Regulation of AMPA Receptor Currents by Mitochondrial ATP-Sensitive K⁺ Channels in Anoxic Turtle Neurons

George Zivkovic¹ and Leslie Thomas Buck¹,²
¹Department of Cell and Systems Biology and ²Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada

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INTRODUCTION

Mammals are extremely anoxia intolerant. Cerebral ischemia causes mammalian neurons to become hypexcitable, leading to a rapid depletion of adenosine 5'-triphosphate (ATP) stores, failure of ATP-driven ion transporters (e.g., Na⁺/K⁺-ATPase), and depolarization of the plasma membrane potential through the loss of ion gradients. Consequently, the release of excitatory amino acids (glutamate, aspartate) and activation of postsynaptic receptors such as the N-methyl-D-aspartate receptor (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) currents are an important part of the turtle’s natural defense. Here we investigate the role of mitochondrial ATP-sensitive K⁺ (mKATP) channels in the regulation of AMPAR. Whole cell AMPAR currents were stable over 90 min of normoxic recording; however, anoxia resulted in a 52% decrease in AMPAR currents. Pharmacological activation of mKATP channels with diazoxide or levcromakalim resulted in a 46% decrease in normoxic AMPAR currents and the decrease was abolished with application of the antagonists 5-hydroxydecanoic acid and glibenclamide, whereas mKATP antagonists blocked the anoxia-mediated decrease. Mitochondrial K⁺ channel modulators responded similarly. The Ca²⁺-uniporter antagonist ruthenium red reduced AMPAR currents by 38% and was blocked with the agonist spermine. The calcium chelator BAPTA in the recording electrode during anoxia or diazoxide perfusion also abolished the reduction in AMPAR currents. We conclude that the mKATP channel is involved in the anoxia-mediated down-regulation of AMPAR activity during anoxia and that it is a common mechanism to reduce glutamatergic excitability.

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Address for reprint requests and other correspondence: L. T. Buck, University of Toronto, Department of Cell and Systems Biology, 25 Harbord Street, RW 329, Toronto, ON, Canada M5S 3G5 (E-mail: les.buck@utoronto.ca).
channel antagonists 5-hydroxydecanoic acid (SHD) and glibenclamide. Alternatively, the m\textsubscript{KATP} agonists diazoxide and levcromakalim could replicate the decrease in NMDAR currents during normoxia. Taken together these results indicate that m\textsubscript{KATP} channel activation is a part of a natural anoxia-tolerance mechanism that ultimately results in decreased whole cell NMDAR currents and neuroprotection (Pamenter et al. 2008b).

NMDARs are generally not active at neuronal resting membrane potential due to the Mg\textsuperscript{2+} ion that rests in the pore, blocking influx of Na\textsuperscript{+} or Ca\textsuperscript{2+} through the channel (Nowak et al. 1984). However, glutamatergic activation of AMPARs produces EPSCs that depolarize the neuronal cell membrane and release the Mg\textsuperscript{2+} block of the NMDAR, permitting Ca\textsuperscript{2+} entry into the neuron (Conti and Weinberg 1999). Evidence of the importance of AMPARs to ECD comes from experiments showing that mice overexpressing AMPARs are more susceptible than wild-type mice to ECD (Le et al. 1997). Therefore a mechanism to reduce AMPAR currents would be a logical part of a mechanism to suppress NMDARs (Siesjö et al. 1991). Indeed, we have demonstrated that anoxic perfusion AMPARs exhibit this decrease (Pamenter et al. 2008a); however, we do not know whether an m\textsubscript{KATP}-based mechanism underlies this response. Since AMPARs are also activated by glutamate and play an important role in regulating NMDAR activity, a common regulatory mechanism would be practical. However, further involvement of AMPARs in neuroprotection and their link to the m\textsubscript{KATP} channel have not yet been investigated.

The aims of this study were to determine: 1) whether pharmacological manipulation of m\textsubscript{KATP} channel activity regulates whole cell evoked AMPAR currents during normoxia and anoxia in turtle cortex and 2) whether the response is Ca\textsuperscript{2+} dependent by blocking changes in intracellular Ca\textsuperscript{2+} with a chelator.

**METHODS**

**Animal ethics approval**

This study was approved by the University of Toronto Animal Care Committee and conforms to the relevant guidelines for the care and handling of experimental animals as outlined in the Guide to the Care and Use of Experimental Animals as determined by the Canadian Council on Animal Care. Adult turtles were obtained from Niles Biological (Sacramento, CA).

