

## Adenosine and ATP-sensitive potassium channels modulate dopamine release in the anoxic turtle (*Trachemys scripta*) striatum

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Submitted 21 September 2004; accepted in final form 14 February 2005

**Milton, Sarah L., and Peter L. Lutz.** Adenosine and ATP-sensitive potassium channels modulate dopamine release in the anoxic turtle (*Trachemys scripta*) striatum. *Am J Physiol Regul Integr Comp Physiol* 289: R77–R83, 2005. First published February 17, 2005; doi:10.1152/ajpregu.00647.2004.—Excessive dopamine (DA) is known to cause hypoxic/ischemic damage to mammalian brain. The freshwater turtle *Trachemys scripta*, however, maintains basal striatal DA levels in anoxia. We investigated DA balance during early anoxia when energy status in the turtle brain is compromised. The roles of ATP-sensitive potassium ( $K_{ATP}$ ) channels and adenosine (AD) receptors were investigated as these factors affect DA balance in mammalian neurons. Striatal extracellular DA was determined by microdialysis with HPLC in the presence or absence of the specific DA transport blocker GBR-12909, the  $K_{ATP}$  blocker 2,3-butanedione monoxime, or the nonspecific AD receptor blocker theophylline. We found that in contrast to long-term anoxia, blocking DA reuptake did not significantly increase extracellular levels in 1-h anoxic turtles. Low DA levels in early anoxia were maintained instead by activation of  $K_{ATP}$  channels and AD receptors. Blocking  $K_{ATP}$  resulted in a 227% increase in extracellular DA in 1-h anoxic turtles but had no effect after 4 h of anoxia. Similarly, blocking AD receptors increased DA during the first hour of anoxia but did not change DA levels at 4-h anoxia. Support for the role of  $K_{ATP}$  channels in DA balance comes from normoxic animals treated with  $K_{ATP}$  opener; infusing diazoxide but not adenosine into the normoxic turtle striatum resulted in an immediate DA decrease to 14% of basal values within 1.5 h. Alternative strategies to maintain low extracellular levels may prevent catastrophic DA increases when intracellular energy is compromised while permitting the turtle to maintain a functional neuronal network during long-term anoxia.

anoxia tolerance; microdialysis; theophylline; GBR-12909; 2,3-butanedione monoxime

UNABLE TO REDUCE ITS HIGH energy requirements, the mammalian brain dies rapidly when deprived of oxygen (27). The uncontrolled release of dopamine (DA) into the extracellular space of the mammalian brain has been identified as one major cause of hypoxic/ischemic brain damage. Although severe hypoxia or ischemia can increase extracellular levels as much as 500-fold (13), even mild hypoxia (cortical arteriole  $P_{O_2}$  = 15 Torr) can cause neuronal damage, with DA increases as high as 200% (21). The increased extracellular DA then contributes to neuronal damage by altering cerebral blood flow and glucose metabolism (13), through the production of reactive oxygen species (ROS) (45), and by modulating the release of excitatory amino acids (EAAs) (37), especially through its interactions with glutamatergic systems (20, 24). Excess DA has also been shown to inhibit the mitochondrial respiratory system, including complex I of the electron transport system

(6). Unlike the widespread release of EAAs, however, which occurs upon brain depolarization (4, 47), DA release is seen before high energy stores are fully depleted (21), with Santos et al. (48) reporting a linear correlation between the ratio of ATP to ADP in synaptosomes and the release of DA, although others have reported hypoxia-dependent decreases in ATP that did not affect DA release (36).

In the hypoxic mammalian brain, increases in extracellular DA are due primarily to decreased reuptake into the cell (1, 21), coupled with increased release from intracellular stores (15). Uptake back into the cell is the primary route of DA removal from the extracellular space (21), as is true of other neurotransmitters, and this occurs via an energy- and sodium-dependent specific transport mechanism (1, 19). Thus conditions that decrease energy availability in the hypoxic brain can induce increases in extracellular neurotransmitter levels well before energy stores are so depleted as to cause neuronal depolarization.

