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

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Zivkovic G, Buck L.T. Regulation of AMPA receptor currents by mitochondrial ATP-sensitive K⁺ (mKATP) channels in anoxic turtle neurons. J Neurophysiol 104: 1913–1922, 2010. First published August 4, 2010; doi:10.1152/jn.00506.2010. Mammalian neurons rapidly undergo excitotoxic cell death during anoxia, whereas neurons from the anoxia-tolerant painted turtle survive without oxygen for hours and offer a unique model to study mechanisms to reduce the severity of cerebral stroke. An anoxia-mediated decrease in whole cell N-methyl-D-aspartate receptor and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) 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 levromakalim 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⁺ 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.

INTRODUCTION

Mammals are extremely anoxia intolerant. Cerebral ischemia causes mammalian neurons to become hyperexitable, 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 (AMPA) are facilitated (Bosley et al. 1983; Ikonomidou and Turski 2002). Excessive Ca⁺² influx via the NMDAR activates proteases, lipases, and endonucleases, which destroy cellular integrity and result in an irreversible process known as excitotoxic cell death (ECD) (Wang and Qin 2010). In certain animal models, blockade of NMDARs and AMPARs is neuroprotective of focal ischemia (Siesjö et al. 1991) but often lead to deleterious side effects under clinical application (Ikonomidou and Turski 2002). The western painted turtle (Chrysemys picta bellii) has the remarkable ability to survive prolonged periods of anoxia (Hochachka 1986): about 1,000-fold longer than a mammal at 25–37°C and as long as 6 mo in decreased temperatures of 1–3°C (Lutz 1992; Nowak et al. 1984; Ultsch and Jackson 1982). This model provides a working model of anoxia tolerance where natural protective mechanisms can be explored and translated into clinically useful situations to improve the outcomes from cerebral stroke and cardiac infarct.

Facultative anaerobes such as the painted turtle use ion channel arrest to prevent ECD (Hochachka 1986), where a decrease in membrane permeability resulting from a down-regulation of ion channel activity prevents the anoxic injury observed in mammals (Bickler and Buck 2002, 2007). Unlike the potentiation of NMDARs and AMPARs observed in mammals during anoxia, turtle NMDARs and AMPARs undergo a reversible ion channel arrest during anoxic episodes that is neuroprotective (Pamenter et al. 2008a; Shin and Buck 2003). In the turtle cortex, the NMDARs and AMPARs undergo a roughly 50% and 60% reduction in evoked postsynaptic current (EPSC) amplitude following anoxic perfusion, respectively, a decrease that is reversible on reoxygenation (Pamenter et al. 2008a; Shin and Buck 2003).

The down-regulation of both ionotropic glutamate receptors may be due to the opening of a mitochondrial ATP-sensitive K⁺ (mKATP) channel, which has also been linked to ischemic preconditioning in some animal models, including: cultured rat cortical models following glutamate toxicity (Kis et al. 2003, 2004), anoxic juvenile mouse brain stem (Muller et al. 2002) and in rat hippocampal and cortical neurons following anoxia/reperfusion injury (Heurteaux et al. 1995; Semenov et al. 2000). The neuroprotective role that the mKATP channel is thought to confer during anoxia is due to its ability to regulate Ca⁺² uptake into the mitochondrial matrix and prevent mitochondrial Ca⁺² overload. The opening of mKATP channels increases K⁺ conductance and facilitates K⁺ influx into the mitochondrion, which activates the K⁺/H⁺ exchanger and in turn dissipates the mitochondrial proton gradient. This mildly depolarizes the mitochondrial membrane potential and decreases Ca⁺² uptake into the matrix through the membrane potential-dependent Ca⁺² uniporter (Holmuhamedov et al. 1999). We have demonstrated that a mild increase in cytosolic Ca⁺² ([Ca⁺²]c) of about 10% occurs during anoxia in the turtle cortex and that it is linked to mitochondrial Ca⁺² release via activation of the mKATP channel (Pamenter et al. 2008b). As mentioned earlier, anoxia also resulted in a 50% decrease in NMDAR currents blocked by the inclusion of the mKATP.
channel antagonists 5-hydroxydecanoic acid (SHD) and glibenclamide. Alternatively, the mKATP agonists diazoxide and levcromakalim could replicate the decrease in NMDAR currents during normoxia. Taken together these results indicate that mKATP channel activation is 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\(^{2+}\) ion that rests in the pore, blocking influx of Na\(^{+}\) or Ca\(^{2+}\) through the channel (Nowak et al. 1984). However, glutamatergic activation of AMPARs produces EPSCs that depolarize the neuronal cell membrane and release the Mg\(^{2+}\) block of the NMDAR, permitting Ca\(^{2+}\) 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 mKATP-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 mKATP channel have not yet been investigated.

