects against ischemic brain damage the increased activity of class III NAD^+-dependent HDACs, Sirts, are required. This is probably because Sirt targets control expression of genes involved in neuroprotection pathways and also Sitr1, Sirt2, and Sirt3–5 modulate activity of non-histone proteins and transcription factors linked to cellular bioenergetic metabolism, inflammation, and autophagy. Since cellular NAD^+ levels are significantly depleted following acute brain injury, the activity of these enzymes is compromised during the recovery period. Thus, by replenishing the NAD^+ levels or administering Sirt activators (such as resveratrol) the acetylation of the target proteins can be restored. Although reports are not available, one would expect that the loss of mitochondrial NAD^+ would lead to the inhibition of Sirt3 activity and increased acetylation of mitochondrial proteins. As mentioned above this could lead to further inhibition of oxidative phosphorylation and the TCA cycle with increased sensitivity of mitochondria to MPT inducing stress and higher ROS production rates. Replenishing the mitochondrial NAD^+ levels could then reverse the negative effect of hyperacetylation due to activation of Sirt3.

4.2. N-acetyl-aspartate as indicator of neuronal damage and source of acetyl-CoA in non-neuronal brain cells

N-acetyl-aspartate (NAA) is the most abundant acetylated brain metabolite synthetized in neuronal mitochondria. Synthesis of NAA is catalyzed by L-aspartate *N*-acetyltransferase (Asp-NAT) via *trans*-acetylation of AcCoA and aspartate. Several studies demonstrated that in adult rat brain NAA synthesis takes place in neuronal mitochondria from AcCoA, generated from glucose and aspartate, a product of TCA cycle [95–97]. The NAA synthesis rate is one to two orders of magnitude slower when compared with the synthesis rate of other brain metabolites [98]. NAA is predominantly localized in neurons, oligodendrocytes-type-2, and myelin, whereas astrocytes and mature oligodendrocytes contain very low levels of NAA [99]. Following the synthesis, NAA is transported down the axons and used by oligodendrocytes for myelin synthesis, repair, and maintenance. NAA provides 30% of

necessary AcCoA for myelin lipid synthesis. Specifically, NAA is taken by oligodendrocytes in axo-glial contact zones and converted into acetate and subsequently AcCoA (see [98]). Although NAA synthesis and turnover is very slow and relies on existing AcCoA and aspartate, following ischemic injury NAA levels decrease quickly and correlate with the fast decrease in ATP [100–102]. This was interpreted that following the acute brain injury like TBI or stroke, during the 'metabolic crisis' due to disrupted oxidative glucose metabolism, the NAA may serve as substrate and provide acetyl moieties to sustain oxidative metabolism and also help to maintain histone proteins acetylation levels.

4.3. Acetyl-carnitine and ketone bodies offer neuroprotection via acetyl-CoA metabolism

The brain is capable of replenishing the AcCoA pool via metabolism of alternative substrates, i.e. ketones, fatty acids, and acetyl-carnitine [103] (Fig. 4). This innate ability of brain to utilize alternative substrates for energy is highly important during pathological conditions such as stress, stroke, and brain trauma, which are characterized by impaired oxidative glucose metabolism and increased lactate production. The ability of the brain to use these substrates has been known for years, however, recent research re-examines these phenomena with specific attention to cell-, and compartment-specific points of view. From circulating ketone bodies represented by β-hydroxybutyrate (βOHB), acetoacetate, and acetone, βOHB is the most abundant ketone body. It is generated by the liver under starvation and is transported into brain cells by the monocarboxylate transporters via a sodium-independent and sodium-dependent manner (for review see [104]). It can enter directly into the mitochondria, however this pathway is yet to be understood. Once in mitochondria, βOHB is converted to acetoacetate via β-hydroxybutyrate dehydrogenase (BDH), which requires NAD^+ as cofactor, thus this reaction results in production of acetoacetate and NADH [74]. It is interesting to note that BDH contains several sites for Sirt3 regulation, but whether the activity of BDH is affected by acetylation remains to be determined [105]. The ability to utilize βOHB for brain energy and metabolism is a subject of regional and developmental regulation. Specifically, βOHB is a preferred substrate for energy and metabolism in the developing brain. However, it is also present in the adult brain during caloric restriction, starvation, and after exogenous administration in high concentration [106]. All brain cells are capable of utilizing βOHB for respiration, however, neurons and oligodendrocytes use βOHB more efficiently than astrocytes [107]. Furthermore, βOHB has been shown to increase mitochondrial respiration, ATP production, and NAD^+/NADH ratio in cortical neurons even in the presence of 1 mM of glucose [108]. Experiments using ^{13}C NMR (nuclear magnetic resonance spectroscopy) showed that βOHB was oxidized to a greater extent in neurons when compared to cortical astrocytes [109].