A few vertebrate species, however, do not share the mammalian central nervous system vulnerability to hypoxia and are in fact able to withstand months of complete anoxia (27). The best-documented anoxia-tolerant vertebrate is the freshwater turtle *Trachemys scripta*, which is able to maintain neuronal ion gradients and ATP levels for at least 48 h of anoxia at room temperature (10) by lowering its metabolic rate to match glycolytic ATP supply (27) and thus avoiding anoxic depolarization and uncontrolled EAA release (39).

Unlike mammals, the *Trachemys* brain is also protected against increases in extracellular DA even during complete anoxia; low DA levels are maintained at least in part by the continued function of reuptake mechanisms (34). The turtle brain is able to continue energy-dependent DA reuptake because, unlike the mammal, the anoxic turtle brain maintains ATP levels during long-term anoxic conditions (29). It is only when a significant imbalance occurs between energy supply and utilization that DA increases above basal levels in the hypoxic or ischemic mammalian brain (54).

ATP levels in *T. scripta* do drop significantly, however, during the first 1–2 h of anoxia when the turtle is in transition from normoxia to the depressed metabolism of complete anoxia (8, 28). During this temporary transition state, metabolic energy demands temporarily outstrip supply, and ATP levels drop ~20%; after the turtle has completed metabolic suppression, ATP concentrations again rise to pre-anoxic levels (29). Although mammalian DA homeostasis is lost during hypoxia, we have shown that striatal DA in the *Trachemys* brain remains at normoxic levels even during this transitional period (34).

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The question is thus raised, by what mechanism(s) does the turtle brain maintain DA balance in the extracellular space that is apparently insufficient or not utilized by the hypoxic mammal? Two possibilities could explain the lack of increase in extracellular DA when intracellular ATP is reduced: DA release may occur along with a continued functioning of reuptake mechanisms (as occurs in long-term anoxia) despite decreased energy supplies, or the turtle may block the release of DA, as it does with other excitotoxins such as glutamate (35).

One potential mechanism to prevent DA release when ATP is low is the activation of ATP-sensitive potassium ( $K_{ATP}$ ) channels. These channels are normally inhibited by physiological levels of ATP but open in response to decreases in intracellular ATP (5, 9). These channels are thought to play a critical protective role during the early stages of ischemia in the mammalian brain (26) by increasing membrane hyperpolarization, increasing cerebral blood flow (53), and decreasing the release of excitatory neurotransmitters including DA (52, 55). These mechanisms collectively reduce energy consumption while preventing depolarization and thus provide a protective effect to the brain under conditions of low oxygen/reduced ATP.

As in the mammal,  $K_{ATP}$  channel activation also protects the turtle brain against depolarization during anoxia. Stimulated  $K_{ATP}$  channels were shown by Pek-Scott and Lutz (42) to be involved in the downregulation of membrane ion ( $K^+$ ) permeability (channel arrest) during the first hour of anoxia in *T. scripta*. This protective effect could be blocked upon addition of  $K_{ATP}$  channel blockers during the first hour of anoxia, but channel blockers had no effect by 4 h of anoxia, consistent with a return to normoxic ATP levels in the long-term anoxic turtle brain and closure of  $K_{ATP}$  channels (42).  $K_{ATP}$  channels are also one mechanism by which the turtle brain decreases glutamate release during the first 1–2 h of anoxia, while stimulation of  $K_{ATP}$  also decreases glutamate release in normoxic animals (35). The known role of  $K_{ATP}$  channels in preventing DA release in the mammalian brain (52) and their involvement in the anoxic tolerance of the turtle brain indicate a prospective mechanism to prevent DA release into the turtle striatum during the initial energy crisis.

Along with  $K_{ATP}$ , adenosine (AD) has also been shown to have protective effects in both the mammalian and turtle brain.  $AD_1R$  activation in the mammal inhibits neurons both pre- and postsynaptically by modulating ionic currents and decreasing neurotransmitter release (16, 49);  $AD_1R$  activation decreases DA release (25), while antagonists increase DAergic neuronal damage due to mitochondrial dysfunction (2). In the anoxic turtle brain, AD acts as a “retaliatory metabolite” to balance energy supply and demand (28) and increase cerebral blood flow (22). As is seen with  $K_{ATP}$ , stimulation of adenosine receptors (ADR) in the normoxic and anoxic turtle brain also decreases glutamate release (35), and ADR stimulation is thus another prospective mechanism to prevent DA release in early anoxia.

