Astrocytic glycogen-derived lactate fuels the brain during exhaustive exercise to maintain endurance capacity

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Brain glycogen stored in astrocytes provides lactate as an energy source to neurons through monocarboxylate transporters (MCTs) to maintain neuronal functions such as hippocampus-regulated memory formation. Although prolonged exhaustive exercise decreases brain glycogen, the role of this decrease and lactate transport in the exercising brain remains less clear. Because muscle glycogen fuels exercising muscles, we hypothesized that astrocytic glycogen plays an energetic role in the prolonged-exercising brain to maintain endurance capacity through lactate transport. To test this hypothesis, we used a rat model of exhaustive exercise and capillary electrophoresis-mass spectrometry–based metabolomics to observe comprehensive energetics of the brain (cortex and hippocampus) and muscle (plantaris). At exhaustion, muscle glycogen was depleted but brain glycogen was only decreased. The levels of MCT2, which takes up lactate in neurons, increased in the brain, as did muscle MCTs. Metabolomics revealed that brain, but not muscle, ATP was maintained with lactate and other glycogenolytic/glycolytic sources. Intracerebroventricular injection of the glycogen phosphorylase inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol did not affect peripheral glycemic conditions but suppressed brain lactate production and decreased hippocampal ATP levels at exhaustion. An MCT2 inhibitor, α-cyano-4-hydroxycinnamate, triggered a similar response that resulted in lower endurance capacity. These findings provide direct evidence for the energetic role of astrocytic glycogen-derived lactate in the exhaustive-exercising brain, implicating the significance of brain glycogen level in endurance capacity. Glycogen-maintained ATP in the brain is a possible defense mechanism for neurons in the exhausted brain.

brain glycogen | lactate transport | ATP | endurance capacity | metabolomics

Glucose derived from blood is the primary energy source for generating ATP in the brain, but an important energy reserve is brain glycogen synthesized from glucose in astrocytes (1). Astrocytic glycogen is broken down through glycogenolysis/glycolysis to produce lactate as a neuronal energy substrate transported by monocarboxylate transporters (MCTs) (2). Indeed, brain glycogen decreases during memory tasks (3) and in some physiologically exhaustive conditions such as sleep deprivation (4) and hypoglycemia (5). The genetic/pharmacologic inhibitions of glycogenolysis and/or lactate transport impair neuronal survival under severe hypoglycemia, as well as axon transmission and hippocampus-related memory formation (6–8). Therefore, astrocytic glycogen-derived lactate is a critical energy source for meeting brain energy demands for neuronal functions and/or survival.

Although less than for exercising muscles, physical exercise activates brain neurons and increases brain energy demand (9). Blood glucose and lactate contribute to brain energetics during moderate or intense exercise (10, 11). Muscle glycogen is an important energy for maintaining muscle contraction during endurance exercise (12), however, the role of brain glycogen during exercise remains uncertain. We have reported a decrease in brain glycogen in the cortex, hippocampus, hypothalamus, cerebellum, and brainstem during prolonged exhaustive exercise associated with lactate elevation (13, 14). Furthermore, prolonged, but not exhaustive, exercise increases levels of hippocampal MCT2 (15), which transports lactate to neurons as MCT1 does to exercising muscles (16). Although untested, it is thus postulated that the lactate derived from astrocytic glycogen plays a role in brain energetics to maintain endurance capacity during prolonged exercise, as is the case for memory formation in the hippocampus (6).

Notably, brain glycogen decreases, but is not fully depleted, under exhaustive conditions such as sleep deprivation (4) and hypoglycemia (5). We observed the same phenomenon in the exercise-exhausted rat brain (13, 14), whereas muscle glycogen was almost fully depleted with ATP reduction (17). In contrast, insulin-induced severe hypoglycemia elicits depletion of brain glycogen and reduces brain ATP, resulting in neuronal death in the hippocampus (18). Epileptic seizures also induce neuronal death caused by brain ATP decrease (19), and lead to hippocampal-related memory dysfunction (20). Further, lactate plays a neuroprotective role against excitotoxic and ischemic damage through ATP production (21, 22). We thus hypothesized that the astrocytic glycogen-derived lactate acts to maintain brain ATP levels during exhaustive exercise, thereby contributing to endurance capacity.

Significance

Muscle glycogen fuels exercising muscles to sustain endurance capacity. The brain also stores glycogen in astrocytes to produce lactate as an energy source transported to active neurons via the monocarboxylate transporter MCT2. Although physical exercise activates brain neurons and increases their energy demand, the energetic role of astrocytic glycogen in the exercising brain remains unknown. To address this issue, we used a rat model of prolonged exhaustive exercise, microwave irradiation of brains, metabolomics, and intracerebroventricular injection of inhibitors of glycogenolysis and MCT2. Our findings provide direct evidence that lactate derived from astrocytic glycogen fuels the prolonged-exercising brain to maintain endurance capacity. This new perspective on brain energetics during endurance exercise could lead to better strategies for endurance performance.

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To test this hypothesis, we used a rat model of prolonged exhaustive exercise and high-power microwave irradiation for accurate detection of brain metabolism (10 kW) (4, 14). First, glycogen and MCT proteins were measured in the brain and skeletal muscles of exhausted rats to confirm the validity of exhaustion. Next, we used metabolomics by capillary electrophoresis-mass spectrometry (CE-MS) to characterize metabolic profiles associated with ATP synthesis (glycolysis, TCA cycle, and purine metabolism) in the brain (hippocampus and cortex) and plantaris muscle. Finally, we examined the inhibitory effects of brain glycogen phosphorylase and lactate transport via MCT2 on brain ATP and endurance capacity during exhaustive exercise.

