REVIEW ARTICLE

Roles and Mechanisms of Lactylation Modification in Hypoxia-exacerbated Neuroinflammation

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Abstract: Lactate is a product of cellular energy metabolism. In the brain, lactate shuttles between astrocytes, microglia, and neurons, playing an important role in coordinating cellular metabolism and signaling. Hypoxia causes a shift in cellular metabolism from oxidative phosphorylation to glycolysis, increasing lactate. In addition to being a metabolic substrate, lactate also acts as a signaling molecule. More interestingly, lactate accumulation can induce protein lactylation modification, a newly identified post-translational modification (PTM). Lactylation modifications occur on both histone and non-histone proteins and are involved in the regulation of numerous cellular processes, including tumorigenesis, immune inflammation, and embryonic development. In the context of neuroinflammation, our recently published report showed that hypoxia activates microglia and exacerbates neuroinflammation in the brain. This review summarizes the effects of hypoxia on lactate metabolism, as well as the process of lactylation and delactylation in microglia. The regulatory mechanisms of protein lactylation in hypoxia-exacerbated neuroinflammation are further discussed.

Keywords: Hypoxia; Lactate; Lactylation; Neuroinflammation; Microglia.

INTRODUCTION

Physiologically, basal brain metabolism requires 20% of the oxygen consumption of the body (1). Most of the oxygen in the brain is consumed by neurons (2). However, in hypoxic environments, cellular metabolism shifts from oxidative phosphorylation to anaerobic glycolysis. The process of glycolysis progressively metabolizes glucose to pyruvate, which ensures a rapid ATP supply. The effects of hypoxic stimulation on brain tissue are manifold. Inhalation of a mixed gas containing 11% O_2 induces vasodilation of the vertebral artery, basilar artery, and forebrain, and increases blood flow and perfusion (3). In early development, low oxygen leads to incomplete myelin development in newborns, resulting in cognitive deficits (4). Hypoxia leads to neuronal metabolic disorders, disrupting neurotransmitter synthesis and release, and affecting nerve signaling (5, 6). Prolonged hypoxic stimulation at 4,300 m for 6 months induces neuronal apoptosis, resulting in irreversible brain tissue damage, causing multiple neurological dysfunctions such as cognitive and motor deficits, and increasing the risk of neurological disorders (7, 8). Brain tissue hypoxia is mainly caused by low oxygen content when access to high altitude (9), hypoxic environments in intracranial tumors (e.g., gliomas) (10, 11), hypoxemia or low brain tissue oxygenation ($PbtO_2$) due to traumatic brain injury (12), decreased brain oxygen

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saturation due to obstructive sleep apnea (OSA) (13, 14), decreased cerebral blood flow perfusion due to ischemic stroke (15) and disruption of cerebral blood flow during neonatal hypoxia-ischemia (HI) (16). This review focuses on the modulation of neuroinflammation due to manipulated lactate production caused by hypoxia alone, either simulated hypobaric hypoxia or plateau hypoxia exposure.

Under normoxia, pyruvate is metabolized by pyruvate dehydrogenase (PDH) to acetyl-coenzyme A (CoA), which subsequently enters the tricarboxylic acid (TCA) cycle in the mitochondria. On the other side, pyruvate is converted to lactate by lactate dehydrogenase (LDH) under hypoxic conditions. Lactate in the brain is involved in a variety of biological processes. Previous theories believed that lactate was a waste product of cell metabolism. Studies in recent years have shown that lactate in the brain is involved in a variety of biological processes in cells (17). For example, lactate serves as an energy source to provide metabolic support for neurons (18) maintain the acidic environment of lysosomes (19) transmit signals between neurons and glial cells (20) and regulate the activation of macrophages by mediating post-translational modification of proteins (21). Microglia are a type of macrophage that reside in the central nervous system (CNS) (22, 23) and play a crucial role in immunosurveillance within the brain (24). Microglia initiate immune defense upon hypoxic stimulation of the CNS, trans-differentiate between an immunosuppressive M2 phenotype and an activated M1 phenotype, and express pro-inflammatory cytokines which cause cognitive impairment (25-27). Moderate supplementation of L-lactate (5 mM) attenuates lipopolysaccharide (LPS)-induced pro-inflammatory activation of microglia and neuroinflammation in mice (28). Supplementation with 20 mM L-lactate exacerbates the LPS-induced inflammatory response in the BV2 microglial cell line (29). Recent studies have shown that lactate-driven histone lactylation modulates the immune status of mouse microglia, resulting in neuroinflammation and cognitive impairment (29, 30). This review summarized the effects of hypoxia on lactate metabolism and recent advances in the regulation of hypoxia-induced neuroinflammation through lactylation modifications.