The purpose of this study was to examine DA balance during the transition period between normoxia and anoxia in the turtle brain and to determine whether activated  $K_{ATP}$  channels and/or ADR play a role in preventing extracellular DA increases during this period.

## MATERIALS AND METHODS

**Materials.** The studies described were approved by the institutional animal care and use committee of Florida Atlantic University. Freshwater turtles (*T. scripta*) were purchased from a commercial supplier (Lemberger, Oshkosh, WI). The dopamine blocker 1-[2-[bis(4-fluorophenyl)methoxyethyl]-4-(3-phenyl-propyl)piperazine dihydrochloride (GBR-12909),  $K_{ATP}$  channel blocker 2,3-butanedione monoxime (BDM), and  $K_{ATP}$  channel opener 7-chloro-3-methyl-2H-1,2,4-benzothiadiazine 1,1-dioxide (diazoxide) were purchased from Tocris Cookson (St. Louis, MO). The specific  $K^+$   $K_{ATP}$  channel blocker 5-chloro-N-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-2-methoxybenzamide (glibenclamide) was purchased from Research Biochemicals International (Natick, MA). All other chemicals and reagents were purchased from Sigma Chemicals (St. Louis, MO), including AD and the general ADR antagonist theophylline.

**Methods.** Experiments were performed at 25°C on the freshwater turtles *T. scripta elegans*. Animals were divided into 10 groups ( $n = 5$  or 6 per group): normoxic controls (no drug), normoxic experimental animals treated with drug, early anoxia (1-h nitrogen exposure) treated with each drug, and long-term (3–4 h) anoxic animals treated with AD, the ADR antagonist theophylline, or the specific DA uptake blocker GBR-12909. Drug treatments included: GBR-12909 (2  $\mu$ M as per Ref. 34), the  $K_{ATP}$  opener diazoxide (500  $\mu$ M as per Ref. 28), the  $K_{ATP}$  blocker BDM (500  $\mu$ M as per Ref. 35), AD (200  $\mu$ M as per Refs. 29, 35) or the general ADR blocker theophylline (100  $\mu$ M as per Ref. 39). BDM is a specific blocker of the  $K_{ATP}$  channel able to reversibly inhibit whole cell  $K_{ATP}$  currents with a  $K_i = 11 \pm 3$  mM for half-maximal inhibition and a Hill coefficient of  $2.5 \pm 0.2$  (51). Data for anoxic control, normoxic, and 4-h anoxic turtles treated with GBR-12909 have been previously reported (34). Additional control, 1-h anoxic, and 4-h anoxic animals ( $n = 5$  or 6 per group) were treated with the  $K_{ATP}$  channel blocker glibenclamide (400  $\mu$ M). All drugs were administered via the microdialysis probe perfusate in turtle artificial cerebrospinal fluid (ACSF).

Turtles were anesthetized with AErrane (Isoflurane, USP, Anaquest) in air. Anesthesia was induced using a 4% isoflurane in air mixture pumped from a 1.5-l rebreathing bag. Animals were maintained on 1.7% isoflurane once a surgical plane was achieved (50).

After exposing the skull, we trephined a 1-cm-diameter hole and removed the skullcap. 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 (5–6 mm depth from the cerebral surface, depending on animal size). The probe was perfused with turtle ACSF solution at 1.2  $\mu$ l/min with a CMA/100 microdialysis pump (Carnegie Medecin, Solna, Sweden); sampling was preceded by a 1-h stabilization period following probe insertion. Previous work in our lab has demonstrated that 1 h is a sufficient stabilization period to reduce extracellular neurotransmitters levels resulting from the damage of probe insertion. Anoxia was induced by changing the breathing mixture to certified 99.99% nitrogen (County Welding, Pompano Beach, FL) and 1.7% isoflurane; previous studies have shown that arterial  $PO_2$  is essentially zero by 1 h of  $N_2$  ventilation (33).

