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Lactate dyscrasia: a novel explanation for amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease) is a progressive debilitating neurodegenerative disease with no cure. We propose a novel molecular model for the pathogenesis of ALS that involves an adenosine triphosphate (ATP)-dependent muscle neuronal lactate shuttle (MNLS) at the neuromuscular junction (NMJ) to regulate the flow of lactate from muscle to neurons and vice versa. Failure of the MNLS due to respiratory chain dysfunction is proposed to result in lactate toxicity and degeneration of nerve endings at the NMJ leading to nerve terminus dysjunction from the muscle cell. At a critical threshold where denervation outpaces reinnervation, a vicious cycle is established where the remaining innervated muscle fibers are required to work harder to compensate for normal function, and in so doing produce toxic lactate concentrations which induces further denervation and neuronal death. This mechanism explains the exponential progression of ALS leading to paralysis. The molecular events leading to the dysregulation of the MNLS and the dismantling of NMJ are explained in the context of known ALS familial mutations and age-related endocrine dyscrasia. Combination drug therapies that inhibit lactate accumulation at the NMJ, enhance respiratory chain function, and/or promote reinnervation are predicted to be effective therapeutic strategies for ALS.
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1. Introduction

Amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease) is a debilitating disease that is characterized by progressive neurodegeneration of motoneurons in the brain and spinal cord. Initial manifestations are weakness of limbs, or weakness in the bulbar region leading to abnormalities of speech, swallowing difficulties, and facial weakness (Schmidt et al., 2009). Eventually the loss of motoneurons results in paralysis of voluntary muscles and to death by respiratory failure within 1–5 years of onset of the disease.

ALS is the most common form of motoneuron disease in humans: a little over 5600 people in the USA are diagnosed with ALS each year (incidence of 1–2 per 100,000 per year). Although most cases of ALS typically develop between the ages of 40 and 70, it is often overlooked as being an age-related disease with ethnic and gender predilections. ALS is slightly more prevalent in men (60%) (Wijesekera and Leigh, 2009).

The etiological mechanisms that underlie ALS are unclear, however 5%–10% of ALS cases are familial (FALS) and in those families, there is a 50% chance that each offspring will inherit the genetic mutation and develop the disease (Beghi et al., 2006; Mitchell and Borasio, 2007). However, the underlying gene defect in most patients with FALS is unknown and 90% of all cases have no family

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history of ALS and are considered sporadic ALS (SALS). The pathophysiology of ALS has been postulated to involve immune mechanisms (neuroinflammation and T-cell responses), glutamate-mediated excitotoxicity, oxidative stress, mitochondrial dysfunction, apoptosis, protein aggregation, and aberrant axonal transport (Holmoy, 2008; Mantovani et al., 2009; Pasinelli and Brown, 2006; Seksenyan et al., 2009; Shaw, 2005; Wang et al., 2004).

1.1. Cellular and molecular pathogenesis of ALS

The neuropathology of ALS has been characterized from postmortem analyses (Leigh et al., 1995). The major pathological features of ALS include: (1) degeneration of the corticospinal tracts and extensive loss of lower motoneurons or anterior horn cells (Garofalo et al., 1995; Ghatak et al., 1986; Hughes, 1982); (2) degeneration and loss of Betz cells and other pyramidal cells in the primary motor cortex (Hammer et al., 1979; Maekawa et al., 2004; Udaka et al., 1986); and (3) reactive gliosis in the motor cortex and spinal cord (Ekblom et al., 1994; Kawamata et al., 1992; Murayama et al., 1991; Schiffer et al., 1996).

In addition to the loss of neurons, various types of inclusion bodies have been identified in degenerating neurons and surrounding reactive astrocytes and are well demonstrated hallmarks of ALS (Barbeito et al., 2004). Ubiquitinated inclusions found in lower motoneurons of the spinal cord and brainstem are the most common and specific type of inclusion in ALS (Matsumoto et al., 1993) and in corticospinal upper motoneurons (Sasaki and Maruyama, 1994). The exact composition of such inclusions, classified as "Lewy body-like inclusions", "Skein-like inclusions" (He and Hays, 2004; Kawashima et al., 1998), and Bunina bodies (Wada et al., 1999) is not known. However, the proteins identified so far can include ubiquitin (Leigh et al., 1991; Murayama et al., 1989), Cu/Zn superoxide dismutase 1 (SOD1) (Shibata et al., 1994, 1996), peripherin (He and Hays, 2004), Dorfin (a RING-finger type E3 ubiquitin ligase) (Niwa et al., 2002), and more rarely synuclein (Sone et al., 2005). Various studies conducted in ALS postmortem tissue in the early nineties found accumulations of intermediate filament proteins (hyperphosphorylated neurofilament subunits and peripherin) in hyaline conglomerate inclusions and axonal "spheroids" in spinal cord motoneurons (Corbo and Hays, 1992; Munoz et al., 1988; Sobue et al., 1990), and pyramidal cells of the motor cortex (Troost et al., 1992). Moreover, cystatin C-containing Bunina bodies are found in the cell bodies of motoneurons in ALS (Okamoto et al., 1993; Sasaki and Maruyama, 1994). Some breakdown products of abnormal proteins caused by oxidative stress called ubiquitinated inclusion bodies (UIBs), are also implied in the pathogenesis of ALS (Alves-Rodrigues et al., 1998). Fragmentation of the Golgi apparatus (Fujita et al., 2000; Fujita et al., 2002; Gonatas et al., 1998), mitochondrial vacuolization (Okamoto et al., 1990) and ultrastructural abnormalities of synaptic terminals (Sasaki and Iwata, 1996) are other neuropathological features of ALS.