Results

Prolonged Exhaustive Exercise Decreases Glycogen and Increases MCT2 Protein in the Brain. Rats were exercised on the treadmill until exhaustion (20 m/min; time to exhaustion 84.4 ± 2.9 min). Blood lactate was significantly increased and glucose levels were significantly decreased compared with the sedentary group ($P < 0.01$) (Fig. 1 *A* and *B*). Blood ketone body (β-hydroxybutyrate) levels increased ($P < 0.01$) (Fig. 1*C*). Exhaustive exercise also caused a depletion (decrease by 97.3%) of muscle glycogen levels ($P < 0.01$) (Fig. 1*D*). Brain glycogen levels in the cortex and hippocampus were decreased by 75.1 and 66.3%, respectively ($P < 0.01$) (Fig. 1*E*), but the depletion seen in skeletal muscle did not occur in the brain (Fig. 1*F*). Concomitantly, MCT2 protein levels in the cortex and hippocampus increased with exhaustive exercise ($P < 0.05$), similar to the increases in MCT proteins observed in skeletal muscle (Fig. 2 *A–E*). GLUT1 and 3 protein levels increased only in the cortex and GLUT4 increased in muscles (Fig. 2).

Lactate Increases in Prolonged Exercise-Exhausted Brains but Not in Muscle. Metabolomics measured 159, 183, and 182 metabolites and revealed that 76, 79, and 72 metabolites were changed significantly in the plantaris muscle, cortex, and hippocampus, respectively, with exhaustive exercise. Principal component analysis and hierarchical cluster analysis clearly indicated the difference in metabolic profiles between sedentariness and exhaustion in all tissues (Fig. S1).

The glycolysis map of the plantaris muscle after exhaustive exercise showed depletion of glycogen and glucose and almost total depletion of glycolytic sources and lactate ($P < 0.05$) (Fig. 3*A*). However, TCA-cycle sources increased ($P < 0.05$) (Fig. 3*B*), suggesting the contribution of β-oxidation of lipids lacking in the brain. Maps of the cortex and hippocampus revealed a decrease in glycogen, glucose, and upstream glycolytic metabolites including fructose-1, 6-bisphosphate (F1-6P) ($P < 0.05$), but none of these metabolites were depleted. Downstream metabolites, such as F1-6P, together with TCA-cycle sources, were sustained and the lactate level was increased ($P < 0.01$) (Fig. 3 *C–F*).

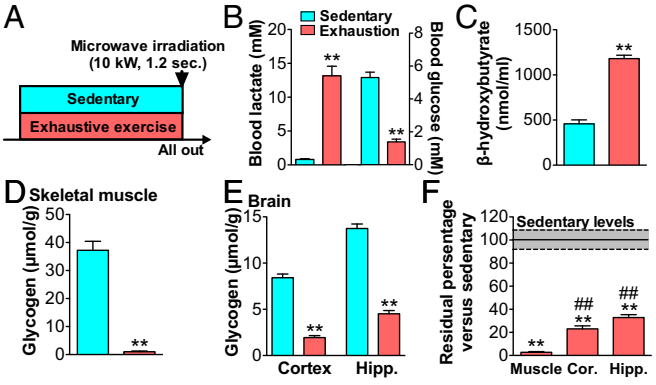

Fig. 1. Prolonged exhaustive exercise completely depletes muscle glycogen but only decreases brain glycogen. (*A*) Experimental design for exhaustive prolonged exercise in rats. (*B*) Blood lactate and glucose. (*C*) Blood β-hydroxybutyrate. (*D*) Glycogen in the plantaris muscle. (*E*) Glycogen in the cortex (Cor.) and hippocampus (Hipp.). (*F*) Residual amount of glycogen in the muscle and brain. Data are expressed as mean ± SE ($n = 5$ per group). $^{**}P < 0.01$ versus sedentary group; $^{\#\#}P < 0.01$ versus muscle.

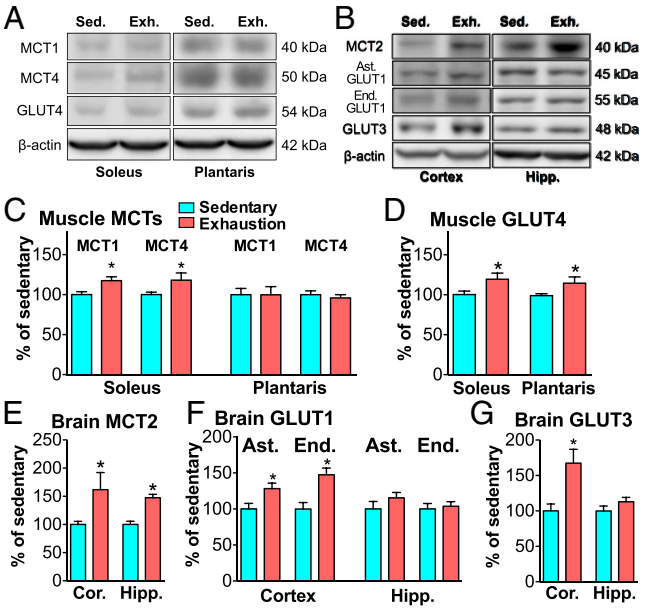

Fig. 2. Exhaustive exercise increases MCT and GLUT protein in muscles and the brain. (*A*) Typical photos of Western blotting bands for MCT1, MCT4, GLUT4, and β-actin in muscles. Exh., exhaustion; Sed., sedentary. (*B*) Typical photos of Western blotting bands for MCT2, astrocytic (Ast.) GLUT1, endothelial (End.) GLUT1, GLUT3, and β-actin in the brain. (*C*) Muscle MCT protein. (*D*) Muscle GLUT4 protein. (*E*) Brain MCT2 protein in the cortex and hippocampus. (*F*) Brain GLUT1 protein. (*G*) Brain GLUT3 protein. Data are expressed as mean ± SE ($n = 5$ per group). $^{*}P < 0.05$.