Dialysate was collected over 30-min intervals and analyzed immediately. Baseline DA levels were determined for all groups as the mean of the three samples (1.5 h) immediately before an experimental insult (drug or anoxia). Drug control groups were sampled for an additional 1.5 h in normoxic animals with drug perfusion. For anoxic animals, turtles were ventilated on  $N_2$  following baseline sample for 0.5 h (short-term anoxic exposures) or 2.5–3.5 h (long-term anoxia) before drug insult. Anoxic animals were then sampled for an additional 1.5 h of drug/anoxic exposure. In all cases, perfusion with the experimental drugs in ACSF was begun in the 0.5-h sample before the time point of interest, to allow time for movement through the system's dead spaces. Probe recovery was determined from known

standards in vitro (21). Dopamine recovery averaged  $14.5 \pm 2.0\%$  (mean  $\pm$  SE); results presented are not corrected for recovery.

Samples (20  $\mu$ l) were analyzed for monoamine content using reversed-phase HPLC with UV detection. The system consists of a Waters 510 HPLC pump (Waters, Milford, MA), a Rheodyne 7725i Manual Injector (also Waters), a  $4.6 \times 100$ -mm reversed-phase catecholamine column, (C18, 3  $\mu$ m; obtained from Alltech Associates, Deerfield, IL), and an SPD-6A UV Spectrophotometric Detector (Shimadzu, Kyoto, Japan). The mobile phase flow rate was 1.3 ml/min and consisted of 97% 20 mM potassium phosphate-3% MeOH. Minimum detectable DA levels with this method are  $\sim 200$  pg/ml. Methylene blue was injected through the microdialysis probe at the end of each experiment to identify probe location; probes were rinsed following each use and reused up to a total of three times per probe. Recovery decreases significantly if probes are utilized more than three times. Data were used only from those turtles in which probe location in the striatum was verified.

Values are expressed as a percentage of baseline  $\pm$  SE due to between-animal variability. Statistical significance of changes was determined nonparametrically (Kruskal-Wallis test for unequal variances utilizing the SAS/JMP statistical package; Cary, NC). In cases of equal variance, one-way ANOVA/Student's *t*-test was used; *P* < 0.05 was considered to be statistically significant.

## RESULTS

**DA reuptake (1 h).** During the first hour of anoxia, addition of the specific DA uptake blocker GBR-12909 into the turtle striatum did not result in any increases in extracellular DA (Fig. 1); the turtle is apparently not releasing DA into the extracellular space during the initial transition period. This is in direct contrast to both the normoxic and 4-h anoxic turtle, where addition of this uptake blocker increased extracellular striatal DA two- to threefold (34).

**K<sub>ATP</sub> channels.** Blocking K<sub>ATP</sub> channels with BDM in the 1-h anoxic turtle resulted in a significant increase in extracellular DA, whereas this treatment had no effect after 4-h anoxia (Fig. 2A). DA levels in the dialysate ([DA]<sub>dia</sub>) rose to a mean of  $227 \pm 172\%$  of baseline after addition of the blocking agent during the initial hour of anoxia but changed <2% compared with basal levels after 4-h anoxia. Significant increases could be observed within 0.5 h of addition of the drug (Fig. 2B). The increase in [DA]<sub>dia</sub> seen at 1-h anoxia is not due to an effect of BDM alone, as addition of the blocker to the normoxic (control) brain did not significantly alter [DA]<sub>dia</sub> ( $76 \pm 6\%$  of basal

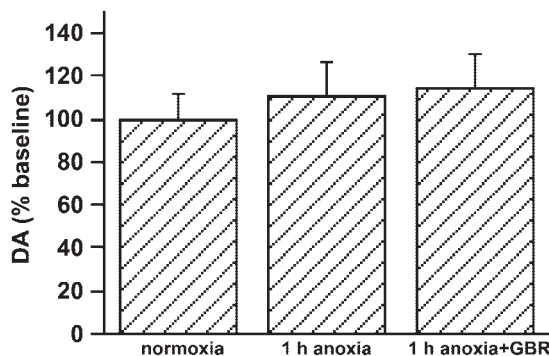


Fig. 1. Extracellular dopamine in the striatum of the turtle does not change significantly over a 1-h anoxic exposure. Administration of the specific dopamine (DA) uptake blocker GBR-12909 (2  $\mu$ M) does not increase extracellular DA, indicating that DA is not being released. Mean DA level in the dialysate ([DA]<sub>dia</sub>) in the normoxic group was  $97 \pm 13$   $\mu$ M.