Approximately 20% of ALS patients also have signs and symptoms of frontotemporal dementia such as cortical atrophy including the frontal and temporal lobes (Nakano, 2000), hippocampus and amygdala (Wilhelmsen et al., 2004), spongiform change in the neocortex, and UIBs in the substantia nigra (Al-Sarraj et al., 2002). Furthermore, the presence of crescent shaped inclusion-type UIBs in the neostriatum has been found to be a feature specific to ALS-frontotemporal dementia, and not occurring in a variety of other neurodegenerative disorders including Pick's disease, Parkinson's disease, and Alzheimer's disease (Kawashima et al., 1998). UIBs are found in ALS patients in the dentate gyrus, frontal and parietal neocortices, anterior cingulate gyrus, hippocampus, parahippocampal gyrus, amygdale and neostriatum. The density and distribution of these inclusions was higher in cognitively-impaired ALS patients (as defined by poor performance on neuropsychological testing) than in unimpaired individuals (Kawashima et al., 1998; Wilson et al., 2001). The cognitively impaired patients also had UIBs in the temporal, occipital, and entorhinal cortices, posterior cingulate gyrus, caudate, and putamen. Computerized morphometry revealed a 25% reduction in the pyramidal neuronal density in layer V of the premotor cortex, dorsolateral prefrontal cortex, and anterior cingulate cortex compared with age-matched controls (Maekawa et al., 2004). This is particularly relevant in the context of findings from positron emission tomography neuroimaging which identified decreased binding of the GABAergic ligand (11C)-flumazenil in the prefrontal cortex (Lloyd et al., 2000; Turner et al., 2005) and increased microglial activation (implicated in mechanisms of neuronal cell death) in the dorsolateral prefrontal cortex (Turner et al., 2004).

1.2. Spatiotemporal changes in ALS neuropathology

Dissecting the spatiotemporal changes in pathology is key to understanding the molecular mechanism(s) involved in ALS. From a spatial perspective, the notion that ALS affects only the motoneurons while sparing the central nervous system was refuted when neuropathological examination showed ubiquitin-immunoreactive but tau-negative inclusions in the frontotemporal cortex, hippocampus, and dentate gyrus (Jackson and Lowe, 1996). To determine where and when the pathological changes of motoneuron disease begins, Fischer and colleagues (Fischer et al., 2004) performed a comprehensive spatiotemporal analysis of disease progression in $SOD1^{G93A}$ mice. Quantitative pathological analysis was performed in the same mice at multiple ages at neuromuscular junctions (NMJs), ventral roots, and spinal cord. Mice became clinically weak at 80 days and died at 131 $\pm$ 5 days. At 47 days, 40% of end plates were denervated whereas there was no evidence of ventral root or cell body loss. At 80 days, 60% of ventral root axons were lost but there was no loss of motoneurons. Motoneuron loss

Fig. 1. Muscle innervation. An illustration of a normal motor unit showing axons leading to motor end plates (neuromuscular junctions) on muscle cells. (Taken from http://www.biology.iastate.edu/Courses/212L/New%20Site/31%20MUscle%20&%20Skeletal%20systems/muscle%20skeletal%20index.htm). Photos and layout by Linda Westgate, Warren Dolphin, and Mark A. Mangum.

was well underway by 100 days. Microglial and astrocytic activation around motoneurons was not identified until after the onset of distal axon degeneration. Thus, in this animal model of human ALS, motoneuron pathology begins at the distal axon and proceeds in a "dying back" pattern. This is supported by the denervation and reinnervation changes in muscle but normal-appearing distal motoneurons following autopsy of a reported ALS patient (Fischer et al., 2004).

1.3. The neuromuscular junction and its dismantling in ALS

The basic unit of movement is comprised of skeleton, muscles connected to skeleton, and nerves connected to the muscles. A motor unit consists of 1 motor neuron in the anterior horn of the spinal cord, its axon, and all the muscle fibers innervated by the branches of the axon (Fig. 1). The axon of the nerve terminates on the muscle fibers at the NMJ. The number of motor units that are active in a muscle at any 1 time determines the level of performance of the muscle. Thus each functional NMJ determines the motor ability.

It has been demonstrated that neuromuscular deficits in ALS do not result from motoneuron cell death but rather from loss of axonal integrity. As mentioned above, in the SOD1^{G93A} transgenic mouse model, motor unit numbers in fast-twitch tibialis anterior, extensor digitorum longus, and medial gastrocnemius muscles decline from 40 days of age, 40–50 days before reported overt symptoms and motoneuron loss (Hegedus et al., 2007; Kennel et al., 1996). Motor unit numbers fall after overt symptoms in the slow-twitch soleus muscle (Hegedus et al., 2007). Similarly, the fact that end plates are denervated much earlier than the axons and the cell body loss during the pathogenesis of ALS as described in SOD1^{G93A} mice (Fischer et al., 2004), gives ample indication that the degenerative process in ALS starts at the NMJ. In canine motoneuron diseases, functional motor unit failure precedes neuromuscular degeneration (Balice-Gordon et al., 2000).

Early muscle-specific decline has been correlated to selective preferential vulnerability of large, fast motor units, innervated by large motoneurons. Large motoneurons appear to be the most vulnerable in ALS with die-back occurring prior to overt symptoms (Hegedus et al., 2007). Subsequently, it was found that disease progression in fast-twitch muscles of SOD1^{G93A} mice involves parallel processes: (1) gradual selective motor axon die-back of the fast fatigable motor units that contain large type IIB muscle fibers, and of fatigue-intermediate motor units that innervate type IID/X muscle fibers; and (2) activity-dependent conversion of motor units to those innervated by smaller motor axons innervating type IIA fatigue-resistant muscle fibers (Hegedus et al., 2009).

