Mechanisms for maintaining extracellular glutamate levels in the anoxic turtle striatum

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Milton, Sarah L., John W. Thompson, and Peter L. Lutz. Mechanisms for maintaining extracellular glutamate levels in the anoxic turtle striatum. Am J Physiol Regulatory Integrative Comp Physiol 282: R1317–R1323, 2002.—The turtle Trachemys scripta is one of a limited group of vertebrates that can withstand hours to days without oxygen. One facet of anoxic survival is the turtle’s ability to maintain basal extracellular glutamate levels; whereas in most vertebrates, anoxia triggers massive excitotoxic glutamate release. We investigated glutamate release and reuptake in the anoxic turtle and the effects of adenosine and ATP-sensitive potassium (K_\text{ATP}) channels on glutamate homeostasis. Striatal extracellular glutamate was measured in anesthetized T. scripta by microdialysis in normoxia and over 2-h anoxia. Glutamate release is decreased by 44% in the early anoxic turtle; this anoxic-induced decrease in glutamate release was prevented when K_\text{ATP} channels and adenosine receptors were blocked simultaneously but not when either mechanism was blocked individually. We hypothesize that the continued release and reuptake of glutamate during anoxia help maintain neuronal tone and aid in the recovery of a functional neuronal network after long periods of anoxia, whereas activation of adenosine and/or K_\text{ATP} conserves energy by reducing glutamate release and lowering transport costs.

microdialysis; adenosine; adenosine triphosphate-sensitive potassium channels; Trachemys scripta

GLUTAMATE HAS LONG BEEN IDENTIFIED as the major excitatory neurotransmitter in the vertebrate nervous system (8), with intracellular concentrations ranging from 50 \(\mu\)M in astrocytes to as high as 10 mM in glutamatergic neurons (9). Basal extracellular glutamate levels, however, are maintained at only 1–3 \(\mu\)M, which provides a high signal-to-noise ratio at the synapse while simultaneously preventing excitotoxicity (9). Under conditions of oxygen deprivation, however, such as in hypoxia or ischemia, the extracellular compartment of the mammalian brain is flooded with high levels of excitatory compounds such as glutamate, aspartate, and dopamine. Pathological increases in extracellular glutamate are thought to play a central role in hypoxic/ischemic neuronal degeneration and reperfusion injuries (15).

Under basal conditions, glutamate levels in the extracellular compartment are determined by both synaptic release and continuous leakage from nonvesicular stores, balanced by high-affinity reuptake mechanisms (1). These energy- and sodium-dependent transporters include both neuronal and astroglial subtypes, with the majority of glutamate uptake occurring through glial transporters such as GLT-1 (9). With a net movement of 3 Na^+ cotransported into the cell and 1 K^+ countertransported for each glutamate anion, energy costs are estimated to be at least 1.5 ATP per glutamate anion (35). Glutamate reuptake may thus constitute a large fraction of the total energy requirement of neurotransmission (35) and is likely to be severely compromised during conditions of energy deficit such as hypoxia or ischemia. Low ATP inhibits these transport processes, leading to glutamate increases in the extracellular space both by continuous glutamate leakage and by membrane depolarization as the sodium pump slows. The breakdown in transmembrane Na^+ and K^+ ion gradients may also cause transporters to run in reverse, releasing additional glutamate into the extracellular space (36). Excess glutamate then activates ionotropic and metabotropic receptors (GluR) that trigger a cascade of events resulting in neuronal death. The majority of hypoxic/ischemic glutamate release to the extracellular space is thought to occur by this reversal of glutamate reuptake transporters (25).

The freshwater turtle Trachemys scripta, however, is able to survive days to months of anoxia (15); the brain maintains ion gradients and avoids anoxic depolarization for at least 48 h. Basal levels of extracellular glutamate (20) and dopamine (19) are also maintained during periods of oxygen deprivation. It has been assumed until now that low extracellular glutamate levels were maintained during anoxia simply by preventing release into the extracellular space. However, recent work on dopamine homeostasis indicates that neurotransmitter balance in the anoxic turtle brain is a much more dynamic process that may use blockage of release as well as continued release and reuptake (19; Milton and Lutz, unpublished observations). The turtle’s ability to prevent pathological increases in extra-
cellular glutamate may then be a combination of 1) decreased synaptic release, 2) decreased glutamate leakage, 3) maintained or increased glutamate re-uptake, and/or 4) prevention of glutamate transporter reversal. The purpose of this study was to investigate the relative roles of these mechanisms in maintaining low extracellular glutamate levels in the early anoxic (1–2 h) turtle brain, when the animal experiences a temporary energy crisis.