**Dissection and whole cell patch-clamp recording protocol**

All experiments were performed at a room temperature of 22°C. The basic protocols for cortical sheet dissection and whole cell patch-clamp recordings under normoxic and anoxic conditions are described elsewhere (Shin and Buck 2003). Briefly, turtles were decapitated and whole brains were rapidly excised from the cranium within 30 s of decapitation. Six cortical sheets were isolated from whole brains and bathed in artificial cerebrospinal fluid (aCSF) containing (in mM): 110 NaCl, 2.6 KCl, 1.2 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 2 NaH\textsubscript{2}PO\textsubscript{4}, 2H\textsubscript{2}O, 26.5 NaHCO\textsubscript{3}, 10 glucose, and 5 imidazole (pH 7.4; osmolality 285–290 mOsm).

Cortical sheets were placed in an RC-26 chamber with a P1 platform (Warner Instruments, Hamden, CT). The chamber was gravity perfused with aCSF at a rate of 2–3 mL min\textsuperscript{-1}. Normoxic aCSF was gassed with 95% O\textsubscript{2}–5% CO\textsubscript{2}, whereas a gas mixture of 95% N\textsubscript{2}–5% CO\textsubscript{2} was used to achieve anoxic conditions. Whole cell evoked AMPAR currents during normoxia and anoxia were recorded for 90 min. Prior to each recording, cortical sheets were added to prevent NMDAR-mediated currents that may result from AMPAR activation. Prior to each recording, cortical sheets were perfused with TTX for 5 min to prevent spontaneous and evoked action potentials. AMPAR current–voltage relationships in the cortex of the painted turtle were previously determined, with a reversal potential of about 4 mV (Pamenter et al. 2008a). For all experiments, the attached cells were clamped at a holding potential of −80 mV and AMPA was applied onto cortical sheets using the fast-perfusion system until a current was measured (2–10 s, depending on the proximity of the drug delivery system to the neuron being recorded from). The same AMPA application time was then used for every recording performed on the same neuron within a single experiment.

**Evoked AMPA current recordings**

Normoxic experiments consisted of an O\textsubscript{2}/CO\textsubscript{2} aCSF perfusion as explained earlier. A fast-step perfusion system (VC-6 perfusion valve controller and SF-77B fast-step perfusion system; Warner Instruments) was used to deliver 1 μM tetrodotoxin (TTX) and 50 μM AMPA. The concentration of AMPA was selected based on previous experiments done in the turtle cortex (Pamenter et al. 2008a). Higher concentrations (AMPA: 100–200 μM) resulted in large currents that became deleterious to the cell, as assessed by membrane potential rundown and cell death. An AMPA concentration of 50 μM produced repeatable currents that did not affect the integrity of the neurons throughout the course of the 90-min experiment. In bulk perfusate, 25 μM (2R)-aminooxy-5-phosphonovaleric acid (APV) was added to prevent NMDAR-mediated currents that may result from AMPAR activation. Prior to each recording, cortical sheets were perfused with TTX for 5 min to prevent spontaneous and evoked action potentials. AMPAR current–voltage relationships in the cortex of the painted turtle were previously determined, with a reversal potential of about 4 mV (Pamenter et al. 2008a). For all experiments, the attached cells were clamped at a holding potential of −80 mV and AMPA was applied onto cortical sheets using the fast-perfusion system until a current was measured (2–10 s, depending on the proximity of the drug delivery system to the neuron being recorded from). The same AMPA application time was then used for every recording performed on the same neuron within a single experiment.

Control evoked AMPAR currents were recorded at the start of the experiment (t = 0 min) and at t = 10 min. The initial current recording at t = 0 min was set to 100% and all subsequent recordings from that experiment were normalized to that control value, whereas the second control value at 10 min was used for statistical analysis. Following the two recordings, the cells were perfused with anoxic aCSF containing specific pharmacologic compounds for 40 min and evoked AMPAR currents recorded at 20-min intervals. Tissue was then reperfused with control normoxic aCSF for 40 min and currents recorded at 20-min intervals following the change in aCSF perfusion.

