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Endogenous reductions in *N*-methyl-D-aspartate receptor activity inhibit nitric oxide production in the anoxic freshwater turtle cortex

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Abstract **Increased nitric oxide (NO) production from hypoxic mammalian neurons increases cerebral blood flow (CBF) but also glutamatergic excitotoxicity and DNA fragmentation. Anoxia-tolerant freshwater turtles have evolved NO-independent mechanisms to increase CBF; however, the mechanism(s) of NO regulation are not understood. In turtle cortex, anoxia or NMDAR blockade depressed NO production by 27 ± 3% and 41 ± 5%, respectively. NMDAR antagonists also reduced the subsequent anoxic decrease in NO by 74 ± 6%, suggesting the majority of the anoxic decrease is due to endogenous suppression of NMDAR activity. Prevention of NO-mediated damage during the transition to and from anoxia may be incidental to natural reductions of NMDAR activity in the anoxic turtle cortex.**

Keywords: Channel arrest; L-NAME; Hypoxia; Cerebral blood flow; Diaminofluorescein; Calcium

1. Introduction

Cerebral blood flow (CBF) is generally elevated with hypoxia or hypercapnea in human and murine models [1,2]. Increased CBF is considered neuroprotective since it allows greater delivery of glycolytic substrate and rapid removal of acidic anaerobic end products from sensitive brain regions. Nitric oxide (NO) is a signaling metabolite that plays a critical role in the regulation of CBF in adult mammalian brain such that: (1) CBF increases coordinately with increased NO production in response to hypoxia and also following reoxygenation; (2) NO scavengers or inhibition of nitric oxide synthases (NOS) decrease CBF and abolish the hypoxic increase in CBF; and (3) NO promoters increase CBF in normoxia and during hypoxia [3]. In murine models, ischemia induces rapid elevations in cerebral NO, increases NOS activity and elevates NOS gene expression, and all of these events contribute to neuroprotective increases in CBF [4,5].

Although NO promotes enhanced CBF, there is considerable debate as to whether NO production is neurotoxic or neuroprotective in ischemic mammalian neurons [6]. For example, in rat, mouse, gerbil and neonatal piglet brain, NO scavengers or inhibition of neuronal or inducible NOS provide significant neuroprotection and prevent DNA fragmentation and release of apoptotic factors [7–10]. Furthermore, decreased NO formation has been linked to improved behavioral testing performance and neuroprotection in gerbils [11]. However, others have reported that NO donors provide neuroprotection against global and focal models of ischemia in adult rat brain, reduce the extent of ischemic infarct damage in neo-natal rat brain and reduce reactive oxygen species (ROS)-mediated neurotoxicity [12–15]. These conflicting reports may be due to the interaction of NO with numerous cellular systems simultaneously. For example, NO can act as a free radical scavenger, ameliorating the deleterious effects of reactive oxygen species [16]; however, NO can also interact with superoxide to produce peroxynitrate, leading to DNA fragmentation and cell degradation via apoptotic pathways [17].

In mammals, the neurotoxic effects of NO are primarily mediated by excessive glutamatergic receptor activity. Ischemic insults promote elevated glutamate release and over-activation of *N*-methyl-D-aspartate receptors (NMDARs), which is the primary entry point of Ca^{2+} during ischemia and whose activation leads to excitotoxic cell death (ECD) in ischemic mammalian neurons [18]. Neuronal NOS (nNOS) is associated with the NMDAR scaffold protein – postsynaptic density 95 (PSD-95), and NMDAR activation leads to cytosolic Ca^{2+} accumulation and stimulation of nNOS-dependent NO production [19,20]. NO is a powerful intra- and intercellular signaling molecule and it enhances glutamate release from presynaptic neurons, contributing significantly to the acceleration of ECD [21,22]. Therefore, although enhanced NO production promotes CBF and provides neuroprotection during ischemic insults, the side effects of this mechanism are complex and may lead to ECD or apoptotic events in afflicted neurons.