SEARCH STRATEGY

Studies cited in this review were published from 1994 to 2024, with a predominant citation from 2019 to 2024. All studies were searched on the PubMed database using the following keywords: hypoxia, brain, high altitude, microglia, astrocyte, neuron, lactate, lactate metabolism, lactylation, delactylation, neuroinflammation, muscle, heart, liver, intestines, blood, TCA cycle, GLUT, glycolysis, lactate dehydrogenases, oxidative phosphorylation and pentose phosphate pathway. Studies targeting lactate and lactylation were limited to those on hypoxia or neuroinflammation. Data from original studies were based only on humans, rats and mice. Studies that focused on microglia or accurately reported lactate doses were preferentially included. The following types of studies were excluded: those where the full text was unavailable; experimental methods were not clear or they were meta-analyses or abstracts.

1. SOURCE OF LACTATE

Lactate in human blood is mainly released from skeletal muscle, the brain, erythrocytes and perivenous hepatocytes (31). Human blood lactate levels are approximately 0.5 mM to 2.2 mM during resting breathing and achieve 12 mM to 25 mM during exercise (32), After 1 and 3 days of exposure to a plateau environment at 4559 m, blood lactate levels gradually increased in the volunteers (33). After inhaling a gas mixture containing 12% O_2 for 20 minutes, the lactate levels in the brains of volunteers increased significantly (34). After inhaling a gas mixture containing 10% O_2 for 60 minutes, brain lactate concentration increased from 0.4 mM to 1.2 mM in volunteers, as detected by magnetic resonance imaging (35). Under hypoxia, part of the lactate in the brain is converted by lactate dehydrogenase B (LDHB) to pyruvate (36), which provides energy for neuronal metabolism (37) and another part of the lactate is transported into the venous blood (38). Most of the lactate in the blood is converted by the liver to pyruvate, which is involved in energy metabolism (32).

1.1. Lactate Production in the Brain

Brain tissue mainly consists of neurons and glial cells. Glial cells include microglia, astrocytes, and oligodendrocytes (39). Astrocytes are the main producers of lactate, while neurons are the main consumers of lactate (40). Under normoxia, lactate is transported out of astrocytes by monocarboxylate transporters MCT1 and MCT4 (MCT1/4), and then into neurons by MCT2 (41). MCT1/4 is also

expressed on the cell membrane of microglia, where it performs the same function (42).

Under normoxia, glucose was transported into the cytosol by the cell membrane glucose transporter protein GLUT1 (43, 44). Glucose is catalyzed to pyruvate by enzymes, such as phosphofructokinase (PFK), phosphoglycerate kinase 1 (PGK1) and pyruvate kinase (PK), which are located in the glycolytic pathway (40). Pyruvate is converted to acetyl-CoA by PDH (40). Glycolytic pathways are active in astrocytes. Pyruvate accumulates in astrocytes since astrocytes highly express fructose-2,6-bisphosphatase 3 (PFKFB3) and pyruvate dehydrogenase kinase isoform 4 (PDK4) (40), therefore, pyruvate accumulates in the cells. Part of the pyruvate is converted to lactate by the enzyme lactate dehydrogenase (LDH). In humans, LDH is a tetramer composed of two subunits, LDHA and LDHB, which are encoded by separate genes. LDHA is more active in driving the conversion of pyruvate to lactate, while LDHB is more active in driving the conversion of lactate to pyruvate. There are five isozymes of LDH, of which LDH1 contains only the LDHB subunit, LDH5 consists only of the LDHA subunit, and three additional isozymes contain both LDHA and LDHB subunits (45). The composition of the subunits determines that LDH1 catalyzes the production of pyruvate and LDH5 catalyzes the production of lactate. LDH5 is highly expressed in astrocytes and converts pyruvate accumulated in these cells to lactate, which means that lactate in the brain is primarily produced by astrocytes (40, 46). LDH1 is highly expressed by neurons. Lactate is converted to pyruvate by LDH1 in neurons and then enters the tricarboxylic acid cycle(40). Thus, lactate is one of the main energy sources for neurons.

Astrocytes are less sensitive to hypoxic stimuli than microglia. A comparative study between primary astrocytes and microglia revealed that after being incubated with 2-Deoxy-D-glucose (2-DG) instead of glucose for 6 h in an anaerobic environment, astrocytes maintained 98% cell viability and simultaneously increased LDH secretion. In contrast, microglia had a survival rate of approximately 12% (47). This suggests that astrocytes are more tolerant to hypoxic stimuli and have a greater capacity for lactate synthesis. The mechanism of lactate production might be attributed to the induction of PGK1 phosphorylation and the inhibition of PDH in the mitochondria. This leads to a blockage in the conversion of pyruvate to acetyl-CoA, consequently resulting in the production of more lactate (48, 49). In a hypoxic environment, the enhanced glycolytic activity of astrocytes and the blocked TCA cycle result in the accumulation of pyruvate that is converted to lactate.