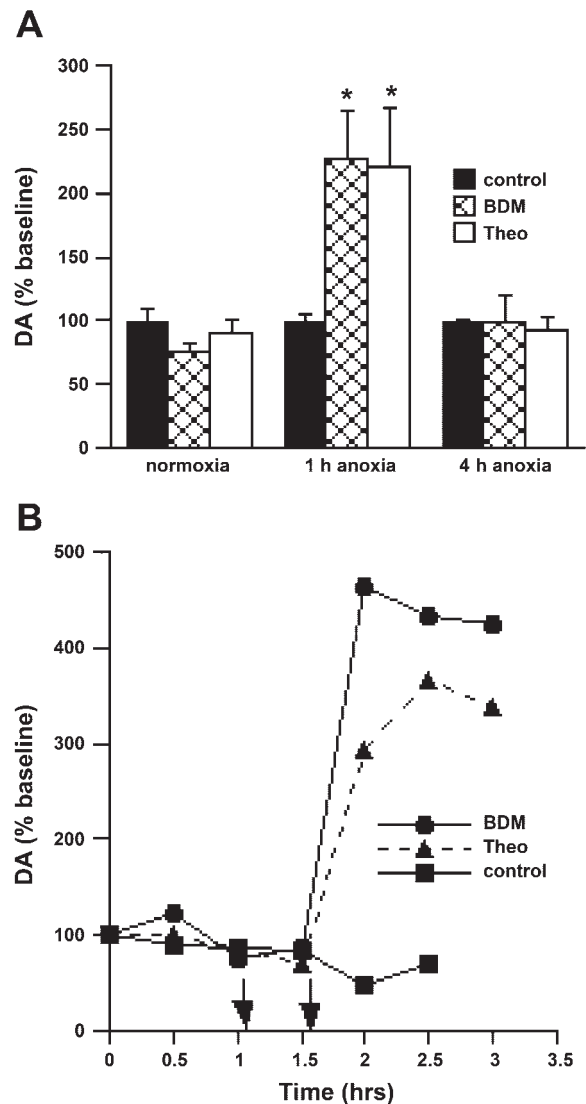


Fig. 2. Blocking ATP-sensitive K<sup>+</sup> channels or adenosine receptors (ADR) in the 1-h anoxia turtle resulted in significant increases in extracellular DA. A: no increases occurred when the drug was administered to control [normoxia/2,3-butanedione monoxime (BDM)] or 4-h anoxic animals. Mean initial [DA]<sub>dia</sub> in anoxic animals was  $380 \pm 34$   $\mu$ M before drug administration; mean initial [DA]<sub>dia</sub> was  $497 \pm 31$   $\mu$ M in normoxic animals. \*Significant difference from control at *P* < 0.05. B: typical time course of DA increases in drug-treated animals. Anoxia is initiated at the 1-h time point (left arrow); drug perfusion begins 0.5 h after the start of anoxia (right arrow). Theo, theophylline.

levels). It thus appears that K<sub>ATP</sub> channels play a role in the turtle brain by preventing DA release during the initial transition period to anoxia, but this effect disappears during long-term anoxia. Similar results were obtained using a second K<sub>ATP</sub> blocker; addition of glibenclamide increased [DA]<sub>dia</sub> to a mean of  $207 \pm 82\%$  of control in the 1-h anoxic turtle but had no effect in normoxic animals (not significant due to variability of time course, data not shown).

Further evidence that K<sub>ATP</sub> channels play a significant role in DA balance in the turtle striatum was obtained with the K<sub>ATP</sub> activator diazoxide. Perfusion of diazoxide into the normoxic brain resulted in an immediate significant decrease in extracellular striatal DA, to <14% of control values within 1.5 h (Fig. 3).