The aims of this study were to determine: 1) whether pharmacological manipulation of mKATP channel activity regulates whole cell evoked AMPAR currents during normoxia and anoxia in turtle cortex and 2) whether the response is Ca\(^{2+}\) dependent by blocking changes in intracellular Ca\(^{2+}\) 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): 107 NaCl, 2.6 KCl, 1.2 CaCl\(_2\), 1 MgCl\(_2\), 2 NaH\(_2\)PO\(_4\), 2 H\(_2\)O, 26.5 NaHCO\(_3\), 10 glucose, and 5 imidazole (pH 7.4; osmolarity 280–290 mOsm). Cell-attached 5- to 20-GΩ seals were obtained using the blind-patch technique described elsewhere (Blanton et al. 1989). Typical access resistance ranged from 10 to 30 MΩ and any patches were discarded if access resistance varied by >20%. All data were collected at 2 kHz using an Axopatch-1D amplifier, a CV-4 headstage, and a Digidata 1200 interface and analyzed using Clampex 7 software (Axon Instruments, Union City, CA).

**Evoked AMPA current recordings**

Normoxic experiments consisted of an O\(_2\)/CO\(_2\) 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 used 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)-amino-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 or 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 to the start of an experiment. To maintain anoxic conditions in the bath a plastic cover with a hole for the recording electrode was placed over the recording chamber and the space between the cover and the bath was gently gassed with 95% N\(_2\)-5% CO\(_2\). The aCSF reservoir and recording chamber were constantly gassed with 95% N\(_2\)-5% CO\(_2\) throughout the entire anoxic experiment. The partial pressure of oxygen (PO\(_2\)) was measured using a Clark-type electrode and decreased from about 610 mmHg PO\(_2\) (hyperoxia) to 0.5 mmHg PO\(_2\) (anoxia) within 5 min of gassing, which is the limit of detection for the PO\(_2\) electrode and not significantly different from the anoxic aCSF reservoir (data not shown).

Whole cell patch-clamp recordings were obtained using 3- to 6-MΩ borosilicate glass electrodes. Electrodes contained a solution containing (in mM): 8 NaCl, 0.0001 CaCl\(_2\), 10 Na HEPES, 110 K-glucuronate, 1 MgCl\(_2\), 0.3 NaGTP, and 2 NaATP (adjusted to a pH of 7.4 using methanesulfonic acid; osmolarity 280–290 mOsm). Cell-attached 5- to 20-GΩ seals were obtained using the blind-patch technique described elsewhere (Blanton et al. 1989). Typical access resistance varied from 10 to 30 MΩ and any patches were discarded if access resistance varied by >20%. All data were collected at 2 kHz using an Axopatch-1D amplifier, a CV-4 headstage, and a Digidata 1200 interface and analyzed using Clampex 7 software (Axon Instruments, Union City, CA).

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with AMPA (50 μM) and blocked with 6-cyano-7-nitroquinolinine-2,3-dione (CNQX, 30 μM). Mitochondrial K_{ATP} channels were activated using levromakalim (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 (Pamenter 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 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 (mK_{Ca}) 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, levromakalim, NS1619, glibenclamide, and paxilline were initially dissolved in dimethylsulphonic acid (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. 1, 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 levromakalim 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 levromakalim-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 levromakalim 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_{Ca} 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^+ (mK_{Ca}) channel. Although the mK_{Ca} 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 mK_{Ca} 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 mK_{Ca} channel antagonist paxilline because AMPAR currents did not significantly change throughout the 40-min perfusion (n = 8, Fig. 3, A and C). The mK_{ATP} channel antagonist 5HD did not abolish the decrease in AMPAR currents when applied with NS1619 because AMPAR currents decreased by 37 ± 10.9% and 58.5 ± 8.1% (n = 8, Fig.