The particular roles of adenosine and ATP-sensitive potassium (KATP) channel activation on glutamate release were also examined, as these mechanisms are known to be neuroprotective in the hypoxic/ischemic mammal as well as in the anoxia-tolerant turtle. Activated KATP channels play an important role in decreasing membrane permeability (channel arrest) in the anoxic turtle (23) and are thought to ameliorate neuronal injury in the hypoxic mammalian brain by hyperpolarizing the neuronal membrane (7) and by inhibiting glutamate and dopamine (DA) release (37, 38). KATP channels, in turn, may be modulated by adenosine (27). Adenosine A1 receptor activation in the mammal inhibits many neurons both pre- and postsynaptically by modulating ionic currents and by decreasing neurotransmitter release (8, 28). Adenosine plays an important role in the anoxic turtle brain as well, acting as a "retaliatory metabolite" to balance energy supply and demand (15, 21). Perfusing the anoxic isolated turtle cerebellum with adenosine receptor blockers causes a rapid depolarization (15), whereas superfusion of the anoxic brain in situ with the adenosine receptor blocker aminophylline prevents the normal anoxia-induced increase in cerebral blood flow (12).

MATERIALS AND METHODS

Materials. The studies described were approved by the institutional animal care and use committee. Freshwater turtles were purchased from a commercial supplier (Lemberger, Oshkosh, WI). The general glutamate transport blocker L-trans-pyroridine-2,4-dicarboxylic acid (PDC) and the specific blocker of the astrocytic GLUT-1 glutamate transporter dihydrokinate (DHK; originally a gift from B. Hassel) were purchased from Tocris Cookson (St. Louis, MO). The specific KATP channel blocker 2,3-butanedione monoxime (BDM) and KATP channel opener 7-chloro-3-methyl-2H-1,2,4-benzothiadiazine 1,1-dioxide (Diazoxide) were purchased from Research Biochemicals International (Natick, MA). All other chemicals and reagents were purchased from Sigma Chemicals (St. Louis, MO).

Methods. Experiments were performed at 25°C on the freshwater turtle Trachemys scripta elegans. Turtles were anesthetized with AErrane (Isoflurane, USP, Anaquest) in air. Anesthesia was induced using a mixture of 4% isoflurane in air pumped from a 1.5-liter rebreathing bag. Animals were maintained on 1.7% isoflurane once a surgical plane was achieved (31).

After the skull was exposed, a 1-cm diameter hole was trephined and the skull cap was removed. A small incision through the dura mater exposed the cerebral hemispheres. A stereotaxic instrument and guide were used to insert a CMA 12 microdialysis probe (3-mm membrane length; Bioanalytical Systems, Acton, MA) into the striatum (5-mm depth from the cerebral surface). After a 1-h stabilization period in which no sampling occurred, the probe was perfused with turtle artificial cerebrospinal fluid (ACSF) solution (104 mM NaCl, 3.5 mM KCl, 26 mM NaHCO3, 1.25 mM NaH2PO4, 2.0 mM CaCl2, 2.0 mM MgSO4, and 2.0 mM glucose, pH 7.4) at 2.0 μl/min with a CMA/100 microdialysis pump (Carnegie Medicin, Solna, Sweden). Anoxia was induced by changing the breathing mixture to certified 99.99% nitrogen (County Welding, Pompano Beach, Florida) and 1.7% isoflurane.

Dialysate was collected over 20-min intervals and analyzed immediately. Probe recovery was determined from known standards in vitro (11); glutamate recovery averaged 18.0 ± 2.8%. Samples were analyzed for glutamate content using HPLC with fluorescence detection. In brief, 20 μl of dialysate were mixed with 30 μl complete o-phthalaldehyde reagent solution (Sigma) at 25°C; after exactly 1 min, this was injected onto the HPLC column. The HPLC system consisted of a Knauer HPLC pump 64 and E-Lab gradient controller (OMS Tech, Miami, FL), a reversed-phase column (Adsorbosphere OPA 5u, 150 × 4.6 mm; Alltech, Deerfield, IL), and an RF-535 fluorescence detector (Shimadzu, Kyoto, Japan).

Each treatment group consisted of five animals. All drugs were administered via the microdialysis probe perfusate in turtle ACSF.