**Pharmacology**

All pharmacological compounds used in the whole cell AMPAR experiments were dissolved in bulk perfusate and applied onto the cortical neurons as specified in RESULTS. AMPARs were stimulated...
with AMPA (50 μM) and blocked with 6-cyano-7-nitroquinolinicline-2,3-dione (CNQX, 30 μM). Mitochondrial K$_{ATP}$ channels were activated using levcromakalim (100 μM) or the channel-specific agonist diazoxide (100 μM) and blocked with glibenclamide (80 μM) or the channel-specific antagonist 5HD (100 μM). We have previously measured that at the concentrations used, diazoxide does not affect state 3 respiration in painted turtle mitochondria (Pammenter et al. 2008b). Diazoxide is nearly 2,000-fold more selective for the mitochondrial K$_{ATP}$ channel than for the plasmalemal K$_{ATP}$ channel and is a not significant activator of the pK$_{ATP}$ channel at the concentrations used (Garlid et al. 1996, 1997). Similarly, 5HD does not have a significant effect on pK$_{ATP}$ channels but is an effective blocker of mK$_{ATP}$ channels (Garlid et al. 1997; McCulloch et al. 1991). The mitochondrial Ca$^{2+}$ uniporter was initially activated by spermine (500 μM) and blocked by ruthenium red (40 nM) (Allshire et al. 1985). For experiments involving calcium chelation, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA, 5 mM) was added to the recording electrode solution (Shin et al. 2005). Finally, mitochondrial Ca$^{2+}$-sensitive K$^+$ channels (mKCa) were activated by NS1619 (50 μM) and blocked by paxilline (1 μM) (Sato et al. 2005).

Chemicals

All chemicals were obtained from Sigma Chemical (Oakville, ON, Canada). Diazoxide, levcromakalim, NS1619, glibenclamide, and paxilline were initially dissolved in dimethylsulfoxide (DMSO) before being dissolved in the bulk aCSF, not exceeding 1% vol/vol. DMSO application alone did not affect AMPAR-mediated currents (data not shown).

Statistical analysis

AMPAR-mediated whole cell current data were analyzed following root arcsine transformation using a two-way ANOVA with a Student–Newman–Keuls (all pairwise) post hoc test to compare within and against treatment and control values. Significance was determined at $P < 0.05$ and all data are expressed as means ± SE.

RESULTS

Pharmacological manipulation of the mK$_{Ca}$ channel modifies AMPAR activity

NMDAR and AMPAR currents were abolished following 30 min of perfusion with the antagonists APV and CNQX, respectively ($n = 4$ for each; Fig. 2, A and B). Under normoxia, evoked AMPAR currents did not change significantly over 90 min of recording, but decreased by 46 ± 6.4% and 52 ± 4.9% after 20 and 40 min of anoxic perfusion, respectively ($n = 7$ for each; Fig. 2, A, B, and C). Activation of the mK$_{ATP}$ channel during normoxia using the specific agonist diazoxide decreased the AMPAR current by 41 ± 7.2% and 46 ± 4.2% after 20 and 40 min of perfusion, respectively ($n = 8$, Fig. 2, A and D). Similarly, activation of the K$_{ATP}$ channel using the general agonist levcromakalim resulted in 47 ± 6.7% and 41 ± 4.8% decreases in AMPAR current amplitude following 20 and 40 min of normoxic perfusion, respectively ($n = 7$, Fig. 2, A and E). Diazoxide- and levcromakalim-mediated decreases in AMPAR activity were not significantly different from the anoxia-mediated decrease ($P > 0.001$). The reduction in AMPAR current amplitude observed after either diazoxide and levcromakalim perfusion was abolished by the perfusion of the general mK$_{ATP}$ channel antagonist glibenclamide or specific antagonist 5HD ($n = 7$ for each; Fig. 2, G, H, and J). The mK$_{ATP}$ antagonized currents were not significantly different from normoxic control recordings. During anoxia, perfusion with either 5HD or glibenclamide blocked the anoxia-mediated decrease in AMPAR currents and these were not significantly different from normoxic control or normoxic currents with either 5HD or glibenclamide ($n = 7$, Fig. 2, A, F, and I).