Oligodendrocytes originate from oligodendrocyte precursor cells (OPCs) are responsible for forming the myelin sheath. Oligodendrocytes take up blood glucose via GLUT1 and metabolize it to lactate, which can be utilized by neurons (50, 51). MCT1 is abundantly enriched in the cell membrane of oligodendrocytes (52). Lactate is translocated to the periaxonal space or the extracellular space via MCT1 (52). On the other hand, lactate is an essential energy substrate for myelin formation (50). Lactate from the periaxonal space is taken up into the neuron by MCT2 on the axon to provide a substrate for energy metabolism (52). LDH release levels from oligodendrocyte-like cells were not altered after 48 hours of incubation in an anaerobic environment (53). Zhang *et al.* found that after 1 h of hypoxic stimulation (1% O_2), MCT1 expression levels in OPCs were elevated, but lactate accumulated intracellularly, which may be attributed to the inhibition of MCT1 activity by hypoxia (54). Current studies have focused on dysmyelination and myelin damage due to hypoxia, rather than on energy metabolism in oligodendrocytes. It cannot be ruled out that hypoxia affects glycolytic pathways, leading to the accumulation of lactate in oligodendrocytes.

Microglia, a type of mononuclear macrophage residing in the CNS, are the predominant neuroimmune cells (22, 23). Microglia initiate immune defenses to maintain homeostasis when the CNS is exposed to stimuli (24). The generation of lactate in microglia is also increased under hypoxic conditions. Wang *et al.* demonstrated that lactate production by the human microglial clone 3 (HMC3) microglial cell line doubled after 12 h of incubation in a gas mixture containing 10% O_2 (55). Similarly, we observed an increase in LDH protein expression and a significant increase in intracellular and medium lactate concentrations after 24 h of induction with 0.3% O_2 in primary microglia and BV2 cell lines (29, 30). Wang *et al.* found that the pentose phosphate pathway (PPP) was upregulated in primary macrophages after 7 days of exposure to 1% O_2 (56). Carpenter *et al.* suggested that glyceraldehyde 3-phosphate and fructose 6-phosphate produced by the pentose phosphate pathway could serve as substrates for lactate production (46). Taken together,

hypoxia induces lactate production in microglia relies on upregulation of the glycolytic pathway and LDH activity, and results in elevated lactate levels in the brain.

1.2. Lactate Production in Hypoxic Peripheral Tissue

Under hypoxia environment, muscles, intestines, kidneys, and the heart produce more lactate via the glycolytic pathway due to insufficient oxygen supply. Hypoxia-inducible factor (HIF) is a key factor in sensing and responding to hypoxic stimuli (57). HIF proteins are composed of α and β subunits, with HIF-1α receiving the most attention. The protein level of HIF-1α is regulated by oxygen concentration (58). Under normoxia, HIF-1α is ubiquitinated by PDH and subsequently degraded by the proteasome (59). In hypoxia, PDH activity is inhibited, and HIF-1α increases pyruvate production by promoting the transcription of GLUT and glycolytic enzymes (e.g. Hexokinase (HK), PFK2, PK) (59). HIF-1α positively regulates LDHA in tumor cells in a hypoxic environment, which catalyzes the conversion of pyruvate to lactate (60, 61). Cobalt chloride ($CoCl_2$) is an inducible molecule that has been used to construct a model of chemical hypoxia in cells cultured in vitro (62). $CoCl_2$ was found to simulate the effects of hypoxic stimuli on HIF-1α and HIF-2α, maintaining HIF protein levels in the presence of sufficient oxygen by preventing protein ubiquitination modification followed by degradation (63). In a $CoCl_2$-induced hypoxia model, HIF-2α upregulated LDHA expression and activity in ileal tissue by binding to the hypoxia response element 1 (HRE1) promoter (64). Under hypoxia, the activity of MCT4 is upregulated by HIF-1α, and MCT4 transports lactate through the basolateral membrane to the extracellular space (65, 66). This resulted in increased levels of lactate in the total contents of the ileum, cecum, and appendix (64, 67). Under anaerobic conditions, the levels of lactate release in the intestinal smooth muscle and thoracic aorta of guinea pigs are elevated 3-4-fold (67). Intestinal tissues are exposed to a hypoxic environment and are accompanied by a large number of bacteria. The ischaemic stimulus affects the metabolism of intestinal lactate bacteria, leading to the production of D-lactate (68, 69). For example, Poeze *et al.* reported that the intestinal barrier is damaged by hypoxic stimuli, resulting in the release of D-lactate into the blood (70).