ATP Levels Are Maintained in Prolonged Exercise-Exhausted Brains but Not in Muscle. Metabolomics-produced purine/pyrimidine maps of the muscle and brain showed that ATP and phosphocreatine (PCr) decreased significantly in the exercise-exhausted group compared with the sedentary group ($P < 0.05$) (Fig. 4*A*) but that brain ATP and PCr levels were unchanged after exhaustive exercise (Fig. 4 *B* and *C*). Several downstream sources of purine metabolism, such as AMP, inosine, IMP, hypoxanthine, and uric acid, increased in both the muscle and brain of exercise-exhausted animals ($P < 0.05$) (Fig. 4). These data are direct evidence that ATP consumption is increased in both the brain and muscle but that only brain ATP levels are maintained during exhaustive exercise.

Blockade of Brain Glycogenolysis and MCT2 Decreases Brain ATP Levels. The intracerebroventricular (icv) injection of 1, 4-dideoxy-1,4-imino-D-arabinitol (DAB) (Fig. 5*A*) did not affect peripheral glycemic conditions (Fig. S2). Concurrently, hippocampal glycogenolysis was inhibited, as well as in the hypothalamus, brainstem, and cerebellum (Fig. S3) but not in the cortex (Fig. 5*B*), likely due to the extent and pattern of DAB diffusion after icv injection (Fig. S4). Lactate production was significantly suppressed in the hippocampus ($P < 0.05$) but not in other brain regions (Fig. 5*C* and Fig. S3). DAB also decreased hippocampal ATP levels at exhaustion ($P < 0.05$) (Fig. 5*D*), and accelerated the onset of exhaustion by 20.6% compared with the vehicle group ($P < 0.05$) (Fig. 5*E*). Similar to DAB, the icv injection of α-cyano-4-hydroxycinnamate (4-CIN), an MCT2 inhibitor (7), decreased hippocampal ATP levels during prolonged exercise ($P < 0.05$) (Fig. 5 *F* and *G*) and accelerated the onset of exhaustion by 34.8% ($P < 0.01$) (Fig. 5*H*).

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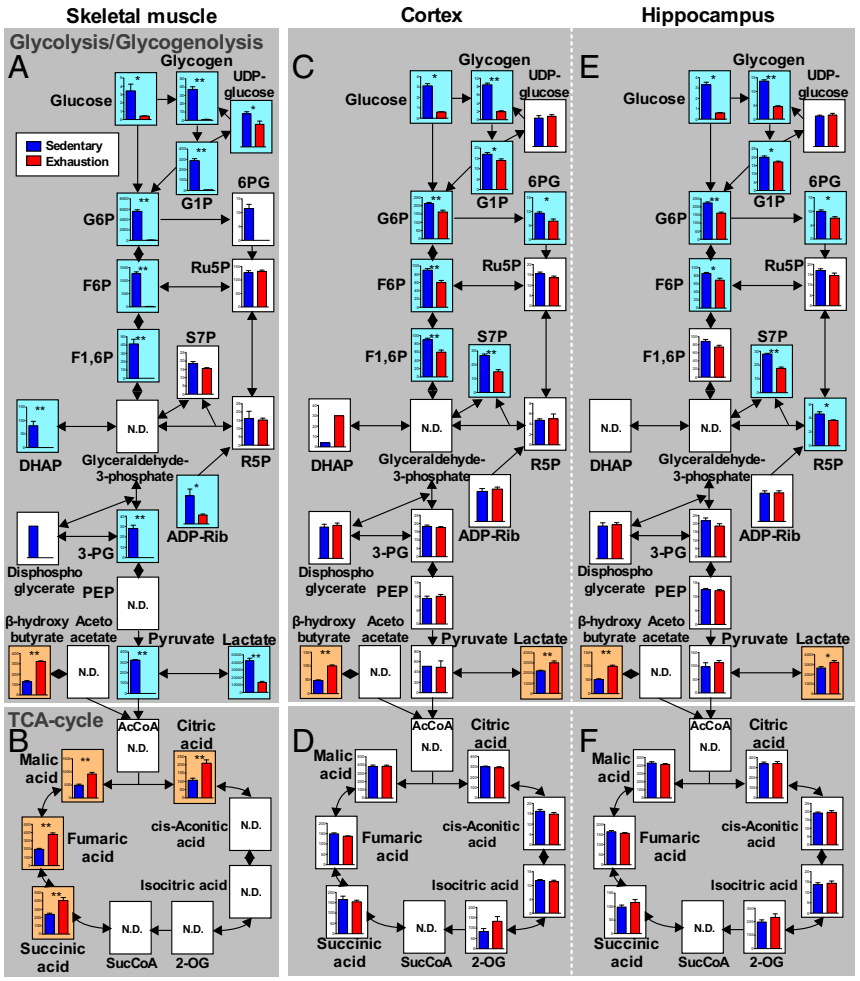

Fig. 3. Lactate increases in the brain but not in muscles during prolonged exhaustive exercise. Glycolytic pathways measured by metabolomics in the plantaris muscle (*A*), cortex (*C*), and hippocampus (*E*). TCA-cycle pathways in the plantaris muscle (*B*), cortex (*D*), and hippocampus (*F*). Glucose and glycogen results are inserted from results of glycogen assays. Data are expressed as mean ± SE ($n = 5$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$ versus sedentary group (Student's *t* test); N.D., not determined. Blue backgrounds indicate significantly decreased sources, and orange backgrounds imply significantly increased sources with exhaustive exercise. Graphs with a *y* axis show absolute detected amounts (nmol/g wet tissue), and graphs without a *y* axis show relative levels. The abbreviated metabolite names are defined in Table S1. The map of plantaris muscle after exhaustive exercise shows a depletion of glycogen and glucose and almost total depletions of glycolytic sources including lactate. Maps of the cortex and hippocampus revealed a decrease in glycogen and glucose and upstream glycolytic metabolites including F1-6P, but none of these metabolites were depleted. Downstream metabolites, such as 3-PG and pyruvate, together with TCA-cycle sources, were sustained and lactate was increased.

