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AMPA receptors undergo channel arrest in the anoxic turtle cortex

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Pamenter ME, Shin DS, Buck LT. AMPA receptors undergo channel arrest in the anoxic turtle cortex. *Am J Physiol Regul Integr Comp Physiol* 294: R606–R613, 2008. First published December 5, 2007; doi:10.1152/ajpregu.00433.2007.—Without oxygen, all mammals suffer neuronal injury and excitotoxic cell death mediated by overactivation of the glutamatergic *N*-methyl-D-aspartate receptor (NMDAR). The western painted turtle can survive anoxia for months, and downregulation of NMDAR activity is thought to be neuroprotective during anoxia. NMDAR activity is related to the activity of another glutamate receptor, the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR). AMPAR blockade is neuroprotective against anoxic insult in mammals, but the role of AMPARs in the turtle's anoxia tolerance has not been investigated. To determine whether AMPAR activity changes during hypoxia or anoxia in the turtle cortex, whole cell AMPAR currents, AMPAR-mediated excitatory postsynaptic potentials (EPSPs), and excitatory postsynaptic currents (EPSCs) were measured. The effect of AMPAR blockade on normoxic and anoxic NMDAR currents was also examined. During 60 min of normoxia, evoked peak AMPAR currents and the frequencies and amplitudes of EPSPs and EPSCs did not change. During anoxic perfusion, evoked AMPAR peak currents decreased 59.2 ± 5.5 and 60.2 ± 3.5% at 20 and 40 min, respectively. EPSP frequency ($EPSP_f$) and amplitude decreased 28.7 ± 6.4% and 13.2 ± 1.7%, respectively, and $EPSC_f$ and amplitude decreased 50.7 ± 5.1% and 51.3 ± 4.7%, respectively. In contrast, hypoxic (P_{O_2} = 5%) AMPAR peak currents were potentiated 56.6 ± 20.5 and 54.6 ± 15.8% at 20 and 40 min, respectively. All changes were reversed by reoxygenation. AMPAR currents and EPSPs were abolished by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). In neurons pretreated with CNQX, anoxic NMDAR currents were reversibly depressed by 49.8 ± 7.9%. These data suggest that AMPARs may undergo channel arrest in the anoxic turtle cortex.

N-methyl-D-aspartate receptor; excitotoxic cell death; spike arrest

THE WESTERN PAINTED TURTLE (*Chrysemys picta belli*) can survive without oxygen for days to months (43). This tolerance is predicated on the turtle's ability to maintain neuronal function by using anaerobically produced ATP. In contrast, mammals cannot survive on anaerobic metabolism alone. Oxygen deprivation induces elevations in excitatory amino acids, which leads to overexcitation of glutamate receptors, ATP loss, anoxic depolarization, excessive Ca^{2+} influx, and rapid excitotoxic cell death (ECD) (6, 11, 24, 36, 41). Two glutamate receptors, the *N*-methyl-D-aspartate receptor (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR), are the principle mediators of ECD. In mammals, the activity of both receptors is increased during hypoxia (14, 29), and blockade of either receptor is neuroprotective against global or focal ischemia (15, 42).

A proposed mechanism used by facultative anaerobes to prolong anoxia tolerance and prevent ECD is a decrease in membrane permeability and downregulation of ion channel or receptor activity termed "channel arrest" (17). Indeed, in contrast to the commonly observed anoxic potentiation of mammalian NMDAR currents, turtle NMDARs undergo reversible channel arrest under anoxic or hypoxic conditions, reducing NMDAR currents (3, 7, 39, 40). Generally, NMDARs are not active at resting membrane potential due to the occupation of Mg^{2+} within its pore region (32). This "Mg^{2+} block" prevents influx of Na^+ and Ca^{2+} through the receptor. However, glutamate activation of postsynaptic AMPARs produces excitatory postsynaptic potentials (EPSPs) that induce neuronal depolarization, Mg^{2+} exclusion and subsequent activation of NMDARs in the postsynaptic cell (12, 32).