Normoxic treatments. Normoxic treatment groups were as follows: 1) normoxic controls (4-h respiration on air, no drugs); 2) non-PDC drug controls: 1-h baseline sampling followed by 1-h individual drug administration with one of the following: 500 μM KATP channel opener Diazoxide (16), 200 μM adenosine (18), 100 μM general adenosine receptor blocker theophylline (24), and 500 μM KATP blocker BDM (Milton and Lutz, unpublished observations); and 3) normoxic PDC or DHK animals: 1-h baseline sampling followed by perfusion of experimental drugs (above) coadministered with glutamate uptake blocker PDC (2.5 mM (22)) or DHK (1 mM (29)). All normoxic treatment groups were respired on air; sampling occurred following a 1-h recovery period after insertion of the microdialysis probe.

Anoxic treatments. Drug concentrations for anoxic treatments were as per normoxic treatments above. Experimental anoxic treatments were as follows.

Anoxic controls. One-hour air respiration was followed by 2-h N2 respiration.

Administration of the KATP blocker BDM. After the 1-h normoxic recovery period, animals were respired on air for 30 min followed by 30-min N2 respiration to obtain baseline glutamate values. The KATP blocker BDM was infused through the microdialysis probe beginning at 30-min N2 respiration; sampling followed for an additional 1.5 h.

L-Trans-PDC administration. Glutamate transport blockade by l-trans-PDC was used to determine rates of glutamate release in anoxic vs. normoxic turtles. After the 1-h normoxic recovery period, animals were respired on nitrogen for 30 min in which no sampling occurred. Samples were collected to determine anoxic baseline glutamate levels for the following hour, after which the general glutamate uptake blocker PDC was administered through the microdialysis probe for three additional 20-min sampling periods.

Coadministration of adenosine and KATP antagonists with PDC. To assess the respective roles of adenosine and KATP channels in preventing glutamate release, the general adenosine receptor antagonist theophylline and/or KATP blocker BDM were coadministered with PDC in the anoxic turtle as described above (1-h baseline sampling followed by 1-h drug exposure in anoxic animals). Because of rapid glutamate uptake by excitatory amino acid (EAA) transporters, changes in extracellular glutamate can be highly variable and diffi-
cull to detect (26); therefore all anoxic experiments other than basal normoxic and anoxic controls were performed by the coperfusion (reverse dialysis) of each drug in the presence of the general glutamate uptake inhibitor l-trans-PDC to provide background glutamate levels sufficiently high to detect changes. PDC possesses strong glutamate transporter affinity and negligible EAA-receptor affinity (2) and reliably increases extracellular glutamate in the mammalian brain without causing neurohistological damage (17) when applied through the microdialysis probe in concentrations such as were used in this study (22, 26). The seemingly high concentrations of drugs typically used in microdialysis studies are necessary because of the incomplete permeability of the dialysis membrane as well as the torturous diffusion pathways through tissue (32), such that only a fraction of the drug infused through a microdialysis probe actually reaches the tissue.

Methylene blue was injected through the microdialysis probe at the end of each experiment to identify probe location. Data were used only from those turtles in which probe location in the striatum was verified.

Baseline levels were defined as the mean of the two samples immediately before an experimental insult (anoxia or drug treatment); all baseline sampling occurred after an initial 1-h presampling period (19). Values are expressed as a percentage of baseline ± SE due to between-animal variability. In all text and figures, the data labeled “anoxia” refer to the mean of samples collected over the period of nitrogen respiration from 1 to 2 h. Statistical significance of changes was determined nonparametrically [Kruskal-Wallis test for unequal variances using the SAS/JMP (Cary, NC) statistical package, Tukey-Kramer post hoc analysis]. In cases of equal variance, one-way ANOVA/Student’s t-test was used. P < 0.05 was considered to be statistically significant.

RESULTS

Glutamate homeostasis. Basal levels of extracellular glutamate averaged 3.4 ± 0.8 μM in the turtle striatum; there was no significant difference between normoxic and anoxic baseline levels. As has been previously described (20), baseline levels of excitatory neurotransmitters (glutamate and aspartate) did not increase over 4 h of anoxia, whereas levels of the inhibitory neurotransmitter GABA increased significantly (Fig. 1).