Discussion

This study tests the hypothesis that astrocytic glycogen-derived lactate acts to maintain brain ATP levels during exhaustive exercise, thereby contributing to endurance capacity. Our metabolomics analysis in the exercise-exhausted rat model shows that brain ATP levels are maintained, along with increased MCT2 protein expression, lactate, and residual glycogen, during prolonged exhaustive exercise (Figs. 1–4). The hippocampus is a particularly sensitive brain region, particularly for memory (6, 7), and we also confirmed the decrease in hippocampal ATP by a targeted icv blockade of hippocampal glycogenolysis during exhaustive exercise (Fig. 5 *A–E*). Furthermore, targeted icv disruption of MCT2 also decreased hippocampal ATP and resulted in lowered endurance capacity (Fig. 5 *F–H*). These findings provide direct evidence that lactate derived from astrocytic glycogen plays an energetic role in the brain during prolonged exhaustive exercise. This mechanism contributes to endurance capacity and complements data showing that exhaustive exercise compromises working memory regulated by the hippocampus in humans (23).

Physiological Validity of the Rat Model for Exhaustive Exercise. We confirmed hypoglycemia, hyperlactatemia, and muscle glycogen depletion in the rat model of exhaustive exercise used in the present study (Fig. 1 *A–D*). These are known fatigue factors established in rodents and humans (12–14, 24), confirming the development of exhaustion in our rat model.

Notably, ATP and PCr were significantly decreased in the exhausted plantaris muscle (Fig. 4). In general, muscle ATP levels are maintained during endurance exercise, and this represents a defense mechanism against muscle rigor and/or necrosis (25). However, short-duration (about a minute) exhaustive exercise induces declines in ATP and PCr levels of over 50 to 90% in type II (fast-twitch) fibers (26). The plantaris muscle consists of over 90% type II fibers (27). Thus, the decreases in ATP, PCr, and glycogen are due to the metabolic character of the plantaris muscle, indicating factors leading to the failure of muscle contraction (muscle fatigue) in type II fiber-enriched muscles.

Although muscle PCr decreased, free creatine did not increase ($P = 0.10$; Fig. 4*A*) and plasma free creatine did increase in exhausted rats (Table S2). Free creatine is a metabolite after dephosphorylation of PCr, and leaks from damaged muscles into the blood during hypodynamia of the heart muscle and during ultramarathons (28, 29). Plasma free creatine is also a biomarker of kidney function (glomerular filtration rate), which is lowered by protein waste (29). Thus, muscle-produced free creatine plasma levels increase due to the decline of glomerular filtration rate with protein wastes caused by muscle damage during exhaustive exercise.

We observed no muscle rigor or necrosis, and the decreased ATP, PCr, and glycogen recovered to basal levels after 6 h of rest (Table S2). Increased plasma free creatine returned to baseline after 6 h of rest (Table S2), whereas 5 d of rest are needed to recover after an ultramarathon (29). Therefore, our rat model of exhaustive exercise is milder than an ultramarathon, indicating its validity.

Energetic Role of Brain Glycogen During Exhaustive Exercise. The reduction of brain ATP induces neuronal death (18, 19). However, we revealed with metabolomics that brain ATP, but not muscle ATP, levels are maintained with increased MCT2 protein expression, lactate, and residual glycogen during prolonged exhaustive exercise (Figs. 1–4). These findings indicate that the

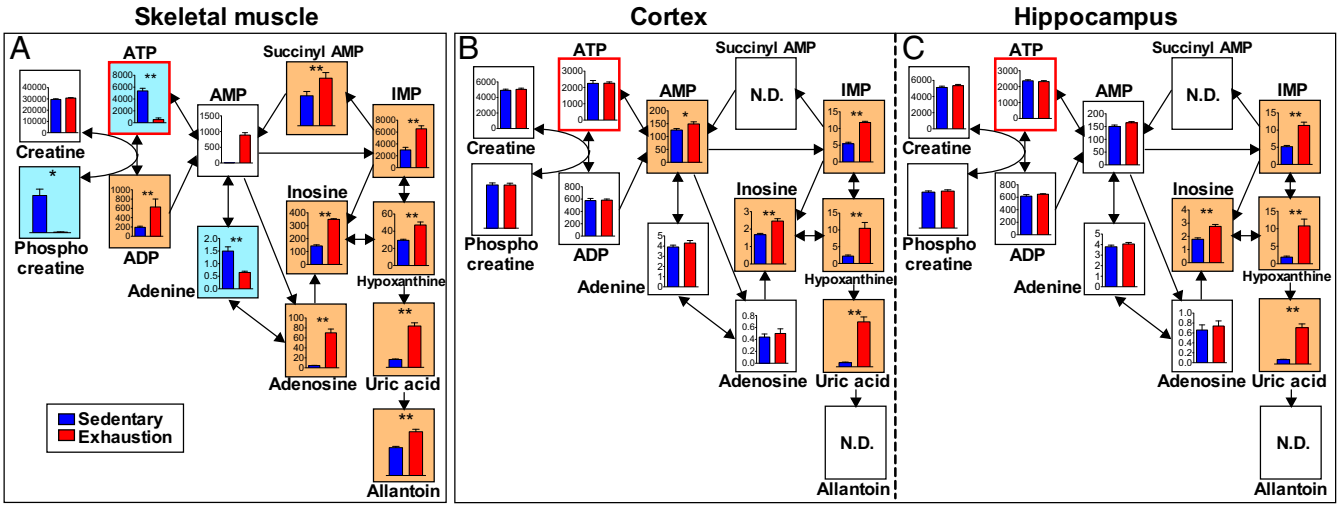

Fig. 4. ATP is maintained in the brain but not in muscles during prolonged exhaustive exercise. Purine metabolism pathways measured by metabolomics in the plantaris muscle (*A*), cortex (*B*), and hippocampus (*C*). $*P < 0.05$, $**P < 0.01$ versus the sedentary group (Student's *t* test). Blue backgrounds indicate significantly decreased sources, and orange backgrounds imply significantly increased sources following exhaustive exercise. Graphs with a *y* axis show absolute detected amounts (nmol/g wet tissue), and graphs without a *y* axis show relative levels. Data are expressed as mean ± SE ($n = 5$ per group). The abbreviated metabolite names are defined in Table S1. Muscle and brain maps show that ATP and PCr were decreased significantly in the exercise-exhausted group compared with the sedentary group ($P < 0.05$), but brain ATP and PCr were unchanged after exhaustive exercise. Several downstream sources of purine metabolism, such as AMP, inosine, IMP, hypoxanthine, and uric acid, were increased in both the muscle and brain of exercise-exhausted animals.

brain, rather than muscle, is protected energetically, likely to avoid neuronal death/dysfunction during exhaustive exercise, supporting the "selfish brain" theory regarding energy competition among organs (30).