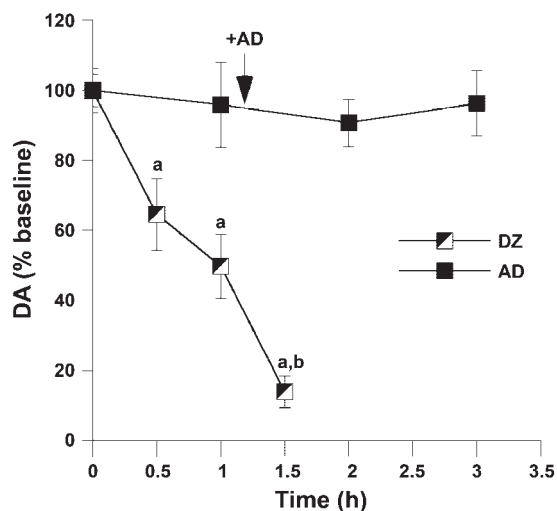


Fig. 3. Stimulation of ATP-sensitive  $K^+$  channels in the normoxic brain resulted in an immediate significant decrease in extracellular striatal DA, which fell to <15% of controls by 1.5 h. Mean initial  $[DA]_{dia}$  was  $389 \pm 71 \mu M$  before drug administration; diazoxide (DZ) administration was begun immediately following initial sample. <sup>a</sup>Significantly different from control at  $P < 0.05$ ; <sup>b</sup>significantly different from previous point,  $P < 0.05$ . Perfusing the normoxic turtle brain with adenosine (AD,  $200 \mu M$ ) had no effect on striatal DA release. Mean initial  $[DA]_{dia}$  was  $244 \pm 39 \mu M$  before drug administration.

**ADRs.** Blocking ADR with the general ADR blocker theophylline also significantly increased striatal  $[DA]_{dia}$  in the first hour of anoxia (Fig. 2A) compared with untreated 1-h anoxic animals.  $[DA]_{dia}$  increased to a mean of  $231 \pm 51\%$  of control vs. untreated striatal DA levels of  $90 \pm 11\%$  of basal; significant increases were observed within 0.5 h of drug administration (Fig. 2B). DA levels in untreated animals at 1- and 4-h anoxia were not significantly different from normoxic levels.

As was observed with  $K_{ATP}$  channels, at 4-h anoxia blocking ADR with theophylline had no effect on extracellular DA levels (Fig. 2). DA in treated animals was  $92 \pm 11\%$  of DA in 4-h anoxic, untreated animals ( $99 \pm 2\%$ ).

In contrast to the dramatic effects of opening  $K_{ATP}$  channels in normoxic animals, however, neither stimulating nor blocking ADR affected normoxic extracellular DA (Figs. 2 and 3).

## DISCUSSION

This study demonstrates the critical roles played by  $K_{ATP}$  channels and ADR in depressing DA release in the early anoxic turtle striatum. Under normoxic conditions, homeostasis is maintained through continuous DA release and reuptake, such that blocking reuptake results in significant DA increases in the extracellular space (34). Blocking reuptake during the initial hour of anoxia, however, does not increase extracellular DA levels (Fig. 1), indicating that release has been greatly reduced. This strategy differs from the long-term (4 h) anoxic turtle, which remains in energy balance (29) and is thus able to continue DA release and reuptake (34). The ability of the turtle to maintain low striatal DA levels during anoxia is in direct contrast to the mammalian brain, where even mild hypoxia can increase DA by 200%, and severe hypoxia/ischemia results in increases up to 500-fold (13, 21); these large increases are closely correlated with ATP depletion (48).