Lactate produced by peripheral tissues can be transported to brain tissue by the circulation system. After a single set of biceps contraction exercises, the lactate concentration in the blood of an athlete increases from 1.4 mM to 4.0 mM and is positively correlated with exercise intensity (71). There is a significant elevation of lactate levels in the blood of athletes after inhaling air with a fraction of inspired oxygen (F_{iO2}) of 0.157 for 5 minutes during cycling (72). In another study, femoral vein lactate levels in cyclists increased from 2.0 mM to 6.0 mM after 3 minutes of exercise in a normobaric hypoxic (F_{iO2}=0.10) environment and reached 12.3 mM after 30 minutes, accompanied by a significant decrease in blood pH (73). A portion of the lactate in human blood is reconverted to pyruvate by the liver at a rate of 320 mM/h for aerobic respiration or gluconeogenesis (74). Part of lactate crosses the blood-brain barrier (BBB) into brain tissue and is transported into neurons by monocarboxylate transporter MCT2 (75) or transported into endothelial cells or glial cells by MCT1 (76). Taken together, lactate levels in the blood are elevated severalfold in response to hypoxic conditions and exercise stimuli. Blood lactate is released from peripheral tissues and then enters the CNS to help regulate the metabolism and homeostasis of neurons and glial cells (Fig. 1).

2. LACTATE MODULATES NEUROINFLAMMATION

Currently, there are conflicting reports on the effects of lactate on the inflammatory response in microglia. Kong *et al.* reported that in the LPS-treated BV2 cells, supplementation with 5 mM L-lactate attenuated the classical activation by blocking the function of MCT1 and inhibiting the glycolytic pathway (28). However, when 20 mM L-lactate was added to this model, the levels of pro-inflammatory cytokines secreted by BV2 cells were significantly increased (29). Human Microglia Clone 3 (HMC3) cells treated with 20 mM lactate for 48 h showed a significant increase in the protein level of the reparative marker (Arginase 1, Arg1) and a decrease in the mRNA content of the inflammatory marker (Tumor necrosis factor, TNF) (77). Thus, different microglial cell lines may respond differently to the same dose of lactate. Low-dose pretreatment with lactate promoted the repair phenotype of microglia. Hong *et al.* reported that lactate pretreatment (10 mM, 1 h) upregulated mRNA levels of anti-inflammatory cytokines (IL-4, IL-10 and CD206) in primary microglia induced by LPS

(1 μg/mL, 8 h) and prevented processes shortening (78). Han *et al.* found that low-dose sodium lactate (25 μM pretreatment for 2 h) prevented the pro-inflammatory activation induced by LPS (200 μg/ml, 24 h) in BV2 cells (79). Interestingly, Zhang *et al.* found that IFNγ and LPS treatment induced proinflammatory activation and production of lactate in BV2 cells; however, supplementation with lactate suppressed the mRNA levels of proinflammatory factors (IL-1β, iNOS and TNF-α) and upregulated repair factors (Arg-1, CD206 and IL-10) (80). In vitro, low doses of lactate are favorable for the repair phenotype of microglia, while high doses of exogenous lactate exacerbate the inflammatory response.