A localized blockade of brain glycogen breakdown (glycogenolysis) inhibited the increases in hippocampal lactate, and decreased hippocampal ATP during exhaustive exercise (Fig. 5 *A*–*D*). A localized disruption of the MCT2 protein also disturbed the maintenance of hippocampal ATP (Fig. 5 *F* and *G*). These data indicate that glycogen-derived lactate transported by increased MCT2 is required for brain ATP maintenance during exhaustive exercise, at least in the hippocampus, which is direct evidence for the energetic importance of brain glycogen during endurance exercise. This evidence provides new insight into the strategies that promote/protect brain functions in animals relating to performance (e.g., endurance capacity and/or cognitive functions).

Astrocytic glycogen provides lactate to neurons but is also needed for ATP synthesis and/or K^+ homeostasis in astrocytes during brain activation (31). Thus, glycogenolysis in the brain following exhaustive exercise could contribute to ATP synthesis and/or K^+ homeostasis in astrocytes. However, blockade of brain glycogenolysis and of MCT2 had similar disrupting effects on hippocampal ATP levels during exhaustive exercise (Fig. 5*G*). Therefore, brain glycogen appears to function as a source of lactate for neurons in the exercising brain.

Other Possible Mechanisms of Brain ATP Maintenance. Glutamate, a key excitatory neurotransmitter, increases protein levels of surface glucose transporter 3 (GLUT3) in neurons (32) and enhances glucose uptake and glycogen synthesis in cultured cortical astrocytes (33). In the present study, brain glutamate was maintained even during exhaustive exercise (Fig. S5). Further, GLUT3 and the astrocytic/endothelial glucose transporter (GLUT1) increased in the cortex, as also did GLUT4 in muscle (Fig. 2). Thus, glutamate may be involved in ATP maintenance by activation of glucose uptake via GLUTs and/or glycogen synthesis, particularly in the cortex.

Neuronal MCT2 transports not only lactate but also ketone bodies (34). The ketone bodies are metabolized as a neuronal energy source during starvation (35), but their metabolism during exhaustive exercise has not been studied. The metabolomics of the present study revealed increased blood and brain levels of β-hydroxybutyrate during exhaustive exercise (Figs. 1 and 3). These data are consistent with the increases in hippocampal MCT2 and cortical β-hydroxybutyrate during prolonged, but not exhaustive, exercise (15). Blood ketone bodies, at least β-hydroxybutyrate, could be transported to neurons by increased MCT2 to spare brain glycogen in exercise-exhausted rats.

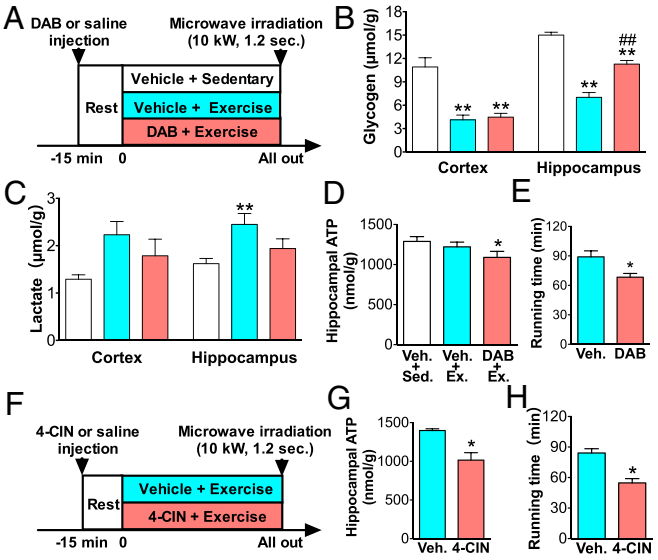

Fig. 5. Blockade of brain glycogenolysis and MCT2 decreases endurance capacity associated with brain ATP. Data are expressed as mean ± SE ($n = 5$ to 7 per group). (*A*) Experimental design for exhaustive prolonged exercise with DAB icv injection in rats. (*B* and *C*) Glycogen (*B*) and lactate (*C*) in the cortex and hippocampus. $**P < 0.01$ versus vehicle (Veh.) + sedentary group; $^{\#\#}P < 0.01$ versus Veh. + exercise (by one-way ANOVA with Tukey's post hoc tests). (*D*) ATP in the hippocampus. (*E*) Running time to exhaustion with DAB icv injection. (*F*) Experimental design for exhaustive prolonged exercise with 4-CIN icv injection in rats. (*G*) ATP in the hippocampus. (*H*) Running time to exhaustion with 4-CIN icv injection. $*P < 0.05$ versus vehicle group (by Student's *t* test).

Although the mechanisms for increases in MCT2 protein are unclear, noradrenaline (NA) is an activator not only for astrocytic glycogenolysis (36) but also for MCT2 expression in neurons (37). NA neurons are activated during prolonged exercise (14). Metabolomics also revealed increases in brain tyrosine, a precursor of NA, which paralleled the decrease in brain glycogen (Figs. S5 and S6). These data point to NA as an inducer of MCT2 expression in the prolonged-exercising brain.