AMPAR-mediated currents are therefore rapid upstream signals that induce downstream NMDAR-mediated Ca^{2+} influx. AMPAR blockade thus decreases excitability earlier than NMDAR blockade and is neuroprotective following oxygen deprivation due to cardiac arrest or following severe global, focal, or repeated ischemic insults (15, 20, 37, 38, 42). AMPAR blockade is also neuroprotective in preventing cell death due to Parkinsonism and seizures (26, 33). Perhaps the most compelling evidence for a role of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) in ECD is that transgenic mice expressing high levels of AMPARs are more susceptible to focal ischemia than wild-type mice (28). Despite this evidence, research into the role of AMPARs in anoxia tolerance has been overlooked, despite extensive research into NMDAR-mediated cell death.

Because AMPARs play an important role in activating NMDARs during normoxia, it follows then to ask whether they play a role in the anoxic regulation of NMDARs. Anoxia-mediated depression of AMPAR activity may contribute to depression of NMDAR activity, decreased electrical excitability, reduced energy expensive Na^+/K^+ ATPase activity, and thus reduced metabolic demand. Because the channel arrest hypothesis has not been investigated in AMPARs, the aim of this study was to determine whether AMPAR activity changes in the hypoxic or anoxic turtle brain and to examine interactions between AMPA and NMDA receptors in anoxic turtle cortical neurons.

MATERIALS AND METHODS

Animals. This study conforms to relevant guidelines for the care of experimental animals and was approved by the University of Toronto

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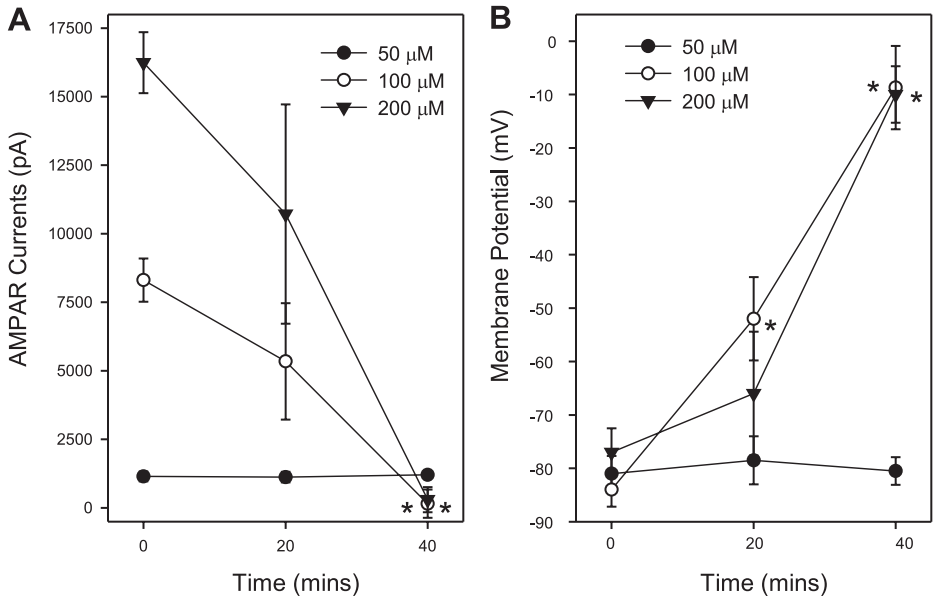

Fig. 1. *A*: dose-response curve of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-elicited peak current magnitude at 0, 20, and 40 min of recordings. *B*: change in resting membrane potential of cells treated with various AMPA doses in Fig. 1*A*. *Significant difference from corresponding normoxic values ($P < 0.05$). Data are expressed as means ± SE from four to six separate experiments.

Animal Care Committee. Adult female turtles were obtained from Niles Biological (Sacramento, CA).