Increased ketone body utilization results in the significant rise of AcCoA and decrease in available Coenzyme A. Thus, βOHB is capable of supporting oxidative metabolism by increasing mitochondrial concentrations of AcCoA and increasing intra-mitochondrial concentrations of NADH (see review [110]).

Although a ketogenic diet has been used for treatment of refractory epilepsy for decades, the mechanisms of neuroprotection offered by ketones are just recently beginning to be understood. Hepatic generation of ketones following mobilization of endogenous

triglycerides, fatty acids, and their subsequent metabolism via β-oxidation has been well studied under starvation, caloric restriction, and exogenous administration in both humans and animals. However, little is known about the brain's endogenous ability to generate ketones. It was demonstrated that in vitro astrocytes are the only cells capable to use fatty acids for oxidative metabolism via β-oxidation, suggesting that astrocytes can generate ketones for neighboring neurons [111]. While Cahoy et al. [112] showed that genes responsible for fatty acid metabolism are present in all cells, however, the comparison of neuronal and astrocytic transcriptional profiles lead to conclusion that fatty acids oxidation is a constitutive metabolic pathway in astrocytes [112,113].

Hence, the astrocytic AcCoA pool can be replenished by fatty acids oxidation and is subjected to regional and developmental regulation [114]. Recent evidence demonstrates that in addition to astrocytes neural stem/progenitor cells are also dependent on fatty acid oxidation in their quiescent state [115]. Astrocytes are capable to upregulate fatty acid oxidation in response to injury and stimulation of this pathway by 3,3,5 triiodo-L-thyronine (T3) resulted in decreased lesion volume in stroke model [116].

Apart from feeding into mitochondrial respiration, ketone bodies also decease the production of ROS by complex I [117], induce BDNF gene expression via activation of the transcription factor NF-kB, and its interaction with the histone acetyltransferase p300/EP300 [108]. Furthermore, βOHB is an inhibitor of class I histone deacetylases (HDACs) [118]. Thus, the neuroprotective effect of βOHB is also exerted via mechanisms similar to pan-HDACs inhibitors TSA and SAHA.

Acetyl-carnitine, the shortest acylcarnitine generated via β-oxidation, doesn't require transferases for intra-mitochondrial transport and provides directly acetyl moieties for the TCA cycle. Using ^{13}C NMR, Scafidi et al., showed that astrocytes utilize acetyl-L-carnitine for energy and neurotransmitter synthesis [119]. Exogenous administration of acetyl-carnitine has been shown to be neuroprotective following ischemia, traumatic brain injury, multiple sclerosis, and peripheral nerve injury [120–123]. Thus, these alternative substrates, βOHB, fatty acids, and acetyl-L-carnitine, provide acetyl moieties for bioenergetics and lipids metabolism. Additionally, by altering AcCoA levels, they can affect histone and non-histone protein acetylation. Thus, they may serve as a therapeutic intervention following the acute brain injury or for chronic neurodegenerative diseases.

5. Conclusions

Both NAD^+ and AcCoA are cellular metabolic intermediates that are essential for amino acids, fatty-acids, and bioenergetic metabolism. Furthermore, they influence gene expression by serving as cofactors for epigenetic modifiers mediating post-translational alterations of histone and non-histone proteins. Thus, the concentrations of AcCoA and NAD^+ affect the acetylation levels of proteins controlling transcriptional regulation and metabolic status. As we discussed both NAD^+ and AcCoA metabolism is disturbed following ischemic stress and there is a complex interplay between downstream effects due to imbalance in NAD^+ and AcCoA homeostasis. The majority of AcCoA is generated via NAD^+ dependent processes from pyruvate resulting in an intimate relationship between the mechanisms involved in

NAD^+, AcCoA metabolism, and mitochondrial function and dynamics. Due to the complexity of postischemic pathology that involves changes in almost every metabolic pathway, a successful treatment strategy will need to comprise of a multi-targeted approach, using compound that affects multiple pathways. Administration of intermediates that can modulate the post-insult NAD^+ and AcCoA levels represents a promising way to manipulate several pathways since these metabolites are involved in many enzymatic reactions and also play a significant role in regulating enzymes activity and gene expression via post-translational modifications.