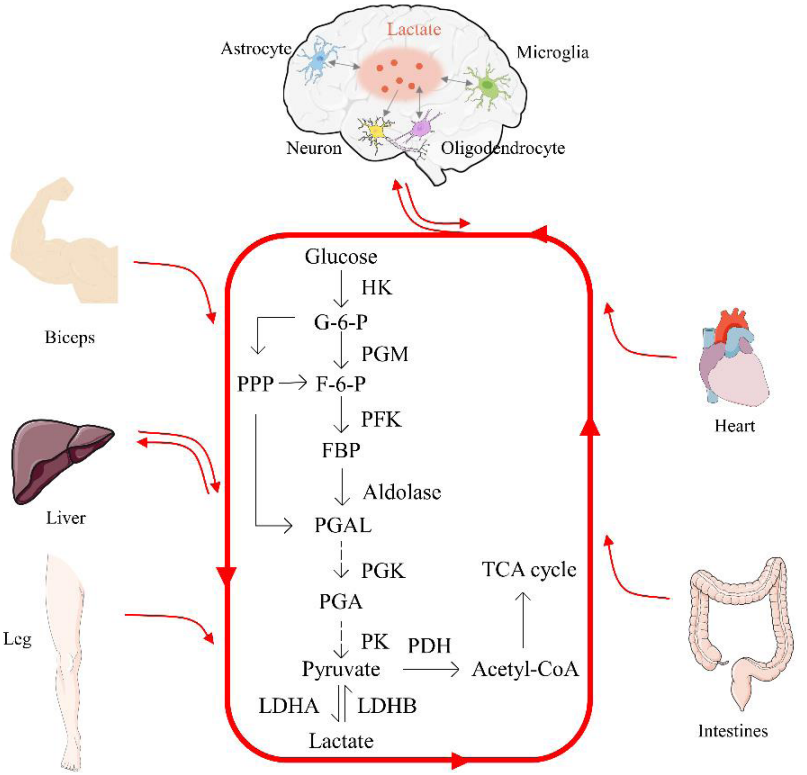

Figure 1. Production and transport of lactate. In the cytoplasm, glucose is gradually converted into G-6-P, F-6-P, FBP, PGAL, etc. through the glycolysis pathway, and is finally metabolized to pyruvate by PK. Part of G-6-P leaves the glycolytic pathway and enters the PPP, where it is metabolized to ribulose-5-phosphate and then converted to F-6-P and PGAL, allowing it to re-enter glycolysis. The PPP does not require oxygen, but hypoxia enhances the non-oxidative pentose phosphate pathway (non-oxPPP) and increases the production of F-6-P and PGAL. The pyruvate produced by the co-regulation of the glycolysis pathway and the PPP is metabolized to acetyl-CoA by PDH, and then enters the mitochondria to participate in the TCA cycle and is ultimately metabolized to NADH. Physiologically, lactate is produced and transported as a metabolic intermediate. Due to hypoxia, exercise, or inflammation, lactate produced by skeletal muscles (such as the biceps and leg muscles), liver, intestines, heart, brain, or other organs is secreted into the blood and participates in metabolic circulation. Part of lactate in blood is metabolized by hepatocytes to pyruvate, which is ultimately converted into glucose or glycogen for storage. Approximately 11% of the lactate in blood is transported into brain tissue via MCT. The other part of lactate in brain tissue originates from astrocytes microglia and oligodendrocytes. Lactate in the brain is taken up by neurons for energy or to mediate glutamatergic signaling. G-6-P: Glucose 6-phosphate; F-6-P: Ructose-6-phosphate; FBP: Fructose-1,6-bisphosphate; PGAL: Glyceraldehyde-3-phosphate; PGA: 3-phosphoglycerate; HK: Hexokinase; PGM: Phosphoglucomutase; PFK: Phosphofructose kinase; PGK: Phosphoglycerate kinase; PK: Pyruvate kinase; LDHA: Lactate dehydrogenase A; LDHB: Lactate dehydrogenase B; PDH: Pyruvate dehydrogenase; PPP: Pentose phosphate pathway; Acetyl-CoA: Acetyl-coenzyme A; TCA cycle: Tricarboxylic acid cycle; NADH, Nicotinamide adenine dinucleotide; MCT: Monocarboxylate transporters.

There are also different conclusions in animal studies, implying that the role of lactate may be dynamic. In a mouse model of neuroinflammation induced by LPS (1 mg/kg, 8 h), classical activation of microglia and expression of inflammatory factors were attenuated by supplementation of 2 μl of 100 mM L-lactate into the brain ventricles (28). Similarly, peritoneal injection of lactate (400 mg/kg for 5 days) before LPS (100 μg/kg) injection prevented the shortening of microglial processes in the prefrontal cortex of mice (78). In D-galactose-gal and $AlCl_3$-induced Alzheimer's disease mice, longer sodium lactate pretreatment (2 g/kg, 5 days/week for 8 weeks) also drove microglia to exhibit a reparative phenotype (79). However, the side effects resulting from long-term interventions using high doses of lactate deserve further investigation. Lactate-driven microglia activation was observed in models of neuroinflammation but not in models of lactate supplementation alone. In a rat model of neuroinflammation induced by LPS (1 mg/kg, 24 h), the inhibition of lactate production attenuated the levels of inflammatory factors in the hippocampus (81). Lactate magnetic resonance spectroscopy (MRS) signal in the extracellular fluid of the core area of the lesion in rats increased up to 20-fold during the 28-day recovery period from cerebral hemorrhage and was accompanied by amoeboid morphologic changes in microglia (82). Lactate levels in hippocampal tissue are approximately fivefold elevated in 12-month-old mice with Alzheimer's disease, inducing an inflammatory response in microglia through histone lactylation modification (83). In the acute phase of LPS treatment, supplemental lactate energizes glial cell metabolism and ameliorates the inflammatory response. In contrast, during the chronic phase, lactate has an activating effect on glial cells and therefore exacerbates the inflammatory response. The fundamental reason for this contradictory phenomenon may stem from the antagonism between the two functions of lactate: as a signaling molecule and as an energy substrate.