Dissection and whole cell patch-clamp recordings. All experiments were conducted at a room temperature of 22°C. Basic methods for turtle cortical sheet dissection and whole cell patch-clamp recordings under normoxic and anoxic conditions are described elsewhere (39). Briefly, turtles were decapitated and whole brains were rapidly excised from the cranium within 30 s of decapitation. Cortical sheets were isolated from the whole brain and bathed in artificial turtle cerebrospinal fluid (aCSF) in mM: 107 NaCl, 2.6 KCl, 1.2 $CaCl_2$, 1 $MgCl_2$, 2 NaH_2PO_4, 26.5 $NaHCO_3$, 10 glucose, 5 imidazole, pH 7.4; osmolarity 280–290 mOsM. Whole cell recordings were performed using 2–4 MΩ borosilicate glass electrodes. Electrodes contained the following (in mM): 8 NaCl, 0.0001 $CaCl_2$, 10 Na HEPES, 110 K gluconate, 1 $MgCl_2$, 0.3 NaGTP, 2 NaATP, adjusted to pH 7.4; osmolarity 295–300 mOsM. For whole cell-evoked current experiments, cells were perfused with 50 μM TTX to prevent action potentials. For evoked AMPAR current experiments, NMDA receptors were blocked with either high extracellular magnesium (4 mM: hypoxic experiments) or 2-amino-5-phosphonopentanoate (APV; anoxic experiments) to isolate AMPA currents. Current-voltage relationships for turtle NMDARs are unaffected by 1 mM Mg^{2+} but are blocked by 4 mM Mg^{2+} (39).

Cortical sheets were placed in a RC-26 chamber with a P1 platform (Warner Instruments, Hamden, CT). Cell-attached 5–20 GΩ seals were obtained using the blind-patch technique, as described elsewhere (5). 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). The chamber was gravity perfused at a rate of 2–3 ml/min. Normoxic aCSF was gassed with 95% O_2/5% CO_2 and anoxic aCSF with 95% N_2/5% CO_2. To maintain anoxic conditions, perfusion tubes from intravenous bottles were double jacketed, and the outer jacket was gased with 95% N_2/5% CO_2. The anoxic aCSF reservoir was bubbled for 30 min before experiments. A plastic cover with a hole for the recording electrode was placed over the perfusion chamber, and the space between the fluid surface and cover was gently gased with 95% N_2/5% CO_2. Throughout the entire anoxic experiment, aCSF was constantly gased with this N_2/CO_2 mixture. The partial pressure of oxygen (P_{O_2}) in the recording chamber decreased from ~610 mmHg P_{O_2} to 0.5 mmHg P_{O_2} (anoxia) within 5 min, which is the limit of detection for the P_{O_2} electrode and not different from that in the N_2/CO_2 bubbled reservoir. P_{O_2} levels were maintained at this level for the duration of anoxic experiments (data not shown). For hypoxic experiments, anoxic aCSF (as above) was mixed with aCSF gassed with room air (~20% [O_2]) to achieve a bath [O_2] of ~5%.

Current-voltage relationships. To determine current-voltage relationships of AMPA receptors, AMPA was applied to neurons voltage-clamped in sequential steps at −80, −50, −30, 0, and +30 mV. Cells were treated with TTX and APV to prevent spontaneous action potentials and NMDAR-mediated contamination, respectively. Cells were allowed to recover for 10 min between each voltage step, and all responses were normalized to the current recorded at −80 mV. Current-voltage relationships for turtle NMDARs have been previously reported in single-channel and whole cell patch-clamp studies (7, 39).

Evoked current recordings. For ligand-elicited experiments, cells were voltage-clamped at −70 mV, and AMPA or NMDA was applied