Brain Glycogen and Endurance Capacity. Blockade of brain glycogenolysis and lactate transport via MCT2 accelerated exhaustion during prolonged exercise (Fig. 5), supporting the hypothesis that brain lactate derived from astrocytic glycogen plays a role in endurance capacity because of its energetic contribution (11, 14, 24). Although DAB affected hippocampal glycogen content, it did not affect cortical glycogen with exhaustive exercise (Fig. 5*B* and Fig. S3). However, icv inhibition of glycogen phosphorylase blocked glycogen depletion in the hypothalamus, brainstem, and cerebellum, although it only significantly reduced lactate levels in the hippocampus, leaving open the question of which brain site(s) signals exhaustion.

DAB inhibited the decrease of glycogen in the hippocampus caused by exhaustive exercise (Fig. 5*B*). Because hippocampal glycogen-derived lactate acts in memory function (6), the decreased glycogen utilization in the exhausted hippocampus might be a cause of exercise-induced cognitive fatigue. However, there is methodological difficulty in detecting cognitive functions of exercise-fatigued animals because they cannot move for given memory tasks because of fatigue. In humans, exhaustive exercise decreases cognitive functions, including working memory (23), but it is unknown whether this reflects hippocampus-based cognitive decline. A new system for determining effects of moderate exercise on pattern separation, a dentate gyrus-specific ability (38), can be applied together with fMRI analysis for fatigue research on this important topic.

Hippocampal neurons also play an important role in the onset of locomotion and exhibit locomotion velocity-dependent firing with theta oscillation (39). Although untested, glycogen-derived lactate might be a contributor to locomotion-dependent hippocampal firing. This postulation could provide novel insight into the significance of the hippocampus not only for memory but also for exercise capacity, implicating the underlying positive relationship between aerobic fitness and cognitive function (40–42).

In addition, the glycogen decrease in the hypothalamus, cerebellum, and brainstem by exhaustive exercise was inhibited with DAB icv injection (Fig. S3). Hypothalamic lactate is an important factor in counterregulation during hypoglycemia (43), and brainstem lactate controls arousal by stimulating NA neurons (44). Therefore, blockade of brain glycogenolysis and lactate transport would result in a lower endurance capacity by suppression of brain region-specific functions (e.g., hippocampus: locomotion; hypothalamus: regulation of energy metabolism; cerebellum: motor control; brainstem: arousal control; etc.).

Biochemical Insight into the Development of Exhaustion and Central Fatigue During Prolonged Exercise. Fatigue induced by prolonged exercise is separated into muscle and central (brain) factors (24). Our metabolomics provides insight into the biochemistry behind fatigue during prolonged exercise (Fig. S7). In the muscles of exercise-exhausted rats, ATP, PCr, and glycogen are significantly decreased, whereas hypoxanthine levels increase due to purine metabolism (Figs. 3 and 4). These findings are consistent with studies on exhausted skeletal muscles (45). Although undetected in the present study, purine metabolism generates ammonia, which is a known muscle fatigue factor (46). Therefore, the depletion of energy sources and accumulation of inhibitors of muscle contraction (e.g., ammonia) are factors for muscle fatigue (failure of muscle contraction).

In the brain, ammonia is essentially detoxified by astrocytes through the glutamate–glutamine cycle derived from muscle and/or the brain itself increases and also inhibits neuronal activity (24). The uptake of tryptophan is also increased due to the elevated ratio of branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs) in the blood, which are precursors of NA and serotonin (5-hydroxytryptamine; 5-HT). Increased brain tryptophan induces elevated 5-HT levels, promoting a "sense of fatigue" and inhibiting neuronal activity (the tryptophan–serotonin hypothesis) (47). Further, hypoglycemia induced by depletion of liver and muscle glycogen creates a lack of energy that further inhibits neuronal activity. NA, 5-HT, and hypoglycemia are also strong activators of astrocytic glycogenolysis. Indeed, increased AAAs, which are likely converted to NA and 5-HT, correlated with decreased brain glycogen (Fig. S6). These factors are also induced not only during fatigue but also by sleep deprivation (48) and hypoglycemia (5, 49), conditions that decrease brain glycogen. Thus, the decrease in brain glycogen is a possible common mechanism for central fatigue.

Central fatigue factors such as ammonia, 5-HT, NA, and/or their precursors derive from muscles and reach the brain via the bloodstream. Ammonia and 5-HT appear to suppress ATP and glycogen consumption through the development of a sense of fatigue and neuronal inhibition. 5-HT and NA activate glycogenolysis and MCT expression for lactate synthesis/transport, thereby maintaining ATP synthesis. These factors would function to maintain brain ATP as the primary outcome through muscle–brain metabolic coupling in exhaustion, implicating central fatigue as a defense mechanism for brain neurons (Fig. S7).

Conclusion

Our findings provide evidence for the energetic role of lactate derived from astrocytic glycogen in the prolonged-exercising brain, thereby contributing to endurance capacity, in keeping with its known role in memory formation involving the hippocampus (6, 7). Shedding light on the mechanism of the positive relationship between endurance and memory, our metabolomics analysis also revealed that the decrease in brain glycogen is a possible factor for exercise-induced central fatigue, which involves muscle–brain metabolic crosstalk. Importantly, the ATP maintenance contributed by brain glycogen at exhaustion likely serves as a neuroprotective mechanism.

Materials and Methods

For a full description of all materials and methods, see *SI Materials and Methods*.

Animals. Adult male Wistar rats (250 to 300 g) (SLC), housed and cared for in an animal facility, were fed a standard pellet diet (MF; Oriental Yeast) and given water ad libitum. Room temperature was maintained between 22 and 24 °C under a 12-h light–12-h dark cycle (lights on, 0700 to 1900 h). All experimental protocols were conducted in accordance with the guidelines of the University of Tsukuba Animal Experiment Committee. The rats were habituated to running on a treadmill (SN-460; Shinano) for five sessions over 6 d, 30 min/d. The running speed was gradually increased from 5 to 25 m/min (13, 14).