Pharmacological manipulation of the mK$_{Ca}$ channel also modifies AMPAR activity

Opening of the mK$_{ATP}$ is proposed to increase mitochondrial K$^+$ uptake; however, the channel has not been cloned and positively identified in the mitochondrial membrane. Therefore we used pharmacological modulators of the mitochondrial calcium-sensitive K$^+$ (mKCa) channel. Although the mKCa channel is not thought to be involved in the anoxic response, this channel has been cloned and sequenced and has a known pharmacology that can serve as a control to confirm the role of mitochondrial K$^+$ influx (Sato et al. 2005). Administration of the mKCa channel agonist NS1619 decreased whole cell AMPAR currents by 44.2 ± 9.1% and 56 ± 3.4% after 20 and 40 min of perfusion, respectively ($n = 8$, Fig. 3, A and B). This decrease was abolished when NS1619 was applied with the mKCa channel antagonist paxilline because AMPAR currents did not significantly change from normoxic control recordings. During anoxia, perfusion with either 5HD or glibenclamide blocked the anoxia-mediated decrease in AMPAR currents and these were not significantly different from normoxic control or normoxic currents with either 5HD or glibenclamide ($n = 7$, Fig. 3, A, F, and I).

FIG. 1. 2-Amino-5-phosphonovaleric acid (APV) and 6-cyano-7-nitroquinolinicline-2,3-dione (CNQX), respectively, abolish N-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptor (AMPAR) currents in the painted turtle. A: representative NMDAR current traces at control ($t = 0$ min) and following perfusion of selective NMDA receptor antagonist APV for 15 and 30 min. B: representative AMPAR current traces at control ($t = 0$ min) and following perfusion of selective AMPAR receptor antagonist CNQX after 30 and 60 min.

J Neurophysiol • VOL 104 • OCTOBER 2010 • www.jn.org
FIG. 2. Mitochondrial ATP-sensitive K⁺ (mKATP) channel agonists and antagonists regulate whole cell AMPAR currents. A: normalized whole cell AMPAR currents at t = 30 min (black bars) and t = 50 min (gray bars) of treatment. Continuous line represents normoxic controls, whereas dashed line represents anoxic controls. Symbols indicate values significantly different from normoxic control (*), anoxic control (†), or drug treatment control (‡). All data are expressed as means ± SE (n = 7). B–I: paired sample trace AMPAR currents at t = 10 min (control) and at t = 50 min following 40 min of anoxic or pharmacological drug perfusion. Anox, anoxia; ATP, adenosine 5'-triphosphate; 5HD, 5-hydroxydecanoic acid; DZX, diazoxide; Lev, levocromakalim; Gb, glibenclamide.
3, A and D). Similarly, the mKATP-mediated decrease in AMPAR currents seen following diazoxide perfusion was not blocked after administration of the mKCa channel antagonist paxilline, where AMPAR currents decreased by 46.8 ± 9.9% and 39.1 ± 7.2% after 20 and 40 min of perfusion, respectively (n = 8, Fig. 3, A and E).

Opening of the Ca²⁺ uniporter abolishes the anoxia-mediated decrease in whole cell AMPAR currents

Mild uncoupling of the mitochondria through the mKATP channel is believed to dissipate the proton gradient and reduce Ca²⁺ uptake through the uniporter, resulting in an increase in [Ca²⁺]c. To confirm that a moderate increase in [Ca²⁺]c mediates a decrease in AMPAR currents during anoxia, we measured whole cell AMPAR currents in the presence of pharmacological manipulators of the mitochondrial Ca²⁺ uniporter. Following perfusion of the Ca²⁺ uniporter agonist ruthenium red (RR), whole cell AMPAR currents decreased by 41.4 ± 7.9% and 37.9 ± 4.8% after 20 and 40 min, respectively (n = 7, Fig. 4, A and B). The decrease in AMPAR currents during anoxia was abolished using the Ca²⁺ uniporter antagonist spermine, where after 40 min of perfusion, whole cell AMPAR currents did not change significantly (n = 7, Fig. 4, A and D). Furthermore, application of spermine for 40 min during normoxia did not alter AMPAR currents (n = 7, Fig. 4, A and C). The diazoxide-mediated decrease (Fig. 2, A and C) in whole cell AMPAR currents was abolished when the drug...
FIG. 4. Opening of the Ca\textsuperscript{2+} uniporter abolishes the anoxia-mediated decreases in whole cell AMPAR currents. A: normalized whole cell AMPAR currents at \( t = 30 \text{ min} \) (black bars) and \( t = 50 \text{ min} \) (gray bars) of treatment. Continuous line represents normoxic controls, whereas dashed line represents anoxic controls. Symbols indicate values significantly different from normoxic control (*), anoxic control (†), or drug treatment control (‡) (refer to Fig. 2). All data are expressed as means ± SE \((n = 7)\).