Blocking glutamate transport in the normoxic brain with the general uptake blocker PDC caused a 570 ± 27% increase in extracellular glutamate (vs. normoxic control levels of 100 ± 5%), indicating that in the normoxic turtle, as in the mammal, low extracellular glutamate levels are maintained by active reuptake (Fig. 2). Blocking reuptake in the anoxic animal, on the other hand, increased glutamate only 300 ± 27% compared with anoxic control levels of 105 ± 6%. Although active reuptake continues in anoxia, the turtle also has decreased glutamate release by 47% relative to the normoxic animal. Perfusion of the normoxic animal with the specific astrocytic GLT-1 transport blocker DHK also increased extracellular glutamate to 250 ± 17% over baseline (Fig. 2). This was significantly lower (56%) than the increase that occurred when general uptake was blocked with PDC, indicating that, as in the mammal, glial cells are important in glutamate clearance, with the GLT-1 transporter accounting for approximately one-half of glutamate uptake. Blockage of the GLT-1 transporter in anoxic animals increased extracellular glutamate 149 ± 8.9% over basal, a decrease of 50% vs. the increase observed in anoxic animals treated with PDC. Thus not only does the GLT-1 transporter remain active during anoxia, but as in air, the glial cells appear to be responsible for a minimum of 50% of glutamate uptake even as overall release and reuptake are decreased.

Factors modulating glutamate release. As activation of both adenosine A1 receptors and KATP channels is thought to play a role in decreasing hypoxic/ischemic...

Fig. 1. Effects of anoxia on extracellular levels of inhibitory and excitatory amino acids measured by intracerebral microdialysis in the striatum of freshwater turtles exposed to 4-h anoxia at 25°C. Time 0 indicates the start of ventilation on 99.99% nitrogen. Values are means ± SE from 5 animals. *Significantly different from normoxia, P < 0.05. Asp, aspartate; Glu, glutamate.

Fig. 2. Perfusion of the anoxic turtle striatum with the general glutamate uptake blocker l-trans-pyrrolidine-2,4-dicarboxylic acid (PDC; 2.5 mM) or the specific astrocytic GLT-1 uptake blocker dihydrokinate (DHK; 1 mM) decreases extracellular glutamate levels compared with normoxic animals. Values are means ± SE, n = 5 animals/group. Anoxic refers to the mean of samples collected between 1- and 2-h nitrogen respiration. O2, normoxic groups; N2, anoxic experimental groups. *Anoxic group significantly different from normoxic group, P < 0.05.
glutamate release by reducing neurotransmitter release (8,38), these two key factors were manipulated to determine their relative potential roles in the observed decrease in glutamate release in the anoxic turtle brain.

**Adenosine.** As shown in Fig. 3, glutamate release in the normoxic turtle can be modulated by adenosine. When the normoxic turtle brains were perfused with adenosine in the presence of PDC, extracellular glutamate levels were significantly reduced below PDC perfusion alone to levels nearly equal to anoxic animals perfused with PDC (290 ± 27 vs. 300 ± 27%; Fig. 2). Adenosine receptor activation, then, reduces glutamate release in the normoxic turtle.

Although the normoxic results showed that adenosine can have a very potent effect to decrease glutamate release, there was, however, little effect on glutamate levels in the 1- to 2-h anoxic turtles in which adenosine receptors were blocked (Fig. 3). Perfusing the anoxic brain with the general adenosine receptor blocker theophylline in the presence of PDC increased extracellular glutamate to only 350 ± 33% of baseline, not statistically different from anoxic turtles perfused with PDC alone. Because blocking adenosine receptors in the early anoxic turtle did not increase glutamate release, it is likely that other modulators are affecting glutamate levels.

**K\textsubscript{ATP} channels.** We next investigated the role of K\textsubscript{ATP} channels, which have been shown to play a protective role in the hypoxic/ischemic mammalian brain, in part, by decreasing glutamate release (38) and have also been demonstrated to inhibit DA release in the 1-h anoxic turtle brain (Milton and Lutz, unpublished observations). As with adenosine, opening K\textsubscript{ATP} channels in the normoxic brain in the presence of PDC decreased extracellular glutamate levels by 49% compared with normoxic glutamate increases in the presence of PDC alone (normoxic/PDC; Fig. 3). K\textsubscript{ATP} activation in normoxia thus decreases glutamate release to levels observed in baseline anoxic PDC animals.

Blocking K\textsubscript{ATP} channels with BDM (in the presence of PDC) in the 1-h anoxic brain, however, did not significantly increase extracellular glutamate above basal anoxic PDC levels (Fig. 3) nor did BDM perfusion (without PDC) increase glutamate when compared with normoxic or anoxic controls (data not shown). The anoxia-induced decrease in glutamate release then is not prevented by inhibition of K\textsubscript{ATP} channels, again indicating the probability that other factors are modulating anoxic glutamate levels.