Certain facultative anaerobes, including freshwater turtles (*Chrysemys picta bellii* and *Trachemys scripta elegans*), goldfish (*Carassius auratus*) and the crucian carp (*Carassius carassius*) are remarkably tolerant to anoxic insult and can survive without oxygen for days at warm temperatures and months at colder temperatures [23]. These organisms rely solely on

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Abbreviations: APV, *-amino-5-phosphonopentanoic* DL-2-amino-5-phosphonopentanoic acid; CBF, cerebral blood flow; DAF-FM, *-5-methylamino-2′, 7′-difluorofluorescein*4-amino-5-methylamino-2′,7′-difluorofluorescein; ECD, excitotoxic cell death; L-NAME, N^G-*arginine* -nitro-L-arginine methyl ester; NMDAR, *N-aspartate* -methyl-D-aspartate receptor; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; PSD-95, Post-synaptic density 95; ROS, reactive oxygen species; SNP, sodium nitroprusside dihydrate

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glycolytic metabolism during anoxic episodes and are thus dependent on maintained delivery of glycolytic substrate [24–26]. Changes in CBF during anoxia and recovery have been reported in both the red-eared slider and the crucian carp, but changes in NO production have not been measured in any facultative anaerobic species. In turtle, CBF increases from 1.7- to 2.6-fold within the first hour of anoxia before returning to pre-anoxia levels, presumably due to the onset of a deeper metabolic depression which limits the need for enhanced glycolytic substrate delivery [27,28]. Furthermore, secondary increases in CBF of up to 80% were observed upon reoxygenation, which may contribute to the removal of acidic anaerobic end products. During normoxia, turtle CBF can be mediated by adenosine or by acetylcholine via the activity of NO; however during anoxia, adenosine alone affects the anoxic increase in CBF while inhibition of NO synthesis or an NO donor have no effect on anoxic CBF [28,29]. Similarly, in the anoxic crucian carp, CBF is increased by anoxia and this increase is not prevented by NOS blockade. NO synthesis has an absolute requirement for oxygen, thus the inability of NO drugs to block the anoxic increase in CBF in these organisms is not surprising.

Although inhibition of NO synthesis during anoxia did not alter turtle CBF, NOS inhibition during normoxia led to a complete cessation of CBF that recovered to control flow rates within 15–20 min. The authors concluded that turtles possess a CBF tonus that is NO-mediated similar to the constant vasodilatory tonus observed in mammals [30], but that upon suppression of this tonus, secondary CBF-regulating mechanisms take over. This secondary mechanism may be responsible for anoxic increases in CBF, which are not susceptible to NO-mediated signaling. Indeed, since these facultative anaerobes survive for months in a zero-oxygen environment, NO-mediated regulation of CBF would be impossible and thus the development of a non NO-based mechanism is an evolutionary necessity. However, in their natural environment turtles undergo frequent prolonged periods of intermittent hypoxia while foraging or diving during which surges in NO production may occur.

As discussed above, increased CBF is neuroprotective, but there is debate as to whether increases in NO production are neuroprotective or neurotoxic during anoxic insult and following reoxygenation in mammals. Thus, the mechanism of CBF regulation in the anoxic turtle is of interest because CBF is greatly elevated during anoxia and following reoxygenation independently of NO production, and because CBF becomes effectively immune to NO signaling during anoxia. It is currently not known how the response of turtle brain to NO is down-regulated during the transition to anoxia and this response could be due to changes in the production of NO, either via direct down-regulation of NOS or as a result of decreased NMDAR-mediated Ca^{2+} influx during anoxia in the turtle cortex. The aims of this study were to (1) determine the changes in NO production from turtle cortex during normoxia and during a normoxic to anoxic transition followed by reoxygenation recovery. Furthermore, (2) we examined the ability of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME: 0.5–5.0 mM) to decrease NO production during normoxia [31]. Finally, (3) we examined the role of NMDAR activity in NO production by blocking NMDARs with DL-2-amino-5-phosphonopentanoic acid (APV: 60 μM) during normoxia and during normoxic to anoxic transitions.