Studies that have used strategies to preserve the NMJ in ALS models invariably show a delay in disease progression (Dobrowolny et al., 2005; Ferri et al., 2003; Gifondorwa et al., 2007; Li et al., 2007; Rouaux et al., 2007; Storkebaum et al., 2005; Suzuki and Svendsen, 2008; Suzuki et al., 2007, 2008). Conversely, prevention of neurite outgrowth promotes denervation in an ALS model (Jokic et al., 2006).

There is conflicting evidence as to whether degenerative signals emanate from the motoneuron itself, or from the muscle. It has been shown that suppression of hSOD1^{G93A} expression within muscle alone is insufficient to maintain grip strength or affect disease onset or survival, but that suppression of hSOD1^{G93A} expression in both motor neurons and muscle is sufficient to maintain grip strength (Miller et al., 2006). Moreover, this group found that follistatin induced inhibition of myostatin, produced sustained increases in muscle mass, myofiber number, and fiber diameter, but these increases did not affect survival. Conversely, a mouse model of muscle restricted mitochondrial defect (similar to the hypermetabolism found in ALS) has been shown to generate motor neuron degeneration (Dupuis et al., 2009). Transgenic mice with muscular overexpression of uncoupling protein 1, a potent mitochondrial uncoupler, displayed age-dependent deterioration of the NMJ that correlated with progressive signs of denervation, grip strength, and a mild late-onset motor neuron pathology.

Together, these data indicate that understanding the molecular events that promote the degeneration and dismantling of NMJ is crucial to understanding the underlying cause of ALS and is a prerequisite for identifying appropriate treatment strategies. The important question therefore is what molecular events lead to the deterioration of the motoneuron terminals at the NMJ? Factors produced by either the muscles or motoneurons that impact the NMJ might be considered prime candidates in the molecular pathology of the disease. What are these factors, and how are they regulated and how do they affect the normal molecular signaling and trafficking at the NMJs?

2. The lactate dyscrasia hypothesis of ALS

What is interesting about many patients with ALS is that the function of the eye muscles is spared. Understanding what is different about the eye muscles compared with other muscles might therefore throw light on what causes skeletal muscles to dysfunction. One noticeable characteristic of the ocular nerves and muscles is that they use lactic acid as a metabolic substrate to sustain function and therefore do not become fatigued by high lactic acid, unlike skeletal muscles (Andrade and McMullen, 2006). Thus, lactate is a metabolic substrate that sustains extraocular muscle function and prevents muscle fatigue suggesting that these muscles have high lactate turnover (i.e., the molecular machinery to convert lactate to pyruvate). Supporting this, lactate dehydrogenase (LDH) activity is detected in oculomotor neurons (Hayashi, 1987) and in eye muscles (Kahan and Juhasz, 1976). Alternatively, it is possible that lactate is also removed from the nerves/muscles via a lactate shuttle (muscle-neuronal lactate shuttle; MNLS), like the recently proposed astrocyte-neuronal lactate shuttle (Erlichman et al., 2008; Mangia et al., 2009). We therefore propose that an MNLS exists to maintain lactate homeostasis between muscles and motoneurons (the neuromuscular unit) and that dysregulation of the MNLS results in lactate assimilation in the NMJ leading to cellular stress, toxicity, and subsequent degeneration. The lack of neurotransmission would be expected to lead to muscular atrophy. Similarly, excess lactate accumulation in myocytes also may promote muscle degeneration, although it might be expected that peripheral motoneurons are more susceptible to high lactate levels than peripheral muscles. Progressive muscular atrophy is another disease where dysregulation of lactate homeostasis could lead to motoneuron degeneration with subsequent rapid muscular atrophy (Ince et al., 2003).

The loss of lactate homeostasis and subsequent death of motoneurons may create a vicious cycle whereby the remaining muscle fibers are required to work harder to compensate for normal muscle function, producing more lactate and/or other toxic radicals inducing further motor neurotoxicity that leads to further neuronal degeneration and death. This would explain the exponential progression of ALS leading to paralysis.

2.1. The molecular model

We propose the existence of an MNLS to maintain lactate homeostasis at the NMJ (Fig. 2). We also anticipate that this shuttle is an adenosine triphosphate (ATP)-dependent shuttle operating at the NMJ between the muscle cell and the nerve terminal to tightly regulate the flow of lactate from the muscle to the neuron (or vice versa). The activity of this shuttle is dependent on both the energy state and the threshold level of lactate tolerance of the muscle cell and the neuron constituting the NMJ. Because the energy state of a cell is dependent upon the generation of ATP, we propose a molecular mechanism involving glycolysis, the tricarboxylic acid (TCA) cycle and the respiratory chain, the main metabolic players of which include lactate, malate, oxaloacetate, citrate, and aspartate. The mechanism can be explained as follows. Under normal cellular conditions, the respiratory chain provides the proton needed for the transport of aspartate from mitochondria to the cytosol via the aspartate shuttle (aspartate is otherwise impermeable to the mitochondrial membrane). The aspartate entering the cytosol can be converted to oxaloacetate by cytoplasmic aspartate aminotransferase. This cytoplasmic oxaloacetate (also impermeable to mitochondrial membrane) is converted to malate by cytoplasmic malate dehydrogenase and the malate thus formed can be converted to pyruvate by malic enzyme. The oxaloacetate can also be converted to phosphoenolpyruvate by phosphoenolpyruvate carboxylase present in the cytoplasm. The phosphoenolpyruvate can then be converted to pyruvate by pyruvate kinase. Pyruvate can be converted to lactate by the activity of cytoplasmic LDH. Pyruvate also can combine with acetyl CoA and can be fed into the TCA cycle along with oxaloacetate via the pyruvate dehydrogenase complex in the mitochondria to generate citrate. Citrate, via a series of reactions in the TCA cycle can regenerate oxaloacetate and malate. Oxaloacetate can be converted to aspartate in the mitochondria by aspartate aminotransferase. The malate and citrate can either diffuse, or are carried, across the mitochondrial membrane by carrier proteins (Fig. 2). This whole series of metabolic reactions occurs in both muscle cells and motoneurons constituting the NMJ. Thus, under normal conditions, lactate levels are kept under tolerable levels at the NMJ by: (1) conversion of lactate to pyruvate; (2) normal mitochondrial function for the generation of protons and ATP; and (3) the activity of the ATP-dependent MNLS.