The effects of endogenous lactate are similar in hypoxia-exposure animal models. In a hypoxic environment (10% O_2, 30 min), increased cortical lactate in rats is accompanied by increased levels of pro-inflammatory cytokines (84). After hypoxia exposure (11.1% O_2, 2 h), lactate levels in rat cerebrospinal fluid increased from 2 mM to 4 mM, activating the G protein-coupled receptor 81 (GPR81)/protein kinase A/cAMP axis, stimulating neurons in the preoptic anterior hypothalamus (PO/AH), and inducing anaphylactic reactions (85). The lactate/GPR81/protein kinase A/cAMP axis may contribute to hypoxia-induced neuroinflammation and structural damage to the retina, resulting in impaired color vision (41, 86). In a brain injury model established by simulated high-altitude hypoxic exposure (9000 m, 23 h), the expression level of LDH in the hippocampus of rats increased, the arrangement of neurons became disordered, and neuronal excitability decreased (87). Taken together, endogenous lactate induced by hypoxia drives the onset and development of neuroinflammation.

3. HYPOXIA-INDUCED PROTEIN LACTYLATION

3.1. Lactylation modification

In 2019, Zhang *et al.* discovered that L-lactate produced during hypoxia-induced macrophage M1 polarization can be used as a substrate to drive the lactylation modification of lysine residues on histones. Histone acetyltransferase p300 may mediate, with the assistance of p53, the transfer of lactyl groups from lactate to lactyl-CoA and ultimately to H3K18 or H4K8, thereby leading to the secretion of reparative cytokines by macrophages (21). Subsequent studies have shown that p300 correlates with protein lactylation (55, 79). YinYang-1 (YY1) lysine 183 is lactylated (YY1K183la) and the level of YY1K183la increases/decreases with p300 overexpression/inhibition in retinal microglial cells of mice reared in a simulated plateau hypoxic environment (55). Han *et al.* found that the lactylation level of proteins in cells was affected by p300 in BV2 cellular model based on β-amyloid (Aβ) protein induction. When the translation of p300 was inhibited by siRNA, the lactylation level of proteins in BV2 cells was reduced, and the expression of Arg1 and VEGF was suppressed (79). However, it is unclear whether p300 binds directly to lactate or lactyl-CoA and induces protein lactylation. Isotope tracing revealed the hypoxia-induced conversion of ^{13}C-labeled glucose to lactate via the glycolytic pathway (88), followed by the transfer of ^{13}C from lactate to the lactyl group of the protein (21). However, the correlation between lactate and lactyl-CoA levels does not represent that carbon transfer has occurred between them (89). It is unclear whether lactyl-CoA serves as an intermediate between lactate and lactylation.

Recent studies have identified lactyltransferase activity in alanyl-tRNA synthetase AARS1 and AARS2 (90, 91). The main function of AARS is to catalyze the synthesis of aminoacyl-AMP and subsequently transfer the aminoacyl group to specific tRNAs, ensuring the precise execution of protein translation (92). Zong *et al.* found that AARS1 in breast cancer cells directly binds sodium lactate, forms lactate-AMP intermediates with the involvement of ATP, and then transfers the lactyl group to the p53 protein (91). Ju *et al.* found that in gastric cancer HGC27 cells, AARS1 drives the lactylation of Yes-associated protein (YAP) at K90 and TEA domain transcription factor 1 (TEAD1) at K108 in the nucleus with the help of ATP energy, promoting tumor cell proliferation (93). In contrast, the effect of p300 on lactylation was found to be weak in this study (93). AARS2 has also been found to possess lactyltransferase activity. In the test tube, AARS2 transfers lactate to the lysine with the help of energy released from ATP phosphate bonds, forming a lactylation modification (94). In human and mouse peritoneal macrophages, Li *et al.* found that AARS2 drove cyclic guanosine-adenylate synthase (cGAS) lactylation, leading to the silencing of the intrinsic innate immune response (90).

A recent study found that in macrophages and microglia stimulated by hypoxia, p300 may mediate protein lactylation via lactyl-CoA due to the correlation between its protein levels and the levels of histone and non-histone lactylation. However, the intracellular concentration of lactyl-CoA is at trace levels (95), which makes p300-driven lactylation difficult to achieve. p300 is a transcription coactivator involved in tumor cell proliferation, invasion, and apoptosis. p300 also possesses acetyltransferase activity and mediates post-translational modifications of proteins such as acetylation and crotonylation (96). The broad biological functions of p300 may result in p300 indirectly influencing lactylation modification. The molecular mechanism by which p300 drives lactylation remains to be verified. In contrast, the process of AARS-driven lactylation modification is more clearly defined and has been detected in human cancer cells and macrophages (Fig. 2). Currently, the lactyltransferase activity of AARS has been found only in non-histone proteins. Targeting the lactylation modification of histones by AARS could help to provide a more complete understanding of the protein lactylation process. Moreover, it cannot be excluded that lactylation modifications are mediated by different enzymes in various cell types, such as cancer cells and microglia.