2. Materials and methods

This study conforms to the University of Toronto Animal Care committee Guide to the Care and Use of Experimental Animals, Volume 2 as determined by the Canadian Council on Animal Care regarding relevant guidelines for the care of experimental animals. Adult western painted turtles (*C. picta bellii*) were acquired from Niles Biological Inc. (Sacramento, CA, USA). Turtles were housed together in an aquatic facility equipped with heat lamps, basking platforms and a flow-through dechlorinated pond maintained at 17 °C. Basic methods of turtle cortical sheet isolation, aCSF preparation and anoxic experimental setup are published elsewhere [32]. Cellular NO was measured using the fluorophore 4-amino-5-methylamino-2′,7′-difluorofluorescein (DAF-FM: Molecular Probes, Eugene, OR, USA). Briefly, freshly dissected cortical sheets were incubated in the dark at 4 °C in 5 μM DAF-FM for two consecutive 1-h loading periods. Sheets were then placed in a flow-through recording chamber and rinsed in dye-free aCSF at 22 °C for at least 20 min before the start of an experiment. The recording chamber was equipped with a custom cuff placed around the objective to provide constant N_2 gas distribution across the surface of the bath during anoxic exposure. DAF-FM was excited for 0.1 s (488 nm) using a DeltaRam X high-speed random access monochromator and a LPS220B light source (PTI, NJ, USA). Fluorescent emissions above 510 nm were isolated using an Olympus DM510 dichroic mirror and fluorescent measurements were acquired (515–530 nm) at 10-s intervals to limit photo bleaching using an Olympus BX51W1 microscope and a QImaging Rolera MGi EMCCD camera (Roper Scientific Inc., IL, USA). Baseline fluorescence was recorded for 10–20 min and then the tissue was exposed to treatment aCSF for up to 80 min. The tissue was then reperfused with control aCSF. For each experiment, 25 neurons were chosen at random and the average change in fluorescence in these neurons 3 min after treatment onset was used for statistical comparison. Data were analyzed using EasyRatioPro software (PTI). Results were analyzed using a repeated measures two-way ANOVA with a Holm–Sidak multiple comparisons test. Significance was determined at $P < 0.05$ unless otherwise indicated, and all data are expressed as mean ± S.E.M. (standard error of mean).

3. Results and discussion

For up to 2 h of normoxic recording DAF-FM fluorescence decreased steadily at a low rate due to photo bleaching or dye leakage but the rate of DAF-FM fluorescence decrease was unchanging ($n = 4$, Fig. 1A and B). The specificity of the dye for NO was confirmed by perfusion of either sodium nitroprusside (SNP: 0.1–10.0 mM) or L-NAME onto normoxic cortical sheets. Perfusion of the general NOS inhibitor L-NAME resulted in slow and small reduction in NO production of 5.8 ± 1.1% ($n = 5$, Fig. 1A and C), consistent with a low rate of endogenous NOS activity in the turtle cortex. The NO-donor SNP induced rapid and repeatable increases in DAF-FM fluorescence of 22.6 ± 3.7% ($n = 4$, Fig. 1A and D). These responses to SNP or L-NAME are consistent with similar control experiments in mammalian brain [33].

In cortical sheets exposed to anoxic aCSF, NO production rapidly decreased 27.1 ± 3.1% with decreasing oxygen and recovered to baseline levels immediately upon reoxygenation ($n = 5$, Fig. 1A and E). These changes were rapid and repeatable in contrast to NO changes in ischemic mammalian brain where low oxygen induces rapid increases in NO, likely due to increases in glutamatergic-connected nNOS activity associated with excitotoxic Ca^{2+} entry and eventual apoptotic cell death [34]. Since inhibition of inducible and neuronal NOS is generally neuroprotective in mammals, we hypothesized that NOS activity is naturally depressed in the anoxia-tolerant turtle brain. To examine this hypothesis, we pre-incubated cortical sheets with anoxic aCSF for 30 min and then treated them