**K\textsubscript{ATP} channels and adenosine.** Despite the fact that neither blocking adenosine receptors nor K\textsubscript{ATP} channels individually significantly elevated anoxic glutamate release, the two mechanisms together exhibit a powerful synergistic effect (Fig. 3). Blocking both K\textsubscript{ATP} channels and adenosine receptors simultaneously in the anoxic brain increased extracellular glutamate levels to 480 ± 63% of basal, significantly higher than anoxic/PDC animals and not statistically different from levels seen in normoxic/PDC animals (570 ± 27%). The inhibition of adenosine receptors and K\textsubscript{ATP} channels at the same time then prevents the anoxia-induced decrease in glutamate release.

**DISCUSSION**

The basal extracellular glutamate levels reported here are in line with previous reports for both the normoxic mammalian brain (6, 9) and the normoxic and anoxic turtle (20). As in the mammalian brain, low extracellular glutamate levels are maintained during normoxia by rapid reuptake into cells surrounding the synaptic cleft; the results reported here indicate that ~50% of this reuptake is astrocytic. Unlike the anoxia-sensitive mammal, however, where extracellular glutamate levels increase dramatically in response to ischemic injury (13), glutamate levels remain low in the anoxic turtle striatum due to a combination of continued glutamate reuptake and a reduction in glutamate release. As in the normoxic brain, glial cell transporters account for approximately one-half (or more) of anoxic glutamate uptake. Other transport mechanisms most probably remain active in anoxia as well, accounting for a similar proportion of glutamate uptake as in normoxia such that the overall pattern of glutamate uptake into neurons and glial cells is maintained.

Our normoxic studies also indicate that activating either adenosine receptors or K\textsubscript{ATP} channels indepen-
dently is sufficient to decrease glutamate release. It should be noted, however, that when adenosine receptors were pharmacologically activated by us in the normoxic brain, K<sub>ATP</sub> channels would likely have remained unstimulated due to high intracellular ATP levels, although a direct link between adenosine and K<sub>ATP</sub> channels has been suggested in ischemic mammalian brain (10, 27). Conversely, when K<sub>ATP</sub> channels were stimulated pharmacologically, adenosine receptors would be inactive due to the high ATP and low adenosine levels of the normoxic brain.

But while adenosine receptor activation decreased glutamate release in the normoxic animal, blocking adenosine receptors alone in the anoxic turtle brain was not sufficient to prevent the observed anoxia-induced decrease in glutamate release. This is in direct contrast to the mammalian brain, where adenosine receptor blockade augments ischemic glutamate release (28), and it suggests that other factors are activated in the anoxic turtle that are able to decrease glutamate release and compensate for adenosine receptor blockade. One such potential mechanism to suppress glutamate release is the stimulation of K<sub>ATP</sub> channels, which are activated during hypoxia and ischemia in response to decreased intracellular ATP. Although the anoxia-tolerant turtle is able to maintain basal ATP levels over long-term anoxia, there is a temporary drop in ATP during the initial hour of anoxia while the turtle is in transition to a hypometabolic state (15). Decreased ATP levels stimulate ATP-dependent potassium channels, an effect reinforced by adenosine A<sub>1</sub> receptor stimulation (30); activation has been shown to modulate DA homeostasis and ion channel downregulation in the early anoxic turtles (Milton and Lutz, unpublished observations; 23). Activated K<sub>ATP</sub> channels have also been shown to hyperpolarize the mammalian neuronal membrane and consequently inhibit neuronal excitability and neurotransmitter release, including the release of glutamate (38). However, this mechanism is probably not operational in the turtle as there is no evidence of hyperpolarization in the anoxic turtle brain (15).