B–F: paired-sample AMPAR current traces at \( t = 10 \text{ min} \) (control) and at \( t = 50 \text{ min} \) following 40 min of anoxic or drug perfusion.

Anox, anoxia; 5HD, 5-hydroxydecanoic acid; DZX, diazoxide.

G. ZIVKOVIC AND L. T. BUCK

J Neurophysiol • VOL 104 • OCTOBER 2010 • www.jn.org
was applied along with the unipporter agonist spermine (n = 7, Fig. 4, A and D). Finally, spermine application along with the mKATP channel-specific antagonist 5HD had no effect on normoxic whole cell AMPAR currents throughout the entire perfusion period (n = 7, Fig. 4, A and E).

**Calcium chelation abolishes the anoxia-mediated decrease in whole cell AMPAR currents**

Increases in [Ca$^{2+}$]$_i$ during anoxia are associated with the decrease in NMDAR activity in the turtle; therefore we investigated whether it has a similar effect on AMPAR currents during anoxia. Normoxic AMPAR currents were not affected by the addition of the calcium chelator BAPTA to the recording electrode (5 mM; n = 7, Fig. 5, A and B); however, inclusion of BAPTA blocked the anoxia-mediated decrease in AMPAR currents (n = 7, Fig. 5, A and C). Furthermore, BAPTA blocked the diazoxide and RR-mediated decrease in AMPAR currents and these data were not significantly different from those observed with BAPTA alone (n = 7, Fig. 5, A, D, and E).

**DISCUSSION**

In this study, we confirm that AMPARs undergo channel arrest during anoxia in a way that is similar to NMDARs and demonstrate that this down-regulation is modulated by the opening of the mKATP channels (Fig. 2). The NMDAR-specific antagonist APV permitted AMPAR currents to be isolated and recorded, thereby determining the effects of the mKATP channel modulators solely on whole cell AMPAR currents (Fig. 1). Similar to anoxic perfusion alone, application of mKATP channel-specific agonists diazoxide or levromakalim resulted in significantly reduced AMPAR current amplitudes (Fig. 2). When either of these agents was used together with the mKATP channel antagonists 5HD or glibenclamide, the reduction was blocked (Fig. 2). Therefore we propose that during anoxia opening of mKATP channels increases K$^+$ conductance, uncouples and depolarizes mitochondria, leading to an increase in [Ca$^{2+}$]$_i$ that subsequently leads to a reduction in AMPAR and NMDAR currents.

There is some concern in the literature over the existence of the mKATP channel since it has not yet been sequenced and its molecular identity remains unclear. However, previous patch-clamp studies of rat liver mitochondria, measurement of mitochondrial matrix volume changes, and flavoprotein oxidation studies have indicated the existence of an inward rectifying K$^+$ channel that is inhibited by high levels of ATP (Ardehali and O’Rourke 2005). Considering this and the consistency of our pharmacological mKATP experiments, we conclude that the mKATP channel is a key mediator in the down-regulation of AMPAR activity in the anoxic turtle cortex. Therefore anoxia brings about changes in the cell that activate secondary messenger pathways that lead to the opening of the mKATP channel, facilitating K$^+$ uptake into the mitochondria and initiating a cascade of events, ultimately leading to a decrease in AMPAR conductance.

Since K$^+$ conductance through the mKATP was not directly measured, we performed several normoxic experiments whereby we increased mitochondrial K$^+$ conductance through activation of a known mitochondrial channel—the mK$_{Ca}$ channel—to investigate whether it would result in reduced AMPAR currents. This channel has been cloned, sequenced, and described in plasma membrane of several cell types, including ventricular myocytes, skeletal myocytes, and neurons (Douglas et al. 2006; Skalska et al. 2008; Xu et al. 2002). However, because there is a lack of evidence for the presence of pore-forming subunits in neuronal mitochondria, it is unclear whether it exists in inner mitochondrial membranes. Gaspar and colleagues (2008) demonstrated that neuronal preconditioning (PC) is independent of K$_{Ca}$ channels. By applying the BK$_{Ca}$ agonist NS1619, they were able to induce immediate PC, although channel antagonism with paxilline or iberiotoxin did not counteract immediate PC, suggesting that either surface K$_{Ca}$ channels or mKATP channels may be involved (Gaspar et al. 2008, 2009). In turtle neurons, we showed that activation of the mK$_{Ca}$ channel using the specific agonist NS1619 reduced AMPAR currents in a similar fashion to anoxia and the mKATP channel antagonists diazoxide and levromakalim. This decrease was abolished when NS1619 was applied with the selective mK$_{Ca}$ channel antagonist paxilline (Fig. 3). Blocking the channel while simultaneously activating the mK$_{ATP}$ channel did not abolish the decrease in AMPAR current, indicating that the mK$_{ATP}$ channel can play a specific role in the anoxic regulation of AMPAR.