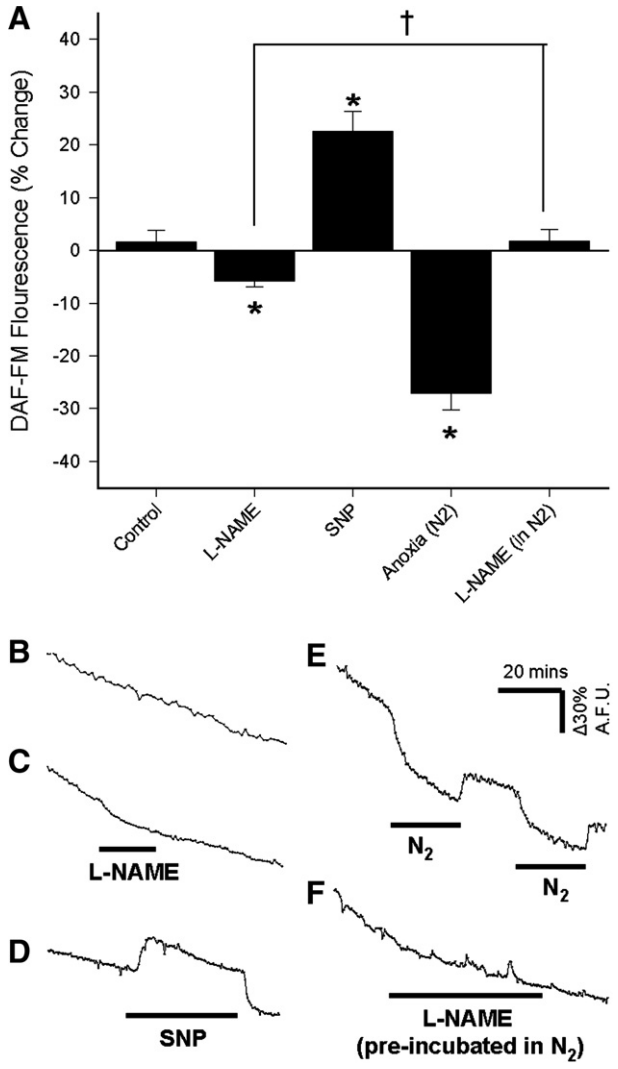

Fig. 1. NO production and NOS activity decrease with anoxia in cortical sheets. (A) Normalized changes in normoxic DAF-FM fluorescence from cortical sheets undergoing a normoxic to anoxic transition or treatment with the general NOS inhibitor L-NAME (0.5–5 mM) or the NO donor SNP (0.1–10 mM). Data are expressed as means ± S.E.M. Asterisks indicate significant changes from control baseline following treatment onset. Dagger indicates significant difference ($P < 0.05$). (B–F) Raw data traces of DAF-FM fluorescence changes from cortical sheets undergoing treatments as specified by the solid bars under the individual traces. Each trace represents the average of >25 neurons from a single cortical sheet.

simultaneously with L-NAME and anoxia. In these experiments, no significant change in NO fluorescence was observed after L-NAME perfusion, suggesting NOS activity is depressed in the anoxic turtle cortex ($n = 4$, Fig. 1A and F).

The decrease in NO formation during the transition to anoxia may be related to glutamatergic receptor activity in the anoxic turtle cortex. In mammals, the toxic effects of NO are mediated by NMDARs, which induce NO formation in a manner dependent on NMDAR-mediated Ca^{2+}-influx. Furthermore, the surge of NO observed in ischemic murine brain is abolished by glutamatergic receptor antagonists [35,36]. The turtle brain expresses a number of adaptations that limit excitotoxic glutamatergic Ca^{2+} entry during anoxia, including reduced glutamate release, depressed NMDAR and α-amino-3-hydroxyl-5-methyl-4-acid receptor currents and removal of NMDARs from the neuronal membrane with prolonged anoxia [37–39]. As a result of these mechanisms, toxic accumulations of cytosolic Ca^{2+} and ECD are avoided during prolonged anoxia. Therefore, regulation of NMDAR-mediated Ca^{2+} entry may underlie the reduced NO production observed during the transition to anoxia in turtle cortex.

To examine the role of NMDAR activity in the regulation of NO production during anoxic transitions, we treated cortical sheets with the NMDAR antagonist APV during normoxia and then subsequently recorded NO fluorescence during a normoxic to anoxic transition in the presence of APV. In normoxic sheets, APV resulted in a 41.2 ± 4.9% depression of NO production ($n = 4$, Fig. 2A and B). Subsequent anoxic exposure of the same tissue resulted in a further small decrease in NO production of 7.1 ± 2.3% that was ~75% smaller than the regular anoxia alone decrease in NO production ($P < 0.01$, $n = 4$, Fig. 2A and B). These data suggest that the decrease in NO production during the transition to anoxia is due largely to depressed NMDAR activity in the cortex. The