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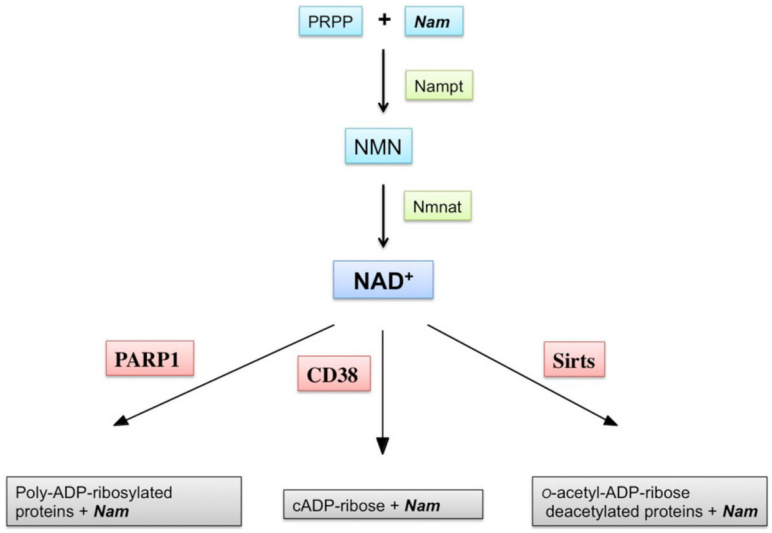

Fig. 1.

NAD^+ catabolism and the NAD^+ salvage pathway. NAD^+-consuming enzymes poly-ADP-ribose polymerase 1 (PARP1), CD38, and sirtuins (Sirts) cleave nicotinamide (Nam) from NAD^+. PARP1 forms complex ADP-ribose polymers that are attached to the target protein. CD38 generates cADP ribose. Sirts conjugate ADP-ribose with an acetyl group removed from a lysine residue of an acetylated protein, generating *o*-acetyl-ADP-ribose. Released Nam is then recycled in the NAD^+ salvage pathway by nicotinamide phosphotransferase (Nampt) that generates nicotinamide mononucleotide (NMN) from Nam and phosphoribose pyrophosphate (PRPP). NMN is then converted to NAD^+ by nicotinamide mononucleotide adenylyl transferase (Nmnat).

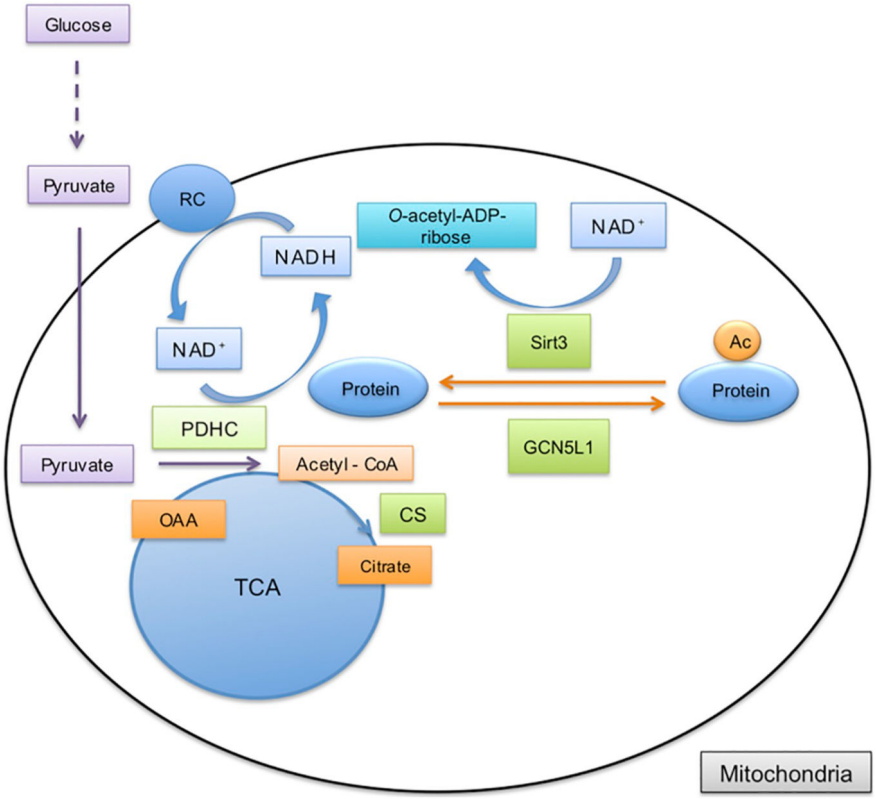

Fig. 2.