Dysfunction of the respiratory chain and the failure to contribute protons required for the translocation of aspartate from the mitochondria to the cell cytoplasm would result in the accumulation of aspartate in the mitochondria. This prevents the conversion of oxaloacetate to aspartate. The accumulated oxaloacetate will therefore be increasingly converted to malate or citrate which either diffuses or is transported into the cytoplasm by nonenergy-dependent carboxylate carriers for conversion to pyruvate (Fig. 2). This continual accumulation of pyruvate leads to its active conversion to lactate by cytoplasmic LDH for the maintenance of (limited) ATP generation via glycolysis (Fig. 2). A decrease in respiratory function would directly impair the energy-dependent transport of lactate, leading to its accumulation in the NMJ, muscle, and/or nerve axon. The accumulation of pyruvate in the cytoplasm also would limit flux through the normal glycolytic pathway for the generation of ATP and protons. Mitochondrial dysfunction and the accumulation of lactate are therefore expected to impact the neuron to a greater extent than the myocyte which is expected to be more tolerant to high concentrations of lactate.

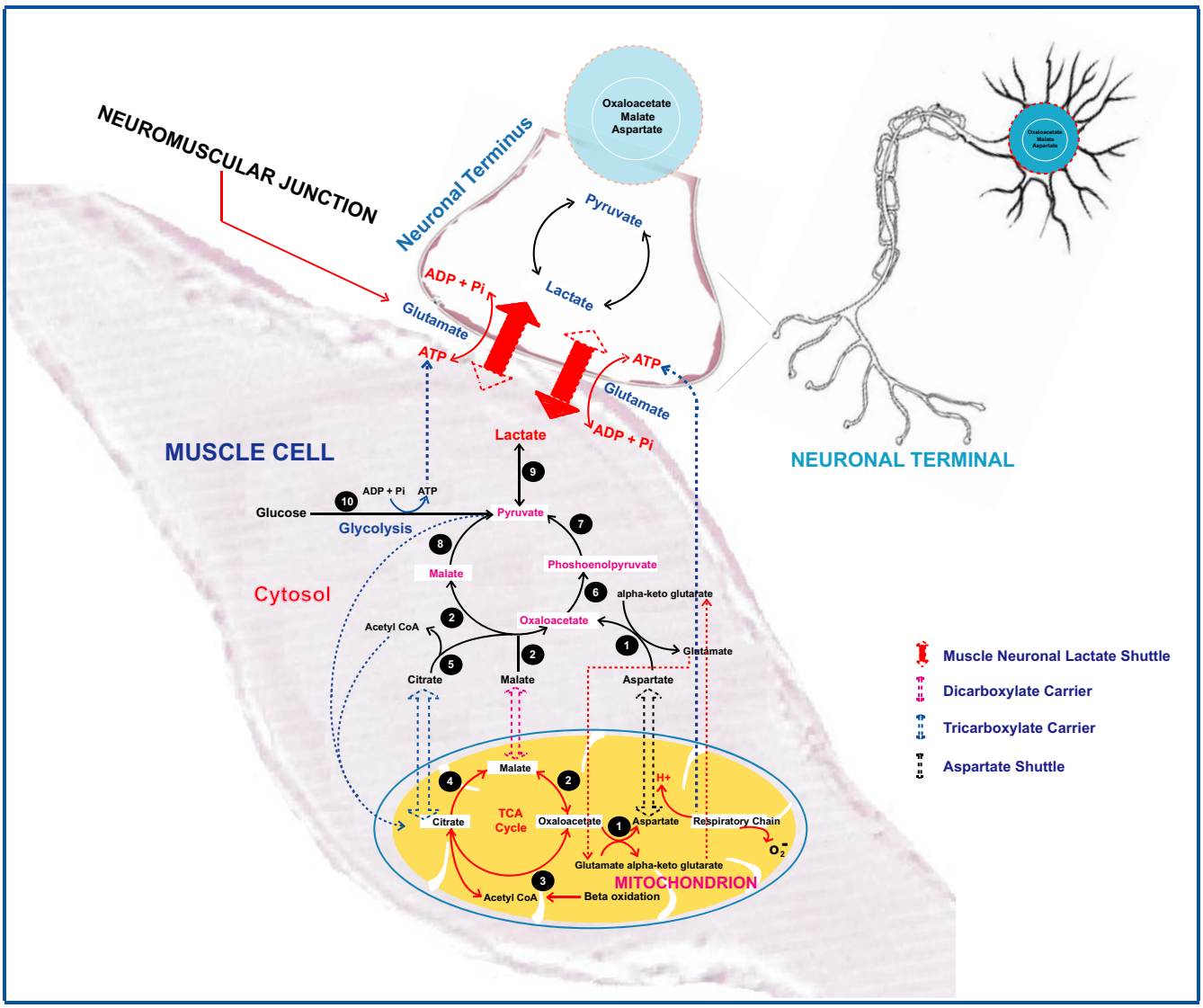

Fig 2. Molecular model for amyotrophic lateral sclerosis (ALS) pathogenesis. The model proposes that failure of the adenosine triphosphate (ATP)-dependent muscle neuronal lactate shuttle (MNLS − hypothetical) due to respiratory chain dysfunction leads to lactate toxicity and consequent dismantling of the neuromuscular junction (NMJ) in ALS. In essence, when the respiratory chain can no longer contribute protons required for the translocation of aspartate from the mitochondria to the cell cytoplasm, aspartate accumulation in the mitochondrion prevents the conversion of oxaloacetate to aspartate by aspartate aminotransferase (1). Accumulated oxaloacetate is increasingly converted to malate by mitochondrial malate dehydrogenase (2) or citrate by citrate synthase (3). Citrate itself can be converted to malate by a series of tricarboxylic acid (TCA) cycle enzymes (4) such as aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinyl CoA synthase, succinate dehydrogenase and fumarase. The malate and citrate formed either diffuse or are carried to the cytoplasm by nonenergy-dependent dicarboxylate and tricarboxylate carriers for conversion to pyruvate. In the cytoplasm, malate is converted to oxaloacetate by malate dehydrogenase (2) and citrate is cleaved to oxaloacetate by citrate lyase (5). The oxaloacetate thus generated in the cytoplasm is converted to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (6) or malate by malate dehydrogenase (2). Cytoplasmic phosphoenolpyruvate and malate are converted to pyruvate by pyruvate kinase (7) and malic enzyme (8), respectively. Pyruvate accumulation will inhibit further glycolysis (10), and anaerobic metabolism will promote pyruvate conversion to lactate by cytoplasmic lactate dehydrogenase (9). The inhibition of the conversion of oxaloacetate to aspartate also will promote glutamate accumulation (because the conversion of glutamate to α-ketoglutarate by mitochondrial glutamate dehydrogenase is coupled to the conversion of oxaloacetate to aspartate; Lehninger et al., 1993). The accumulation of glutamate may promote excitotoxicity. These reactions occur in both nerve and muscle cell. Thus, when the energy-dependent shuttles can no longer operate, toxic levels of lactate and glutamate accumulate in the nerve terminal and NMJ, leading to dysfunction, degeneration of nerve endings and subsequent nerve terminus dysjunction or dismantling from the muscle cell at the NMJ. This loss of NMJs places further 'strain' on remaining muscle-nerve units that attempt to compensate to provide normal function, leading to a vicious cycle of increased lactate production and neurotoxicity.