One mechanism that prevents DA release during the initial transition phase of anoxia is the activation of  $K_{ATP}$  channels. Whereas the turtle remains in energy balance over long-term anoxia, there is a moderate decrease in ATP levels in the turtle brain during the initial 1- to 2-h exposure (8) that appears sufficient to stimulate  $K_{ATP}$  channels and prevent DA release. Blocking  $K_{ATP}$  channel activity more than doubled extracellular DA over the initial 1–2 h of anoxia (Fig. 2) but had no effect in the normoxic brain nor in the 4-h anoxic brain. In both the normoxic and long-term anoxic turtle, on the other hand, ATP levels are high (29) and  $K_{ATP}$  channels are closed and thus unaffected by the blocking agent. Further support for this hypothesis is demonstrated by the pharmacological activation of  $K_{ATP}$  channels under normoxic (unstimulated) conditions, which caused an immediate decrease (within the first 30-min sample) in extracellular DA. After 1.5-h diazoxide perfusion, striatal DA levels were only 14% of basal (Fig. 3). Interestingly, although AD levels are known to affect both normoxic blood flow (22) and glutamate release (35) in *T. scripta*, neither the stimulation nor the blockade of AD receptors in normoxia affected extracellular DA levels. Adenosine, at  $200 \mu M$ , in the microdialysis perfusate has been shown to be sufficient to alter extracellular acetylcholine in rats (31) and extracellular glutamate in the freshwater turtle (35), thus we expect that any effects of normoxic AD exposure on extracellular DA would have been revealed.

However, the fact that diazoxide, but not AD, induces inhibition of normoxic DA release is not surprising. As  $K_{ATP}$  channels in many brain cells are normally closed (3), they provide a straightforward functional switch between a closed, inactive state and the open, active state. ADR, on the other hand, are functional during normoxia as well as during periods of metabolic stress; AD-dependent events are thus likely to have several layers of regulation that allow differential functions under varying metabolic states, including the opposing actions of A1 and A2a type receptors on DA release (see below).

Although the activation of  $K_{ATP}$  channels in mammals has been demonstrated to extend anoxic/ischemic survival by hyperpolarizing neuronal membranes and thus decreasing DA and EAA release (52, 55), these benefits are soon offset by the continued loss of  $K^+$  that eventually results in cell depolarization and excitotoxin release (17). The cost to the turtle of maintaining open  $K_{ATP}$  channels, however, is lower than in the mammal.  $K_{ATP}$  channels are present in the *Trachemys* brain at only 10–30% of levels in the rat, in line with their overall reduced metabolic rate (23), and the glibenclamide-inhibited population of  $K_{ATP}$  channels are not a major route of  $K^+$  loss in the turtle brain as they appear to be in some mammalian preparations (42). In addition, other ion leak channels are inhibited in the anoxic turtle brain (10); this channel arrest may decrease overall ion loss during anoxia to the point where any  $K^+$  loss through  $K_{ATP}$  channels can be offset by  $K^+$  uptake mechanisms even under conditions of reduced ATP production.  $K^+$  loss upon ouabain depolarization is reduced vs. normoxia in the 4-h anoxic turtle brain whether  $K_{ATP}$  channels are blocked or not (42). Unlike the mammalian brain, in fact, turtle neurons survive 180 min of both anoxia and pharmacological anoxia with no noticeable change in membrane resistance (11). By contrast, rat pyramidal neurons respond to anoxia with a loss of membrane resistance, followed by a

transient hyperpolarization and subsequent depolarization (11). As Purkinje cells of the isolated turtle cerebellum also do not hyperpolarize during anoxia (43), the mechanism by which K<sub>ATP</sub> channels modulate DA homeostasis is not likely to be via cellular hyperpolarization; however, an alternative method is yet unknown. Research has suggested that K<sub>ATP</sub> channels may be modulated by a variety of signaling pathways, including neurotransmitter release, H<sub>2</sub>O<sub>2</sub>, and cAMP levels (3, 7); it is quite possible then that K<sub>ATP</sub> channels, in turn, can act through second messenger systems directly, without cellular hyperpolarization. The turtle may provide an interesting model to investigate such mechanisms, as in the mammal, the opening of K<sub>ATP</sub> channels is invariably associated with hyperpolarization, and the possibility of more direct effects has not been examined.