Mitochondrial acetyl-CoA metabolism and protein acetylation. Pyruvate formed during glucose metabolism in the cytosol is transported into the mitochondria. Acetyl-CoA is then generated by pyruvate dehydrogenase complex (PDHC) from pyruvate. During this reaction NAD^+ is reduced to NADH that donates electrons to complex I in the respiratory chain (RC). In the TCA cycle citrate is produced by citrate synthase (CS) from acetyl-CoA and oxaloacetate (OAA). Acetyl-CoA can also be used by mitochondrial acetyltransferase, GCN5L1, to acetylate mitochondrial proteins. The acetyl group is removed from the target protein by mitochondrial deacetylase, Sirt3, which uses NAD^+ and releases the deacetylated protein and *o*-acetyl-ADP-ribose.

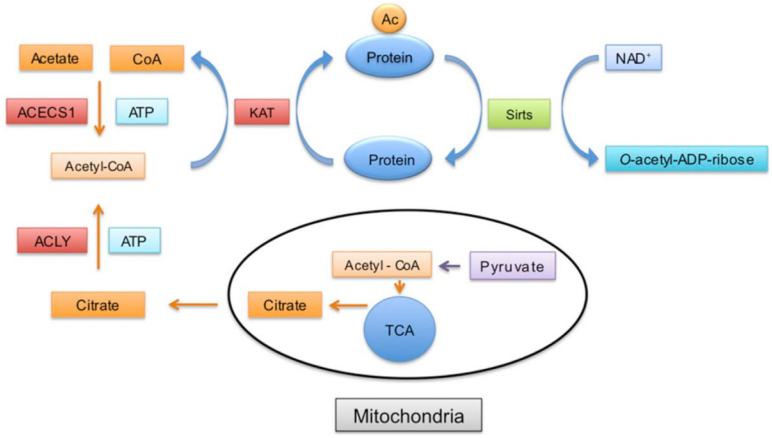

Fig. 3.

Cytosolic acetyl-CoA metabolism and protein acetylation. Citrate generated from acetyl-CoA is transported into the cytosol where it is a substrate for ATP citrate lyase (ACLY). This enzyme converts citrate back to acetyl-CoA in the presence of ATP. Acetyl-CoA can be also synthetized by cytosolic acetyl-CoA synthetase (ACECS1) from acetate, CoA and ATP. Similarly, as in mitochondria, acetyl-CoA is used for acetylation of proteins by acetyltransferases (KAT). The acetylated proteins are deacetylated by sirtuins where the deacetylation is coupled to NAD^+ hydrolysis and *o*-acetyl-ADP-ribose is released.

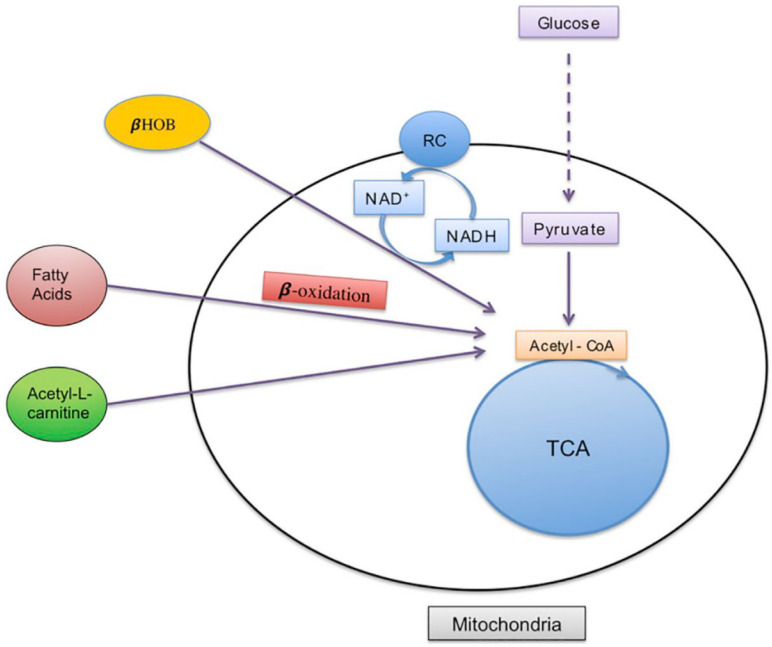

Fig. 4.

Acetyl-CoA synthesis supported by β-hydroxybutyrate (βOHB), fatty acids β-oxidation, and acetyl-L-carnitine metabolism. Acetyl-CoA can be generated from pyruvate, βOHB, β-oxidation of fatty acids (particularly in astrocytes), and from acetyl-L-carnitine. During these metabolic reactions NAD^+ is reduced to NADH.