3.2 Delactylation

The complete process of post-translational modification of proteins involves writing, reading, and erasing. It has been found that sirtuin1-3 (SIRT1-3) and histone deacetylases1-3 (HDAC1-3) possess delactylase activity (97, 98). Mao *et al.* found that SIRT1 reversed the inhibition of oxidative phosphorylation by hypoxia-induced lactylation of pyruvate dehydrogenase A1 (PDHA1) and carnitine palmitoyltransferase 2 (CPT2) in mouse muscle cells (94). SIRT2 and SIRT3 were found to mediate histone delactylation in the cytoplasm and mitochondria of human embryonic kidney (HEK) 293T cells, respectively (97). It is also reported that HDAC1-3 removed lactylation modifications of histones H3K18 and H4K5 in the nucleus of HEK293T cells (97). Members of the SIRT and HDAC families exhibit different delactylase activities for L-lactyl-lysine ($K_{L\text{-}la}$) and D-lactyl-lysine ($K_{D\text{-}la}$) (97). HDAC1/3 is more active against H5K5la, and HDAC3 exhibits a lower delactylase activity in vitro (97). Compared to HDAC1/2, HDAC3 preferred $K_{D\text{-}la}$. Similarly, SIRT1 had a higher delactylase activity for $K_{L\text{-}la}$. D-lactyl-lysine is rarely found in the human body because the production of D-lactate is less than 1/1000th that of L-lactate (89, 97). Therefore, studies of enzymatic activities targeting $K_{L\text{-}la}$ are more important. At present, research on the activities of lactyltransferases and delactylases is mainly completed in vitro. The studies in vivo will help to systematically elucidate the delactylation process.

3.3. Role of Lactylation in Neuroinflammation

We recently found that hypoxic stimulation upregulated global protein lactylation levels in BV2 microglial cells and primary microglia (30). Multiple levels of lysine lactylation of p53 are significantly upregulated in a BV2 microglia model constructed under hypoxic conditions (0.3% O_2) combined with LPS stimulation (29). Hypoxia-induced lactate is a repressor of p53 and promotes the lactylation modification of p53 (p53Kla) (91). p53 regulates the cell cycle and has a high mutation frequency in tumor tissue. Lactylation-modified lysines of p53 are located in conserved sequences (29, 99). p53Kla

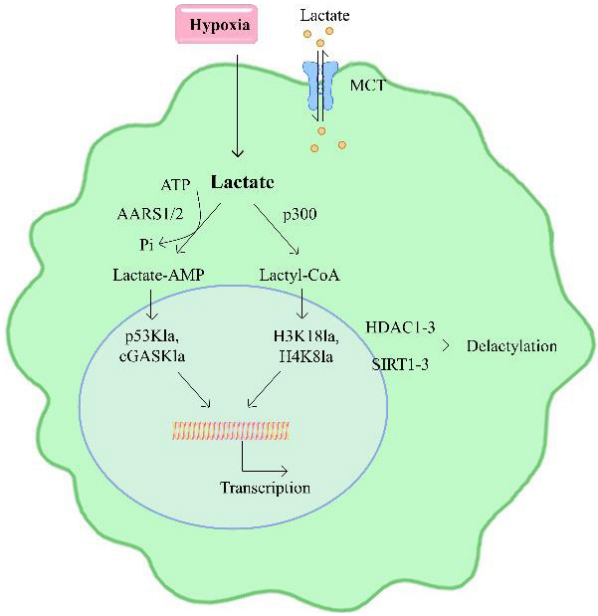

Figure 2. The "write" and "erase" hypotheses of lactylation. Intracellular lactate is produced by hypoxia-induced glycolysis or transported into the cell by MCT from outside. Lactate, as a substrate, binds to AMP to form the lactate-AMP intermediate, which is catalyzed by AARS1 or AARS2. Subsequently, the lactyl group is transferred to the lysine residue of the protein. The breakage of the high-energy phosphate bond of ATP provides the energy for the process described above and produces AMP. Another hypothesis for lactylation suggests that p300 drives lactate to form lactyl-CoA, which transfers the lactyl group to histones. The process of delactylation is recognized to be mediated by HDAC1-3. In vitro studies, SIRT1-3 is also thought to possess delactylase activity. MCT: Monocarboxylate transporter; ATP: Adenosine triphosphate; AMP: Adenosine monophosphate; AARS: Alanyl-tRNA synthetase; Pi: Phosphate group; HDAC: Histone deacetylases; SIRT: Sirtuin; H3K18la: H3K18 lactylation; H4K8la: H4K8 lactylation; cGASKla: Cyclic guanosine-adenylate synthase lactylation; p53Kla: p53 lactylation; Lactyl-CoA: Lactyl-coenzyme A.

translocates to the nucleus and activates p65, which drives the transcription of cytokines such as IL-6, TNF-α, and IL-1β. We also detected the lactylation status of nucleosome remodeling and deacetylase (NuRD) complex, which mainly consists of HDAC1, HDAC2, Metastasis-associated protein MTA1, MTA2, MTA3, and GATA zinc finger domain-containing protein 2 (30). HDAC1 possesses histone and non-histone delactylase activities and affects microglial inflammation by regulating lactylation modifications of H3K9 and H3K27 (100). On the other hand, HDAC2 and MTA2 negatively regulate the expression of inflammatory signaling pathways (101).