Prolonged Exhaustive Exercise. Rats were fasted for 2 h before exercise to obtain stable metabolic conditions ($n = 5$ in each group). They were exercised to exhaustion on a treadmill at 20 m/min. Exhaustion was considered by the standard in previous studies (13, 14).

Inhibition of Glycogenolysis and Lactate Transport in the Brain. A steel guide cannula was inserted into the lateral cerebral ventricle of rat brain (50). Rats were placed on the treadmill for at least 30 min, and randomly injected via an icv catheter with a glycogen phosphorylase inhibitor (DAB; 150 mM in 10 μL of 0.9% saline, pH 7.2) or dose-specific MCT2 inhibitor (4-CIN; 36 mM in 10 μL of 40% DMSO and 0.9% saline, pH 7.2) (Sigma-Aldrich). Fifteen minutes after the icv injection, rats were subjected to exhaustive exercise ($n =$ 5 to 7 in each group).

Sample Collection. Blood samples were collected during exercise through a catheter inserted into the jugular vein. Following the exhaustive exercise, the rats were killed using focused microwave irradiation (MI) (10 kW, 1.2 s; NJE-2603; New Japan Radio). After MI, brain tissues and skeletal muscles were collected. All samples were stored at −80 °C until analysis.

Blood Glucose and Lactate Assays. Blood glucose and lactate levels were measured using an automated glucose/lactate analyzer (2300 Stat Plus; Yellow Springs Instruments).

Brain and Muscle Glycogen Assay. The glycogen assay was performed in 96-well plates using a coupled-enzyme assay method modified from previous studies (13, 14).

Western Blot Analysis. The tissues were lysed in urea-based lysis buffer, and sample proteins were loaded on an SDS/polyacrylamide gel. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane, blocked, and then incubated with primary and secondary antibodies. Proteins were visualized and their signals were quantified with β-actin normalization using image analysis software (GE Healthcare Life Sciences).

Metabolomics. Metabolomics was conducted by Human Metabolome Technologies (19). Each frozen sample was homogenized in methanol, and metabolites were extracted for analysis by CE-MS. CE-MS experiments were performed using Agilent CE systems equipped with a time-of-flight mass spectrometer and a built-in diode-array detector (Agilent Technologies). The identified metabolites were quantified by comparing their peak areas with those of authentic standards using ChemStation software (Agilent Technologies).

Brain Lactate and ATP Assay. Lactate measurements were obtained according to the method described by Matsui et al. (14). ATP levels were analyzed using the ATPlite Kit with luciferin/luciferase activity (6016736, PerkinElmer), and luminescence was measured with a microplate reader (ARVO X4, PerkinElmer).

Statistical Analysis. Data are expressed as mean ± SE and were analyzed by Student's *t* test and a one-way ANOVA with Tukey's post hoc tests using Prism 5 (MDF). Statistical significance was assumed at P values <0.05.