The inhibition of the conversion of oxaloacetate to aspartate also leads to the accumulation of glutamate (as the conversion of glutamate to α-ketoglutarate by glutamate dehydrogenase in the mitochondria is coupled to oxaloacetate to aspartate conversion; Lehninger et al., 1993). Glutamate is known to induce lactate production (Mendelowitsch et al., 2001) by driving aspartate production and subsequently pyruvate and lactate production as discussed above

(Fig. 2). Glutamate also is known to compromise the respiratory function of mitochondria (i.e., inducing Ca^{2+} accumulation and increasing reactive oxygen species (ROS) (Urushitani et al., 2001) and inhibiting proton generation for aspartate transport which leads to excitotoxicity. Thus, when the MNLS fails to shuttle lactate due to a lack of ATP, lactate and glutamate accumulation would lead to degeneration of the nerve endings (Vijayvergiya et al., 2005), dysfunction of the NMJ, and axonal toxicity. The loss of NMJ would lead to a compensatory overactivity of the remaining NMJ and muscle fibers, resulting in further increases in lactate production and/or other toxic radicals, with this vicious cycle inducing further motor neurotoxicity that leads to muscle atrophy and eventually chronic paralysis.

2.2. Evidence for the molecular model

Evidence supporting the MNLS model includes: (1) the concentration of serum lactic acid is higher in persons with ALS (2.77 ± 0.79 mmol/L) and chronic denervated non-ALS patients (2.79 ± 1.29 mmol/L) compared with controls (1.48 ± 0.49 mmol/L) (Siciliano et al., 2001); (2) lactic acid can induce death in neurons (Nedergaard et al., 1991); (3) increased lactate concentrations have been reported in other neurodegenerative conditions such as Huntington disease (Bowling and Beal, 1995) as well as in models of severe and mild brain injury (Ramonet et al., 2004); (4) neuroprotective drugs like nizofenone that block lactate accumulation are used to treat other neurodegenerative diseases (Matsumoto et al., 1994); (5) lactate metabolism in ALS is associated with glutamate excitotoxicity related to neuronal degeneration (Shobha et al., 2007); (6) mitochondrial oxidative phosphorylation is dysfunctional in $SOD1^{G93A}$ transgenic mice (Jung et al., 2002; Mattiazzi et al., 2002; Vijayvergiya et al., 2005); (7) the malate-aspartate shuttle is inhibited in $hSOD1^{G93A}$ expressing cells (Mali and Zisapels, 2008) and this may explain the elevated levels of lactate and the damage to neurons (Mali and Zisapels, 2008); (8) $hSOD1^{G93A}$-expressing cells showed increased concentrations of cytoplasmic malate dehydrogenase messenger ribonucleic acid (mRNA), malate, and lactate compared with noninduced or wild-type-hSOD1-expressing cells (Mali and Zisapels, 2008); (9) the mitochondrial NADH/NAD+ ratio is elevated in $hSOD1^{G93A}$-expressing cells indicating an increased conversion of oxaloacetate to malate in the mitochondria by NADH-dependent mitochondrial malate dehydrogenase MDH (Mali and Zisapels, 2008); (10) impairments in the malate-aspartate shuttle which controls the brain mitochondrial $NADH/NAD^+$ balance is known to drive anaerobic metabolism (particularly damaging to neurons) as well as vulnerability to impairments of glycolytic pathways (Mali and Zisapels, 2008); (11) impaired oxidative metabolism and accumulation of lactate was reported in exercising ALS patients (Siciliano et al., 2001); and (12) functional motor unit failure precedes neuromuscular degeneration in motoneuron disease (Balice-Gordon et al., 2000; Fischer et al., 2004).