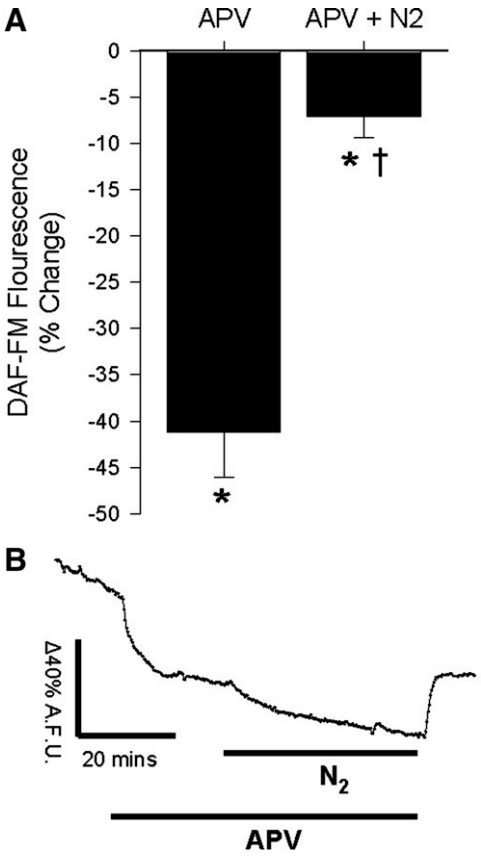

Fig. 2. Anoxic changes in NO production and NOS activity in cortical sheets. (A) Normalized changes in DAF-FM fluorescence from cortical sheets undergoing NMDAR blockade with APV (60 μM) or a normoxic to anoxic transitions in the presence of APV. Data are expressed as means ± S.E.M. Asterisks indicate significant changes from control baseline following treatment onset. Dagger indicates data significantly different from anoxic control (see Fig. 1, $P < 0.05$). (B) Raw data trace of DAF-FM fluorescence changes from a cortical sheet undergoing treatments as specified by the solid bars under the individual traces. Each trace represents the average of >25 neurons from a single cortical sheet.

observation that the magnitude of the decrease of NO production with APV was greater than with anoxia alone is likely due to complete NMDAR inhibition by APV compared to the ~50% endogenous reduction in NMDAR activity in the anoxic cortex [39].

The primary neuroprotective mechanism mediated by depressed glutamatergic activity is the prevention of toxic Ca^{2+} accumulation [40], but it is interesting to note that in the anoxic turtle cortex, depressed NMDAR activity may preclude neuronal damage via a second mechanism: by limiting NO accumulation and associated DNA fragmentation. In mammals, NO contributes to detrimental feed-forward mechanisms that accelerate ECD: NO production is promoted by NMDAR-mediated Ca^{2+} entry and then acts as a retrograde signal to enhance glutamate release and increase the over-activation of NMDARs [21,22]. Therefore, the evolution of NO-independent mechanisms to increase CBF in the turtle cortex allows neuroprotective increases in CBF without deleterious elevations in NO and NMDAR activity that occur concomitantly in mammals.

In summary, some facultative anaerobes utilize increased CBF to enhance neuronal survival during anoxic exposure. Enhanced CBF is also neuroprotective in mammals where it is NO-dependent, however increased NO production leads to elevated glutamate release and DNA fragmentation. Here we measured for the first time in a facultative anaerobe changes in NO production in response to anoxic perfusion and reoxygenation and NOS or NMDAR inhibition. The evolution of NO-independent mechanisms of increased CBF allows these organisms to suppress the production of NO and thus reduce free radical damage and NO-dependent glutamate release without compromising increased blood flow during the transition to and from anoxia. Furthermore, an NO-independent mechanism would allow for regulation of CBF during prolonged anoxia when oxygen is not available and NO cannot be formed.

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References

[1] Van Mil, A.H., Spilt, A., Van Buchem, M.A., Bollen, E.L., Teppema, L., Westendorp, R.G. and Blauw, G.J. (2002) Nitric oxide mediates hypoxia-induced cerebral vasodilation in humans. J. Appl. Physiol. 92, 962–966.

[2] Ioroi, T., Yonetani, M. and Nakamura, H. (1998) Effects of hypoxia and reoxygenation on nitric oxide production and cerebral blood flow in developing rat striatum. Pediatr. Res. 43, 733–737.

[3] Namiranian, K., Koehler, R.C., Sapirstein, A. and Dore, S. (2005) Stroke outcomes in mice lacking the genes for neuronal heme oxygenase-2 and nitric oxide synthase. Curr. Neurovasc. Res. 2, 23–27.

[4] Kader, A., Frazzini, V.I., Solomon, R.A. and Trifiletti, R.R. (1993) Nitric oxide production during focal cerebral ischemia in rats. Stroke 24, 1709–1716.