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1. Bélanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: Focus on astrocyte-neuron metabolic cooperation. *Cell Metab* 14:724–738.
2. Mächler P, et al. (2016) In vivo evidence for a lactate gradient from astrocytes to neurons. *Cell Metab* 23:94–102.
3. O'Dowd BS, Gibbs ME, Ng KT, Hertz E, Hertz L (1994) Astrocytic glycogenolysis energizes memory processes in neonate chicks. *Brain Res Dev Brain Res* 78:137–141.
4. Kong J, et al. (2002) Brain glycogen decreases with increased periods of wakefulness: Implications for homeostatic drive to sleep. *J Neurosci* 22:5581–5587.
5. Herzog RI, et al. (2008) Effect of acute and recurrent hypoglycemia on changes in brain glycogen concentration. *Endocrinology* 149:1499–1504.
6. Suzuki A, et al. (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144:810–823.
7. Newman LA, Korol DL, Gold PE (2011) Lactate produced by glycogenolysis in astrocytes regulates memory processing. *PLoS One* 6:e28427.
8. Swanson RA, Choi DW (1993) Glial glycogen stores affect neuronal survival during glucose deprivation in vitro. *J Cereb Blood Flow Metab* 13:162–169.
9. Secher NH, Seifert T, Van Lieshout JJ (2008) Cerebral blood flow and metabolism during exercise: Implications for fatigue. *J Appl Physiol (1985)* 104:306–314.
10. Vissing J, Andersen M, Diemer NH (1996) Exercise-induced changes in local cerebral glucose utilization in the rat. *J Cereb Blood Flow Metab* 16:729–736.
11. Larsen TS, Rasmussen P, Overgaard M, Secher NH, Nielsen HB (2008) Non-selective beta-adrenergic blockade prevents reduction of the cerebral metabolic ratio during exhaustive exercise in humans. *J Physiol* 586:2807–2815.
12. Gollnick PD, Piehl K, Saltin B (1974) Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J Physiol* 241:45–57.
13. Matsui T, et al. (2012) Brain glycogen supercompensation following exhaustive exercise. *J Physiol* 590:607–616.
14. Matsui T, et al. (2011) Brain glycogen decreases during prolonged exercise. *J Physiol* 589:3383–3393.
15. Takimoto M, Hamada T (2014) Acute exercise increases brain region-specific expression of MCT1, MCT2, MCT4, GLUT1, and COX IV proteins. *J Appl Physiol* 116: 1238–1250.
16. Coles L, Litt J, Hatta H, Bonen A (2004) Exercise rapidly increases expression of the monocarboxylate transporters MCT1 and MCT4 in rat muscle. *J Physiol* 561:253–261.
17. de Haan A, Koudijs JC (1994) A linear relationship between ATP degradation and fatigue during high-intensity dynamic exercise in rat skeletal muscle. *Exp Physiol* 79:865–868.
18. Suh SW, Hamby AM, Swanson RA (2007) Hypoglycemia, brain energetics, and hypoglycemic neuronal death. *Glia* 55:1280–1286.
19. Sugiura Y, Taguchi R, Setou M (2011) Visualization of spatiotemporal energy dynamics of hippocampal neurons by mass spectrometry during a kainate-induced seizure. *PLoS One* 6:e17952.
20. Inostroza M, Brotons-Mas JR, Laurent F, Cid E, de la Prida LM (2013) Specific impairment of "what-where-when" episodic-like memory in experimental models of temporal lobe epilepsy. *J Neurosci* 33:17749–17762.
21. Berthet C, et al. (2009) Neuroprotective role of lactate after cerebral ischemia. *J Cereb Blood Flow Metab* 29:1780–1789.
22. Jourdain P, et al. (2016) L-lactate protects neurons against excitotoxicity: Implication of an ATP-mediated signaling cascade. *Sci Rep* 6:21250.
23. Perciavalle V, Maci T, Perciavalle V, Massimino S, Coco M (2015) Working memory and blood lactate levels. *Neurol Sci* 36:2129–2136.
24. Nybo L, Secher NH (2004) Cerebral perturbations provoked by prolonged exercise. *Prog Neurobiol* 72:223–261.
25. Myburgh KH (2004) Protecting muscle ATP: Positive roles for peripheral defense mechanisms—Introduction. *Med Sci Sports Exerc* 36:16–19.
26. Sant'Ana Pereira JA, Sargeant AJ, Rademaker AC, de Haan A, van Mechelen W (1996) Myosin heavy chain isoform expression and high energy phosphate content in human muscle fibres at rest and post-exercise. *J Physiol* 496:583–588.
27. Enoki T, Yoshida Y, Lally J, Hatta H, Bonen A (2006) Testosterone increases lactate transport, monocarboxylate transporter (MCT) 1 and MCT4 in rat skeletal muscle. *J Physiol* 577:433–443.
28. Ventura-Clapier R, Vassort G (1980) The hypodynamic state of the frog heart. Further evidence for a phosphocreatine-creatine pathway. *J Physiol (Paris)* 76:583–589.
29. Arakawa K, et al. (2016) Changes in blood biochemical markers before, during, and after a 2-day ultramarathon. *Open Access J Sports Med* 7:43–50.
30. Peters A, et al. (2004) The selfish brain: Competition for energy resources. *Neurosci Biobehav Rev* 28:143–180.
31. Mangia S, Giove F, Dinuzzo M (2013) K^+ homeostasis in the brain: A new role for glycogenolysis. *Neurochem Res* 38:470–471.
32. Ferreira JM, Burnett AL, Rameau GA (2011) Activity-dependent regulation of surface glucose transporter-3. *J Neurosci* 31:1991–1999.
33. Hamai M, Minokoshi Y, Shimazu T (1999) L-glutamate and insulin enhance glycogen synthesis in cultured astrocytes from the rat brain through different intracellular mechanisms. *J Neurochem* 73:400–407.
34. Koehler-Stec EM, Simpson IA, Vannucci SJ, Landschulz KT, Landschulz WH (1998) Monocarboxylate transporter expression in mouse brain. *Am J Physiol* 275:E516–E524.
35. Owen OE, et al. (1967) Brain metabolism during fasting. *J Clin Invest* 46:1589–1595.
36. Sorg O, Magistretti PJ (1992) Vasoactive intestinal peptide and noradrenaline exert long-term control on glycogen levels in astrocytes: Blockade by protein synthesis inhibition. *J Neurosci* 12:4923–4931.
37. Pierre K, Debernardi R, Magistretti PJ, Pellerin L (2003) Noradrenaline enhances monocarboxylate transporter 2 expression in cultured mouse cortical neurons via a translational regulation. *J Neurochem* 86:1468–1476.
38. Suwabe K, et al. (2017) Acute moderate exercise improves mnemonic discrimination in young adults. *Hippocampus* 27:229–234.
39. Fuhrmann F, et al. (2015) Locomotion, theta oscillations, and the speed-correlated firing of hippocampal neurons are controlled by a medial septal glutamatergic circuit. *Neuron* 86:1253–1264.
40. Erickson KI, et al. (2009) Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus* 19:1030–1039.
41. Hyodo K, et al. (2016) The association between aerobic fitness and cognitive function in older men mediated by frontal lateralization. *Neuroimage* 125:291–300.
42. Kramer AF, et al. (1999) Ageing, fitness and neurocognitive function. *Nature* 400: 418–419.
43. Chan O, Paranjape SA, Horblitt A, Zhu W, Sherwin RS (2013) Lactate-induced release of GABA in the ventromedial hypothalamus contributes to counterregulatory failure in recurrent hypoglycemia and diabetes. *Diabetes* 62:4239–4246.
44. Tang F, et al. (2014) Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat Commun* 5:3284.
45. Sahlin K, Tonkonogi M, Söderlund K (1999) Plasma hypoxanthine and ammonia in humans during prolonged exercise. *Eur J Appl Physiol Occup Physiol* 80:417–422.
46. Broberg S, Sahlin K (1989) Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. *J Appl Physiol* 67:116–122.
47. Cotel F, Exley R, Cragg SJ, Perrier JF (2013) Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. *Proc Natl Acad Sci USA* 110:4774–4779.
48. Lopez-Rodriguez F, Wilson CL, Maidment NT, Poland RE, Engel J (2003) Total sleep deprivation increases extracellular serotonin in the rat hippocampus. *Neuroscience* 121:523–530.
49. Heyes MP, Papagapiou M, Leonard C, Markey SP, Auer RN (1990) Brain and plasma quinolinic acid in profound insulin-induced hypoglycemia. *J Neurochem* 54: 1027–1033.
50. Ohiwa N, et al. (2007) Possible inhibitory role of prolactin-releasing peptide for ACTH release associated with running stress. *Am J Physiol Regul Integr Comp Physiol* 292: R497–R504.