3. What derails lactate homeostasis leading to neuromuscular junction toxicity?

ALS is an age-related disease; the cause of ALS must therefore be related to the aging process. Because the downstream cause of ALS is the loss of NMJ and motoneurons, understanding what signals regulate the balance between the formation of motoneurons (innervation) and loss of motoneurons (denervation) is critical to identifying the upstream signals that promote ALS. It is known that denervated muscle is readily reinnervated, whereas innervated muscle cannot be hyperinnervated (Frank et al., 1975; Sanes and Covault, 1985). Recent evidence suggests that a compensatory increase in neuromuscular synapse regeneration in ALS mediated by the skeletal muscle-specific micro ribonucleic acid (microRNA), miR-206 that modulates fibroblast growth factor signaling and myogenin expression, is insufficient to compensate for the damage induced by mutant SOD1 (Williams et al., 2009). We propose that at a critical threshold where denervation outpaces reinnervation, a vicious cycle is established where the remaining innervated muscle fibers are required to work harder to compensate for normal function, and in doing so produce toxic lactate concentrations which induces further denervation and neuronal death.

The question therefore becomes what signals promote innervation and what promote denervation, and how do these change with aging? Because innervation is driven by sex steroids (in particular estrogens and progestagens), changes in sex steroids with aging may prevent appropriate reinnervation in those individuals who produce elevated lactate/glutamate (i.e., those containing a mutation in SOD1 or other associated metabolic pathway genes as described above for example). This section will present evidence for the genetic and geno-gerontological (i.e., the genetic regulation of aging processes) regulation of lactate homeostasis that can explain the neuromuscular pathophysiology of FALS and SALS.

3.1. Genetic-linked alterations in lactate homeostasis underlying FALS

Genetic alterations in *SOD1* have been identified in 20%–25% of those with FALS (Cudkowicz et al., 1997). Although loss of catalytic hSOD1 activity has been implicated and mitochondrial ROS is associated with the mechanism underlying denervation-induced atrophy (Muller et al., 2007) in familial ALS, the nature of the toxicity is poorly understood (Carri et al., 1997; Kruman et al., 1999; Rizzardini et al., 2005). Evidence for mutations in SOD1 as driving the pathogenesis of ALS has been reported in cell systems and mice overexpressing the $hSOD1^{G93A}$ protein as described above (Jung et al., 2002; Mali and Zisapels, 2008;

Mattiazzi et al., 2002; Vijayvergiya et al., 2005). These systems suggest that FALS-related mutations alter lactate homeostasis leading to the pathophysiology of FALS.

Suppression of hSOD1^{G93A} expression within muscle alone is insufficient to maintain grip strength or affect disease onset or survival (Miller et al., 2006). Suppression of hSOD1^{G93A} expression in both motor neurons and muscle was sufficient to maintain grip strength. The requirement for reduction of the expression of hSOD1^{G93A} in both muscle and motoneurons to effect an increase in muscle strength suggests that decreases in the aberrant activity of this protein in all cell types is required to reverse the dysfunctional mitochondrial oxidative phosphorylation (Jung et al., 2002; Mattiazzi et al., 2002; Vijayvergiya et al., 2005) and malate-aspartate shuttle inhibition in hSOD1^{G93A} expressing cells (Mali and Zisapels, 2008), and that lactate production in the motoneuron may preferentially effect disease progression.

An analysis of genetic alterations in enzymes involved in pyruvate and lactate production and removal, and the TCA cycle, including α-ketoglutarate dehydrogenase complex along with the glycolytic partner LDH might give further insights into the key molecular candidates involved in the development of FALS.

3.2. Genetic and age-linked alterations in lactate homeostasis underlying SALS

The mean age of onset for SALS is $\sim$ 60 years (Wijesekera and Leigh, 2009), and is strongly suggestive of an aging component in the onset of this sporadic form of the disease. This is supported by studies in the mouse model of ALS (hSOD1^{G93A} mutant mouse). The magnetic resonance imaging signal intensities of nucleus V, VII, XII, and nucleus ambiguus of these mice show an age-dependent increase starting around day 60, parallel to the first behavioral signs of motoneuron disorder (Angenstein et al., 2004). Also, the age-related progression of motor unit loss, adaptive sprouting (reinnervation of the denervated end plates), maladaptive sprouting, and continuing recession of nerve terminals during normal aging is extremely rapidly accelerated in ALS (Gordon et al., 2004).