[5] Iadecola, C., Xu, X., Zhang, F., el-Fakahany, E.E. and Ross, M.E. (1995) Marked induction of calcium-independent nitric oxide synthase activity after focal cerebral ischemia. J. Cereb. Blood Flow Metab. 15, 52–59.

[6] Iadecola, C. (1997) Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci. 20, 132–139.

[7] Fang, S.Y., Tseng, C.C., Yang, Y.L., Lee, E.J., Chen, H.Y., Bhardwaj, A. and Chen, T.Y. (2006) Nitric oxide scavenger carboxy-PTIO reduces infarct volume following permanent focal ischemia. Acta Anaesthesiol. Taiwan 44, 141–146.

[8] van den Tweel, E.R., van Bel, F., Kavelaars, A., Peeters-Scholte, C.M., Haumann, J., Nijboer, C.H., Heijnen, C.J. and Groenendaal, F. (2005) Long-term neuroprotection with 2-iminobiotin, an inhibitor of neuronal and inducible nitric oxide synthase, after cerebral hypoxia-ischemia in neonatal rats. J. Cereb. Blood Flow Metab. 25, 67–74.

[9] Haga, K.K., Gregory, L.J., Hicks, C.A., Ward, M.A., Beech, J.S., Bath, P.W., Williams, S.C. and O'Neill, M.J. (2003) The neuronal nitric oxide synthase inhibitor, TRIM, as a neuroprotective agent: effects in models of cerebral ischaemia using histological and magnetic resonance imaging techniques. Brain Res. 993, 42–53.

[10] Zubrow, A.B., Delivoria-Papadopoulos, M., Ashraf, Q.M., Ballesteros, J.R., Fritz, K.I. and Mishra, O.P. (2002) Nitric oxide-mediated expression of Bax protein and DNA fragmentation during hypoxia in neuronal nuclei from newborn piglets. Brain Res. 954, 60–67.

[11] Li, D.Q., Duan, Y.L., Bao, Y.M., Liu, C.P., Liu, Y. and An, L.J. (2004) Neuroprotection of catalpol in transient global ischemia in gerbils. Neurosci. Res. 50, 169–177.

[12] Kakizawa, H. et al. (2007) Neuroprotective effect of nipradilol, an NO donor, on hypoxic–ischemic brain injury of neonatal rats. Early Hum. Dev. 83, 535–540.

[13] Banerjee, R., Saravanan, K.S., Thomas, B., Sindhu, K.M. and Mohanakumar, K.P. (2008) Evidence for hydroxyl radical scavenging action of nitric oxide donors in the protection against 1-methyl-4-phenylpyridinium-induced neurotoxicity in rats. Neurochem. Res. 33, 985–995.

[14] Martinez-Murillo, R., Fernandez, A.P., Serrano, J., Rodrigo, J., Salas, E., Mourelle, M. and Martinez, A. (2007) The nitric oxide donor LA 419 decreases brain damage in a focal ischemia model. Neurosci. Lett. 415, 149–153.

[15] Serrano, J., Fernandez, A.P., Martinez-Murillo, R., Alonso, D., Rodrigo, J., Salas, E., Mourelle, M. and Martinez, A. (2007) The nitric oxide donor LA 419 decreases ischemic brain damage. Int. J. Mol. Med. 19, 229–236.

[16] Wink, D.A., Hanbauer, I., Laval, F., Cook, J.A., Krishna, M.C. and Mitchell, J.B. (1994) Nitric oxide protects against the cytotoxic effects of reactive oxygen species. Ann. N.Y. Acad. Sci. 738, 265–278.

[17] Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P. and Lipton, S.A. (1995) Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc. Natl. Acad. Sci. USA 92, 7162–7166.

[18] Choi, D.W. (1994) Calcium and excitotoxic neuronal injury. Ann. N.Y. Acad. Sci. 747, 162–171.

[19] Scorziello, A. et al. (2004) Neuronal NOS activation during oxygen and glucose deprivation triggers cerebellar granule cell death in the later reoxygenation phase. J. Neurosci. Res. 76, 812–821.

[20] Ishii, H., Shibuya, K., Ohta, Y., Mukai, H., Uchino, S., Takata, N., Rose, J.A. and Kawato, S. (2006) Enhancement of nitric oxide production by association of nitric oxide synthase with *N*-methyl-D-aspartate receptors via postsynaptic density 95 in genetically engineered Chinese hamster ovary cells: real-time fluorescence imaging using nitric oxide sensitive dye. J. Neurochem. 96, 1531–1539.