We have previously demonstrated that increased K$^+$ uptake through the mK$_{ATP}$ channel uncouples the mitochondria by 10–20%, a range that is similar to the measurements performed in mammalian mitochondria (Murata et al. 2001; Pamenter et al. 2008b). Uncoupling increases activity of the H$^+$/K$^+$ exchanger to maintain electrochemical gradients and reduces the driving force for mitochondrial Ca$^{2+}$ uptake via the Ca$^{2+}$ uniporter, leading to mildly elevated [Ca$^{2+}$]$_i$ levels. Since the mitochondria are a major Ca$^{2+}$ sink, decreased uptake would lead to increased release and a rise in [Ca$^{2+}$]$_i$. Our experiments confirmed that the Ca$^{2+}$ uniporter is involved in this response. Ruthenium red is a potent uniporter antagonist that prevents Ca$^{2+}$ uptake into the mitochondria and therefore increases [Ca$^{2+}$]$_i$. As predicted, normoxic application of ruthenium red resulted in a decrease in AMPAR current similar to the anoxia-mediated decrease (Fig. 4). Furthermore, activation of the uniporter with spermine prevented the anoxia-mediated decrease in AMPAR currents, as did diazoxide/levromakalim treatment. Thus during anoxia a dissipated proton gradient across the mitochondria leads to decreased Ca$^{2+}$ uptake through the uniporter, which ultimately leads to an increase in [Ca$^{2+}$]$_i$ of about 10% (Pamenter et al. 2008b). Unlike the damaging accumulation that occurs during ECD in mammalian neurons (Choi 1992), mildly elevated [Ca$^{2+}$]$_i$ levels in the painted turtle neurons are neuroprotective (Bickler and Buck 2007).

Increased [Ca$^{2+}$]$_i$ levels appear to be critical for the down-regulation of AMPAR currents in the anoxic turtle brain, since sequestering Ca$^{2+}$ with BAPTA abolished the anoxia and diazoxide mediated decreases in whole cell AMPAR currents (Fig. 5). Although the mechanism is not yet known, in the case of the NMDAR we previously hypothesized that protein phosphatases A1 (PP1) and 2A dephosphorylate a serine residue on the calmodulin binding site (CBS1) of the NR1 C-terminus, permitting Ca$^{2+}$-activated calmodulin to bind to CBS1 (Shin et al. 2005). This facilitates NMDAR dissociation from the cytoskeleton and a decrease in NMDAR currents. AMPARs...
FIG. 5. Intracellular BAPTA prevents decreases in whole cell AMPAR currents during anoxia. A: normalized whole cell AMPAR currents at $t = 30$ min (black bars) and $t = 50$ min (gray bars) of treatment. Continuous line represents normoxic controls, whereas dashed line represents anoxic controls. Symbols indicate values significantly different from anoxic control (*), DZX control (†), or RR control (‡). All data are expressed as means ± SE ($n = 7$). B–E: paired-sample trace AMPAR currents at $t = 0$ min (control) and following 40 min of anoxic or drug perfusion ($t = 50$ min). Anox, anoxia; DZX, diazoxide; RR, ruthenium red.
AMPAR channel arrest and anoxia tolerance in the western painted turtle

The anoxia-mediated depression of AMPAR that occurs in the turtle cortex contributes not only to a reduced excitatory input, but also to decreased NMDAR activity, ATP savings, and reduced Na\(^+\) influx through the AMPAR. Studies in rat hippocampal slices have demonstrated that modulation of AMPAR results in an opposing change in NMDAR currents through a Ca\(^{2+}\) -independent mechanism, further stating that since glutamate is an activator for both receptors it may be beneficial to have some sort of regulatory mechanism between the receptors (Bai et al. 2002).

Both NMDARs and AMPARs are also tethered to the postsynaptic density or synaptic region by association with a postsynaptic density protein PSD-95 (Colledge et al. 2003; Dosemeci et al. 2007). This is likely the case for the association of AMPARs with PSD-95 (Colledge et al. 2003; Dosemeci et al. 2007).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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