Nearly 3 decades ago Appel (1981) hypothesized that ALS may be related to steroid hormone/receptor deficiencies and that neurotrophic hormones acting at the synapse may be critical in maintaining the neural networks that are affected in ALS. Little research has been performed since on the role of hormonal signaling with regard the pathogenesis and progression of ALS. We summarize below what is known about sex-linked hormonal involvement in ALS.

The slight male prevalence (male:female ratio approximately 1.5:1; Wijesekera and Leigh, 2009) in the etiology of ALS supports the possible involvement of a sex-linked hormonal component to the pathogenesis of ALS, as proposed for other neurodegenerative diseases (Atwood et al., 2005; Vadakkadath Meethal and Atwood, 2005). An early study indicated a gender-related specificity in the ability of thyrotropin-releasing hormone to potentiate the monosynaptic reflex (Miller and Warnick, 1989). While castration in male neonatal rats lowered the sensitivity to thyrotropin-releasing hormone, testosterone treatment restored that sensitivity. In this respect, there is a significant decrease in free testosterone levels in ALS patients (Militello et al., 2002). Interestingly, the female advantage toward ALS disappears with age (Haverkamp et al., 1995). In animal studies using the hSOD1^{G93A} transgenic mouse, treatment of ovariectomized females with 17β-estradiol did not delay the onset of disease, but prevented progression of ALS motor dysfunctions as shown by extension reflex test for a limited time window (Choi et al., 2008). Importantly, 17β-estradiol treatment rescued the life spans in ovariectomized females (Choi et al., 2008). Moreover, the presence of the "slow Wallerian degeneration" (Wld(S)) gene, shown to be protective in numerous models of axonal degeneration, prolongs survival preferentially in female mice carrying SOD1^{G93A} (Fischer et al., 2005). These observations reinforce the role of sex steroids in the maintenance of normal motoneuron function and throw light on their potential to avert ALS pathogenesis during aging.

That sex steroids are involved in the etiology of ALS is supported by numerous studies indicating that sex steroids are essential for normal brain function; they are neuroprotective, and promote neurogenesis, neuronal survival, and normal cognitive function (reviewed in Bates et al., 2005; Gleason et al., 2005; Simpkins et al., 2005; Vadakkadath Meethal and Atwood, 2005). In particular, progestagens have been demonstrated to promote embryonic neurogenesis (neurulation) (Gallego, et al., 2009; Gallego, et al., unpublished results available at: http://hdl.handle.net/10101/npre.2008.2671.1) and neural differentiation in vitro (Brewer et al., 1993) and in vivo (Wang et al., 2005). Progestagens and estrogens are primary hormonal signals that regulate neuronal growth and differentiation (Gould et al., 2000), promoting neurite development and migration that lead to changes in synaptogenesis (Leranth et al., 2002; Masumoto et al., 1991; McEwan et al., 1996; Simerly, 2002). As part of these differentiation processes, sex steroids are known to modulate growth of dendrites and dendritic spine density, with the loss of sex steroids generally resulting in decreased spine density (Leranth et al., 2003; Woolley and McEwen, 1993).

A decline in peripheral (i.e., gonads, adrenals, or other tissues) and/or local (central nervous system) sex steroid production might therefore be expected to promote motoneuron degeneration. Dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis with aging has been postulated to drive aging-related diseases via alterations in cell signaling throughout the body (Atwood et al., 2005; Bowen and Atwood, 2004; Vadakkadath Meethal and Atwood, 2005). This altered cell cycle signaling, resulting from the decline in sex steroid and inhibin production and elevation in gonadotropin-releasing hormone, gonadotropin, and activin signaling, has been shown to drive postreproductive

degenerative mechanisms involved in Alzheimer's disease (Bowen et al., 2004; Casadesus et al., 2006), osteoporosis (Sun et al., 2006), and blood-brain barrier integrity and associated diseases such as stroke (Wilson et al., 2008).

The abrupt loss of serum sex steroids with reproductive senescence not surprisingly correlates with an increased prevalence of cognitive disease in women (Brookmeyer et al., 1998; Jorm et al., 1987; McGonigal et al., 1993), while sex steroid replacement therapy decreases the incidence (Henderson et al., 1994) and delays the onset of cognitive decline in women and men (reviewed in Gleason et al., 2005). Interestingly, ALS is associated with the loss of cognitive-behavioral competency with progressive involvement of the prefrontal cortex and, in a few instances, profound dementia (Montgomery and Erickson, 1987), supportive of a hormonal component to the disease process.

That these hormones could impact the NMJ is evidenced by the fact that receptors for sex steroids are localized in various muscles, neurons, and neuromuscular junctions of vertebrate and invertebrates (Kimura et al., 1993; Pelletier, 2000). Sex steroid receptors are expressed in smooth muscle cells (Haynes et al., 2002) and respiratory motoneurons of both male and female rats (Behan and Thomas, 2005). Interestingly, sex hormone receptors are present in motor neurons of the rat embryo spinal cord (Rakotoarivelo et al., 2004). There is an enrichment of androgen receptor (+) myonuclei and fibroblasts proximate to neuromuscular junctions in the skeletal muscle of the rat, suggesting that androgen receptors at muscle synapses may selectively regulate synapse-specific genes important for the survival and growth of motoneurons (Monks et al., 2004). Castration reduced the proportion of androgen receptor fibroblasts in muscles and testosterone treatment prevented these effects of castration. There are also evidences for the androgen regulation neuromuscular junction structure and function in some of the sexually dimorphic muscles of the frog *Xenopus laevis* (Brennan and Henderson, 1995). In addition, there is strong evidence for the fact that testosterone via its receptor may regulate the coupling mechanisms between Ca v2.2 channels and neurotransmitter release at the neuromuscular junctions of bulbocavernosus and levator ani muscles motoneurons (Nudler et al., 2005). Earlier results have shown that testosterone deprivation reduces the junctional acetylcholine receptor density and androgens modulate endplate size and acetylcholine ACh receptor density at synapses in rat levator ani muscle (Bleisch and Harrelson, 1989; Bleisch et al., 1982; Souccar et al., 1991). Finally, in *Xenopus laevis*, females have stronger laryngeal synapses than males, and synapse strength is estrogen dependent and the laryngeal neuromuscular synapse is the final effector for sexually differentiated song production. Immunocytochemistry and Western blots confirmed the presence of estrogen receptor protein in laryngeal muscle fibers (Bleisch et al., 1982). Together, these observations suggest a prominent role for sex steroids in the normal functioning of neurons and muscles in the NMJ.