[21] Katchman, A.N. and Hershkowitz, N. (1997) Nitric oxide modulates synaptic glutamate release during anoxia. Neurosci. Lett. 228, 50–54.

[22] Montague, P.R., Gancayco, C.D., Winn, M.J., Marchase, R.B. and Friedlander, M.J. (1994) Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. Science 263, 973–977.

[23] Bickler, P.E. and Buck, L.T. (2007) Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. Annu. Rev. Physiol. 69, 145–170.

[24] Chih, C.P., Feng, Z.C., Rosenthal, M., Lutz, P.L. and Sick, T.J. (1989) Energy metabolism, ion homeostasis, and evoked potentials in anoxic turtle brain. Am. J. Physiol. 257, R854–R860.

[25] Johansson, D. and Nilsson, G. (1995) Roles of energy status, KATP channels and channel arrest in fish brain K+ gradient dissipation during anoxia. J. Exp. Biol. 198, 2575–2580.
[26] Johansson, D., Nilsson, G. and Tornblom, E. (1995) Effects of anoxia on energy metabolism in crucian carp brain slices studied with microcalorimetry. J. Exp. Biol. 198, 853–859.
[27] Davies, D.G. (1989) Distribution of systemic blood flow during anoxia in the turtle, *Chrysemys scripta*. Respir. Physiol. 78, 383–389.
[28] Hylland, P., Nilsson, G.E. and Lutz, P.L. (1994) Time course of anoxia-induced increase in cerebral blood flow rate in turtles: evidence for a role of adenosine. J. Cereb. Blood Flow Metab. 14, 877–881.
[29] Bickler, P.E. (1992) Effects of temperature and anoxia on regional cerebral blood flow in turtles. Am. J. Physiol. 262, R538–R541.
[30] Fernandez, N., Garcia, J.L., Garcia-Villalon, A.L., Monge, L., Gomez, B. and Dieguez, G. (1993) Cerebral blood flow and cerebrovascular reactivity after inhibition of nitric oxide synthesis in conscious goats. Br. J. Pharmacol. 110, 428–434.
[31] Rees, D.D., Palmer, R.M., Schulz, R., Hodson, H.F. and Moncada, S. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746–752.
[32] Pamenter, M.E., Shin, D.S., Cooray, M. and Buck, L.T. (2008) Mitochondrial ATP-sensitive K+ channels regulate NMDAR activity in the cortex of the anoxic western painted turtle. J. Physiol. 586, 1043–1058.
[33] Kojima, H., Hirotani, M., Nakatsubo, N., Kikuchi, K., Urano, Y., Higuchi, T., Hirata, Y. and Nagano, T. (2001) Bioimaging of nitric oxide with fluorescent indicators based on the rhodamine chromophore. Anal. Chem. 73, 1967–1973.
[34] Peeters-Scholte, C. et al. (2002) Neuroprotection by selective nitric oxide synthase inhibition at 24 hours after perinatal hypoxia-ischemia. Stroke 33, 2304–2310.
[35] Garthwaite, J., Charles, S.L. and Chess-Williams, R. (1988) Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336, 385–388.
[36] Lin, S.Z., Chiou, A.L. and Wang, Y. (1996) Ketamine antagonizes nitric oxide release from cerebral cortex after middle cerebral artery ligation in rats. Stroke 27, 747–752.
[37] Pamenter, M.E., Shin, D.S. and Buck, L.T. (2008) AMPA receptors undergo channel arrest in the anoxic turtle cortex. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R606–R613.
[38] Milton, S.L., Thompson, J.W. and Lutz, P.L. (2002) Mechanisms for maintaining extracellular glutamate levels in the anoxic turtle striatum. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282, R1317–R1323.
[39] Bickler, P.E., Donohoe, P.H. and Buck, L.T. (2000) Hypoxia-induced silencing of NMDA receptors in turtle neurons. J. Neurosci. 20, 3522–3528.
[40] Bickler, P.E. and Buck, L.T. (1998) Adaptations of vertebrate neurons to hypoxia and anoxia: maintaining critical Ca2+ concentrations. J. Exp. Biol. 201, 1141–1152.