Although stimulating K<sub>ATP</sub> channels in the normoxic brain decreased glutamate release nearly 50%, as was the case with adenosine, blocking K<sub>ATP</sub> channel activation alone in the early anoxic brain did not prevent the decrease in glutamate release. As with adenosine, it would appear that other factors in the anoxic turtle are able to compensate for the pharmacological blockade of K<sub>ATP</sub> channels. The increase in extracellular adenosine as intracellular ATP breaks down remains a possible important factor. Although blocking adenosine receptors in the early anoxic turtle did not affect glutamate release, increases in adenosine are known to play an important role in anoxic survival. Striatal extracellular adenosine levels in the anoxic turtle increase significantly from the onset of anoxia as ATP levels fall, peaking at 12 times basal levels by 90 min (21). Adenosine is critical for survival in both the anoxic turtle, where blocking A<sub>1</sub> receptors in the anoxic cerebellum induces depolarization (15), and in the ischemic mammal, where adenosine receptor blockade increases excitotoxin release (28). Our results indicate that activation of either adenosine receptors or K<sub>ATP</sub> channels in the normoxic or anoxic turtle brain is sufficient to block glutamate release. This may be the result of a general neuronal inhibition of the cell brought about by decreased ATP levels (activating K<sub>ATP</sub> channels) and/or the release of adenosine and activation of A<sub>1</sub> receptors. Blocking adenosine receptors as we did would enhance the activation of K<sub>ATP</sub> channels by increasing energy demand and lowering ATP levels. Blocking K<sub>ATP</sub> channels could similarly enhance the effectiveness of A<sub>1</sub> receptor activation by increasing adenosine release. Thus, when either mechanisms is blocked in the early anoxic turtle, the other is sufficiently stimulated by high adenosine or decreased ATP to compensate and induce the ~50% decrease in glutamate release.

This hypothesis is supported by our results, in that blocking adenosine receptors and K<sub>ATP</sub> channels simultaneously in the presence of PDC greatly increased glutamate release in the anoxic turtle to levels not significantly different from normoxic/PDC turtles, i.e., the anoxia-induced decrease in glutamate release was almost completely abolished when both potential pathways were blocked. Similar synergistic interactions with adenosine have been reported for both GABA and acetylcholine, while de Mendonca and Ribiero (4) reported that the contribution of group 3 metabotropic glutamate receptors to hypoxia-induced synaptic depression could only be detected when adenosine A<sub>1</sub> receptors were blocked. Lucchi et al. (14) suggested that adenosine and GABA may act in a redundant or perhaps overprotective manner, with GABA acting as a substitute for some of adenosine’s actions in hypoxia when adenosine was not operative. Only when adenosine receptors were blocked was the cosuppression by GABA revealed, which accounted for ~20% of the hypoxic decrease in synaptic transmission. Similarly, Coelho et al. (3) reported a muscarinic receptor-induced depression of synaptic transmission during hypoxia that was only apparent when adenosine A<sub>1</sub> receptors were blocked, suggesting a link through a common transduction signal. Although a direct link between the A<sub>1</sub> receptor and K<sub>ATP</sub> channels has also been suggested for the mammalian brain (10, 27), the present experiments suggest that these mechanisms are functioning independently in the early anoxic turtle, in that blocking either mechanism alone did not alter glutamate release.

The continued release and reuptake of glutamate in the anoxic turtle, albeit at a significantly reduced rate, suggest that the anoxic animal may be attempting to retain neuronal function while simultaneously cutting energy expenditures. As glutamate uptake occurs via a Na<sup>+</sup>/glutamate cotransport mechanism maintained by Na<sup>+</sup>/K<sup>+</sup>-ATPase, decreasing glutamate release conserves energy by decreasing uptake requirements while continuing to maintain basal extracellular glutamate levels. A similar process has been demonstrated for DA homeostasis in the anoxic turtle brain.
where $K_{\text{ATP}}$ stimulation decreases release during early anoxia (Milton and Lutz, unpublished observations) while DA release and reuptake continue over long-term anoxia such that basal extracellular DA levels are maintained (19). The dependence of glutamate transport on the Na$^+$/K$^+$ gradient also implies that while membrane permeability has been shown to decrease in the anoxic turtle brain (5), the near-complete channel arrest reported in the anoxia-tolerant turtle hepatocytes (13) will not be an option for turtle neurons. If the turtle must maintain a degree of ion flux to support glutamate reuptake, energetic costs as high as 1.5 ATP per glutamate may represent a significant fraction of the energy expenditure required under anoxic conditions and thus put a limit on the degree of metabolic reduction that can be achieved. We speculate that the maintenance of basal extracellular glutamate levels, as with the stable DA levels reported previously, acts to maintain the functional integrity of the turtle neuronal network, preserving neural tone and allowing the full recovery of operational activity after hours or even months of anoxia. The maintenance of neural tone by low levels of neurotransmitters has recently been described in the mammalian brain (34). Further studies will determine the pathways of glutamate release (vesicular vs. nonvesicular stores) as well as investigate the mechanisms that control glutamate homeostasis over long-term anoxic exposure.

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