3.3. Glutamate/lactate metabolism and the sex hormones

Increased glutamine synthetase, plasma glutamate levels, defective glutamate metabolism, and glutamate excitotoxicity have been strongly implicated in the pathogenesis of ALS (Andreadou et al., 2008; Bos et al., 2006; Ionov, 2007). The exposure of SALS-serum to rat motoneurons increases their LDH activity and depletes the glutamate transporter GLT-1 and the cells subsequently die presumably due to increased levels of glutamate triggering glutamate-mediated toxicity (Shobha et al., 2007; Vijayalakshmi et al., 2009). Interestingly, this observation ties glutamate toxicity to lactate levels. One of the earlier studies showing increased LDH activity in the hypothalamic nuclei of adult neonatally androgenized female rats (Packman et al., 1977) clearly gave evidence for the linkage of androgen signaling to lactate oxidation in brain. In this regard, the endogenous steroid hormones such as aldosterone, progesterone, and testosterone showed neuroprotective effects from glutamate neurotoxicity in various neuronal cell cultures (Bhavnani et al., 2003; Ogata et al., 1993; Zhao et al., 2002). Interestingly, Mendelowitsch et al. (2001) showed that glutamate induces lactate production, intake of lactate by neurons, and that the neuroprotective effect of 17β-estradiol requires the activities of lactate transporters. Also, the lactate transfer between the astrocyte and neurons is demonstrated to be potentiated by glutamatergic activity (Pellerin, 2008). In addition, we propose that the inhibition of the conversion of oxaloacetate to aspartate can lead to the accumulation of glutamate as the conversion of glutamate to α-ketoglutarate by glutamate dehydrogenase in the mitochondria will not occur because this reaction is coupled to oxaloacetate to aspartate conversion (Lehninger et al., 1993). Thus, it is reasonable to conclude that: (1) glutamate and lactate production and their metabolism are tightly linked; (2) age-dependent changes in reproductive hormones can play a role in glutamate/lactate homeostasis in NMJs; (3) the functions of sex hormones are linked to the lactate metabolism and lactate transporters; (4) lactate transporters are important in normal neuronal function; and (5) cellular energy failure can result in the loss of MNLS function and the consequent accumulation of lactate can lead to neurotoxicity and dismantling of the NMJ.

4. A multidrug therapy for the treatment of ALS

Based on the above model along with the other evidence described above, therapeutic strategies for the treatment ALS should incorporate drugs that: (1) maintain lactate homeostasis in NMJs; (2) maintain mitochondrial function; (3) halt damage to peripheral nerves; and (4) promote regeneration of peripheral nerves. Thus, combinations of drugs that inhibit lactate accumulation at the NMJ, enhance

respiratory chain function, and that are neurotrophic should be most effective at halting the progression of ALS.

The existing treatment modalities partly support this idea. Rilutek (riluzole; Sanofi-Aventis, Bridgewater, NJ), the popular "antiglutamatergic" agent, remains the only Food and Drug Administration (FDA) approved drug for ALS treatment at present, decreasing the progression of ALS, and increasing the survival of ALS patients by 4–19 months (Radunovic et al., 2007). However, riluzole may not be effective during advanced stages of the disease (Radunovic et al., 2007). Memantine, a noncompetitive N-methyl-D-aspartate receptor antagonist, was recently found to prolong the survival of $SOD1^{G93A}$ mouse model (Wang et al., 2005), supporting glutamate toxicity in the pathogenesis of ALS.

There is generally no consensus on the effects of exercise and ALS. While moderate exercise has been shown to have beneficial effects in ALS, strenuous exercise (i.e., which induces lactic acid) may have adverse effects (reviewed in Miller et al., 2006). The balance between lactate production and enhanced signaling for innervation following exercise may explain these discrepancies.

5. Conclusions

We present a novel molecular model for the molecular pathogenesis of ALS that involves an ATP-dependent MNLS to maintain lactate homeostasis at the NMJ by tightly regulating the flow of lactate from muscle to neurons and visa versa. Failure of this shuttle is proposed to lead to lactate assimilation in the NMJ leading to cellular stress, toxicity, and subsequent degeneration. Future studies should focus on the identification and characterization of the MNLS and the mutational and endocrine factors that regulate MNLS function and dysfunction in ALS. Moreover, age-related changes in hormones should be considered in the etiology of the disease. Combination therapy composed of drugs that inhibit lactate accumulation at the NMJ, enhance respiratory chain function, and that are neurotrophic are predicted to be the most effective therapeutic strategy for halting the progression of ALS.

Disclosure statement

There are no actual or potential conflicts of interest.

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