![Fig. 1](https://example.com/fig1.png) 2-Amino-5-phosphonovaleric acid (APV) and 6-cyano-7-nitroquinolinine-2,3-dione (CNQX), respectively, abolish N-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic 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.

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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 $\pm$ SE ($n = 7$).

B–J: 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.
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 Ca\(^{2+}\) 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 Ca\(^{2+}\) uptake through the uniporter, resulting in an increase in [Ca\(^{2+}\)]\(_{c}\). To confirm that a moderate increase in [Ca\(^{2+}\)]\(_{c}\) mediates a decrease in AMPAR currents during anoxia, we measured whole cell AMPAR currents in the presence of pharmacological manipulators of the mitochondrial Ca\(^{2+}\) uniporter. Following perfusion of the Ca\(^{2+}\) uniporter antagonist 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 Ca\(^{2+}\) uniporter agonist 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

![Graph showing AMPAR currents normalized]
FIG. 4. Opening of the Ca$^{2+}$ uniporter abolishes the anoxia-mediated decreases in 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 (‡) (refer to Fig. 2). All data are expressed as means ± SE ($n = 7$). B–F: paired-sample AMPAR current traces at $t = 10$ min (control) and at $t = 50$ min following 40 min of anoxic or drug perfusion. Anox, anoxia; 5HD, 5-hydroxydecanoic acid; DZX, diazoxide.
was applied along with the uniporter 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\(^{2+}\)]\(_c\) 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 levcromakalim 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\(^{2+}\)]\(_c\) 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 (Ardehal 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 mKCa 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 BK\(_{ca}\) 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 mKCa channel using the specific agonist NS1619 reduced AMPAR currents in a similar fashion to anoxia and the mKATP channel antagonists diazoxide and levcromakalim. This decrease was abolished when NS1619 was applied with the selective mKCa channel antagonist paxililne (Fig. 3). Blocking the channel while simultaneously activating the mKATP channel did not abolish the decrease in AMPAR current, indicating that the mKATP channel can play a specific role in the anoxic regulation of AMPAR.

We have previously demonstrated that increased K\(^+\) uptake through the mKATP 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 leads to increased 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\(^{2+}\)]\(_c\) levels. Since the mitochondria are a major Ca\(^{2+}\) sink, decreased uptake would lead to increased release and a rise in [Ca\(^{2+}\)]\(_c\). 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\(^{2+}\)]\(_c\). 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/levcromakalim 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\(^{2+}\)]\(_c\), of about 10% (Pamenter et al. 2008b). Unlike the damaging accumulation that occurs during ECD in mammalian neurons (Choi 1992), mildly elevated [Ca\(^{2+}\)]\(_c\) levels in the painted turtle neurons is neuroprotective (Bickler and Buck 2007).

Increased [Ca\(^{2+}\)]\(_c\) 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, reduced Na\(^{+}/K^{+}\)-ATPase activity, and overall reduced metabolic demand (Bickler and Buck 2007). Along with AMPARs and NMDARs, voltage-gated Na\(^{+}\) and K\(^{+}\) channels are known to undergo ion channel arrest in the anoxic turtle cortex (Pamenter et al. 2008b; Pek and Lutz 1997; Perez-Pinzon et al. 1992). The western painted turtle’s remarkable anoxia tolerance is therefore attributed to a number of strategies and each of these mechanisms seems to be important in the turtle’s survival during anoxia. The rationale for the down-regulation of these receptors is an increased excitatory input that would lead to ATP rundown and potentially deleterious neuronal effects. The fact that anoxia-mediated decreases in both AMPAR and NMDAR currents are mediated by the mK\(_{\text{ATP}}\) channel suggests that mitochondria are an important component of the cascade of events leading up to channel arrest.

In conclusion, we show that AMPAR down-regulation during anoxia is regulated by the mK\(_{\text{ATP}}\) channel. This mechanism is fundamental to the turtle’s remarkable anoxia tolerance, in that the down-regulation of AMPAR and NMDAR through the mK\(_{\text{ATP}}\) channel may indicate a common mechanism of decreasing excitatory input in turtle neurons that prevents ECD and allows for extended survival. Elucidating the mechanism by which \([\text{Ca}^{2+}]_{i}\) ultimately brings about channel arrest in both receptors will provide insights to potential protective mechanisms against ischemic insult in humans.


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