Microglia in the mouse retina underwent immune activation in response to hypoxic stimuli. After 5 days in a hypoxic (15.75% O_2) environment, the cytosol of mouse microglial cells became enlarged, and their protrusions shortened and thickened (55). The lactylation level of lysine 183 (K183) of the transcription factor YY1 was increased, driving the expression of the pro-inflammatory cytokine IL6, the transcription factor STAT3, and the chemokine CCL5, which collectively induced visual function impairment (55, 102, 103). In Alzheimer's disease mice, H3K18la activates the NFκB signaling pathway in hippocampal microglia by binding to the p65 and p50 subunits, leading to the secretion of the pro-inflammatory cytokine IL-6 from microglia (104). However, exogenous lactate (25 μM)-driven H3K18la regulated the transition of BV2 cells to repair phenotype (79). In Alzheimer's disease mice, H4K12la promotes M1 polarization of microglia and impairs cognition through a positive feedback loop involving pyruvate kinase M2 (PKM2) and glycolysis (105). Studies on different brain regions have shown that microglia in hippocampal tissue exhibit higher levels of lactylation compared to those in the cortex (104). These data suggest that hypoxia-induced protein lactylation produced by microglia is crucial for the development of neuroinflammation.

4. INTERVENTION STRATEGIES TARGETING PROTEIN LACTYLATION IN NEUROINFLAMMATION

Hypoxia-induced neuroinflammation is a contributing factor in the onset and progression of altitude sickness (25, 30). Reduced oxygen concentration leads to the production of pyruvate via anaerobic glycolysis, which is subsequently metabolized to lactate by LDH. Lactate serves as a substrate for lactyltransferases, which mediate the protein lactylation modification. Regarding protein lactylation modification, several strategies were proposed to modulate neuroinflammation:

1. Inhibiting the production of pyruvate, theprecursor of lactate. Pan *et al.* found that specific knockdown of PKM2 in mouse microglia reduced H4K12la levels, shifted microglia from an immune-activated state to a resting state, and mitigated cognitive impairment in mice (105).
2. Downregulation of protein lactylation by inhibiting lactate production. Our recent study showed that the pre-treatment of BV2 cells with an LDH inhibitor (sodium oxamate) or the inhibition of LDHA through gene editing can downregulate lactate production, leading to reduced levels of pro-inflammatory cytokines such as IL6 and TNFα (29, 30). Zhang *et al.* found that sodium oxamate supplementation reduced H3K18la levels and enhanced $CD8^+$ T cell activity (106).
3. Targeting lysine lactyltransferases. It has been reported that p300 has lactyltransferase activity. Inhibiting p300 prevents extensive protein lactylation. Huang *et al.* inhibited p300 in microglia both in vitro and in vivo, which led to the downregulation of YY1 lactylation and pro-inflammatory factors produced by microglia (102).
4. Modulation of lactylated proteins by precise intervention strategy. In BV2 cells, before hypoxia-complex LPS stimulation, point mutations of lysine of p53 to arginine led to a significant decrease in the expression of pro-inflammatory cytokines in p53 mutant cells compared to wild-type cells (29). Wang *et al.* alleviated proliferative retinopathy by preventing YY1 lactylation through the YY1K183R mutation in microglia (55). Therefore, precise regulation of protein modification sites may represent a more effective strategy for targeted intervention of neuroinflammation.

5. CONCLUSION

Hypoxia induces elevated levels of lactate in brain tissue. Under hypoxic conditions, the main sources of lactate in the brain are astrocytes and microglia. Additionally, a portion of lactate originates from peripheral tissues and crosses the BBB into the brain via circulation. Hypoxia-induced lactate serves as a substrate for protein lactylation. Lactylation modification regulates the inflammatory response of microglia by modulating the expression of downstream genes and influencing the progression of neuroinflammation. Current studies have demonstrated that protein lactylation in microglia plays a role in the regulation of hypoxia-induced neuroinflammation. Therefore, lactate, as well as its-mediated protein lactylation represent promising targets for interventions in various neurological disorders.

Founding

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Conflict of interest

The authors have no conflicts of interest. ♦

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