The decrease in cellular ATP levels during the initial anoxic exposure, which opens striatal K<sub>ATP</sub> channels, results in turn in increased extracellular AD (40) and the stimulation of ADR. In addition, a direct link between A<sub>1</sub> receptors and K<sub>ATP</sub> channels that appears critical for ischemic preconditioning has been suggested for the mammalian brain (18, 46). As in the mammal, AD is a potent protective neuromodulator in the turtle, where AD increases cerebral blood flow (22), modulate channel arrest (41), and decrease glutamate release (35). But while it is necessary to block both K<sub>ATP</sub> channels and ADR simultaneously to detect increases in glutamate in the 1–2 h anoxic turtle striatum (35), blockade of either one individually increases DA release. A similar link between increased AD during an energy crisis and decreased DA release has also been reported in the rat brain, where the administration of an A<sub>1</sub> agonist significantly decreases extracellular DA (25), while A<sub>1</sub> antagonists increase DA-related damage during mitochondrial dysfunction (2). A<sub>1</sub> receptors are also thought to have a tonic inhibitory effect in the rat nucleus accumbens to decrease both DA and glutamate release (40). Although A<sub>2A</sub> receptors appear to increase DA and glutamate release (14, 44), the inhibitory effects of A<sub>1</sub> stimulation apparently outweigh the effects of A<sub>2A</sub> receptors. Conversely, it has been reported that the selective blockade of DA D<sub>2</sub> receptors produces a significant increase in extracellular AD, presumably due to increased energy demands in dopaminergic cells (38).

The turtle brain, then, appears to use two different strategies to avoid increased extracellular DA under anoxic conditions. During the initial energy crisis of anoxia, the turtle brain reacts in a manner similar to the hypoxic/ischemic mammal brain. K<sub>ATP</sub> channels and ADR are activated by an initial decline in intracellular ATP and consequent increase in extracellular AD, and these, in turn, prevent a catastrophic increase of DA into the extracellular space by blocking its release during this period of energy crisis.

During later anoxia, when ATP levels have been restored, the turtle brain relies on DA reuptake to maintain homeostasis. At this time K<sub>ATP</sub> channels and ADR are no longer involved in DA release, as K<sub>ATP</sub> channels have closed (42) and extracellular AD levels are reduced (40).

Unlike the mammal, however, the turtle brain is able to avoid depolarizing while downregulating metabolism to the hypometabolic state. Upon return to basal ATP levels in later anoxia, K<sub>ATP</sub> channels close (42) while extracellular AD levels

fall (40); during such long-term anoxia, the turtle brain relies on DA reuptake to maintain homeostasis.

Of course, it could also be that the anoxic turtle brain is simply unable to completely prevent DA loss, and the 1-h transition period is too brief to observe any significant increases in extracellular DA due to a decreased but still occurring DA leakage from the cell. Even stimulation of the K<sub>ATP</sub> channels during the transition period is not sufficient to completely prevent DA loss, as occurs in normoxic animals stimulated with diazoxide, as extracellular DA remains at basal levels during this transition period. Our previously reported continued reuptake over long-term anoxia, then (34), would be an adaptation to prevent large DA increases in the face of continuous, long-term loss.

Thus the apparent requirement to maintain low extracellular DA levels may be for either or both of two reasons: protection against excessive DA levels, which are likely to be toxic to the turtle brain, as in mammals (32), and/or the maintenance of basal intra- and extracellular levels through continued long-term release and reuptake. The latter could be critical to maintain the neuronal circuitry of the long-term anoxic brain, as suggested by the continued low-level activity of other neuronal measures. The continued release and reuptake of glutamate also occur in the anoxic turtle brain, for example, despite an estimated energetic cost as high as 1.5 ATP per glutamate (35). The latter could be critical to maintain the neuronal circuitry of the long-term anoxic brain. The anoxic turtle brain also still exhibits frequent, periodic bursts of electrical activity despite being electrically quiescent overall (12); we suggest that continued neuronal activity may be important for maintaining functional integrity in the metabolically depressed brain and allowing recovery when oxygen is again available. Such continued energy expenditures, however, as well as the need to maintain ion gradients for gradient-dependent transport mechanisms, put a limit on the degree of ion channel arrest and metabolic reduction that can be achieved even over long-term anoxia.

#### ACKNOWLEDGMENTS

Special thanks is given to Robert Delaney for technical assistance.

#### GRANTS

Partial support for this research was provided by the National Science Foundation and the Florida Atlantic University Foundation.

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