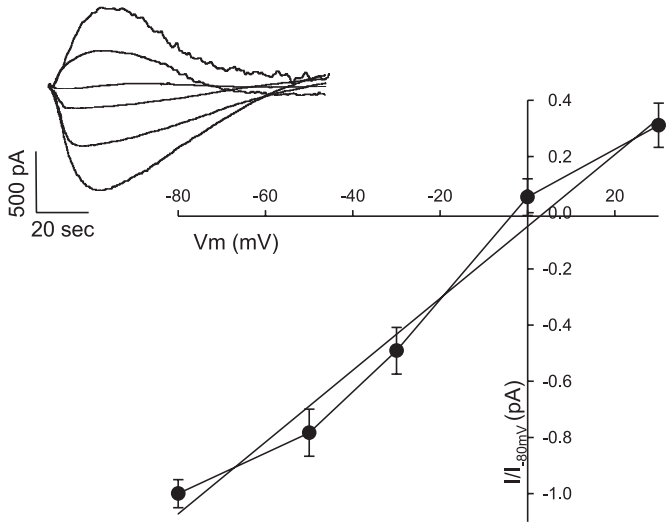

Fig. 2. Normoxic current-voltage relationship of AMPA-elicited currents (50 μM). Cells were voltage-clamped in 20- or 30-mV steps from −80 to +30 mV and normalized to recordings at −80 mV. All cells were perfused with TTX and 2-amino-5-phosphonopentanoate (APV) to prevent action potentials and *N*-methyl-D-aspartate receptor (NMDAR) contamination. Data are presented as means ± SE from eight separate experiments. The slope conductance was 11.9 ± 0.8 pS.

using a fast-step perfusion system (VC-6 perfusion valve controller and SF-77B fast-step perfusion system; Warner Instruments) to puff 50–200 μM AMPA or 300 μM NMDA onto cortical sheets. Control currents were recorded in normoxic aCSF at the start of the experiment ($t = 0$ min) and after 10 min. Cortical sheets were then exposed to anoxic aCSF or aCSF-containing specific receptor modulators for 40 min and then reperfused with normoxic aCSF. AMPA- or NMDA-evoked peak currents were recorded at 20-min intervals following the initial change in perfusion and during reperfusion. Control peak currents were set at 100%, and subsequent peak currents from the same cell were normalized to the control value. Separate control experiments consisting of normoxic aCSF perfusions and NMDAR and AMPAR currents sampled at 0, 10, 20, 40, 60, and 80 min were also performed. The NMDA concentration was selected on the basis of previous experiments in turtle cortex (39, 40). At higher concentrations (100–200 μM), AMPA resulted in large currents that were deleterious to the cell, as assessed by loss of membrane potential and cell death. All whole cell AMPA experiments used 50 μM AMPA, as this concentration resulted in repeatable and consistent currents and did not lead to membrane potential loss (Figs. 1, *A* and *B*).

Spontaneous activity recordings. EPSC and EPSP activity was recorded for up to 2 h from pyramidal neurons at a sampling frequency of 5 kHz. AMPA-mediated EPSC activity was assessed by voltage clamping the cell at −70 mV and recording spontaneous currents. This potential is near the reversal potential of GABA receptors, thus eliminating GABAergic contamination. Cells were perfused with APV to prevent NMDA-mediated currents. Spontaneous EPSC and EPSP activities were recorded in cells undergoing the same experimental protocol as the whole cell current experiments. EPSC and EPSP frequencies and amplitudes were assessed using waveform template analysis in Clampfit 9 software (Axon Instruments). For statistical analysis, spontaneous activity recorded during the final 10 min of 40-min anoxic exposures or of the corresponding time period ($t = 40$–50 min) of normoxic experiments were compared with control recordings from the first 10 min of the experiment.

Pharmacology. AMPA receptors were stimulated with AMPA (50–200 μM) and blocked with CNQX (30 μM). NMDA receptors were stimulated with NMDA (300 μM) and blocked with APV (25 μM) or high Mg^{2+} (4 mM). All chemicals were obtained from Sigma Chemical (Oakville, ON, Canada).

Statistical analysis. AMPAR and NMDAR whole cell current and EPSP and EPSC data were analyzed following root arcsine transformation using two-way ANOVA with a Student-Newman-Keuls test (all pairwise) post hoc test to compare within and against treatment and normoxic values. Significance was determined at $P < 0.05$, unless otherwise indicated in results, and all data are expressed as the means ± SE.

RESULTS

Normoxic and anoxic whole cell AMPAR activity. The current-voltage curve of AMPA-elicited currents had a slope conductance of 11.9 ± 0.8 pS and a reversal potential of 3.4 ± 2.9 mV ($n = 8$, Fig. 2), similar to mammalian AMPARs (30). Summary data of whole cell current traces following AMPA application are shown in Fig. 3*A*. Whole cell AMPAR currents did not change significantly over 80 min of normoxic perfu-

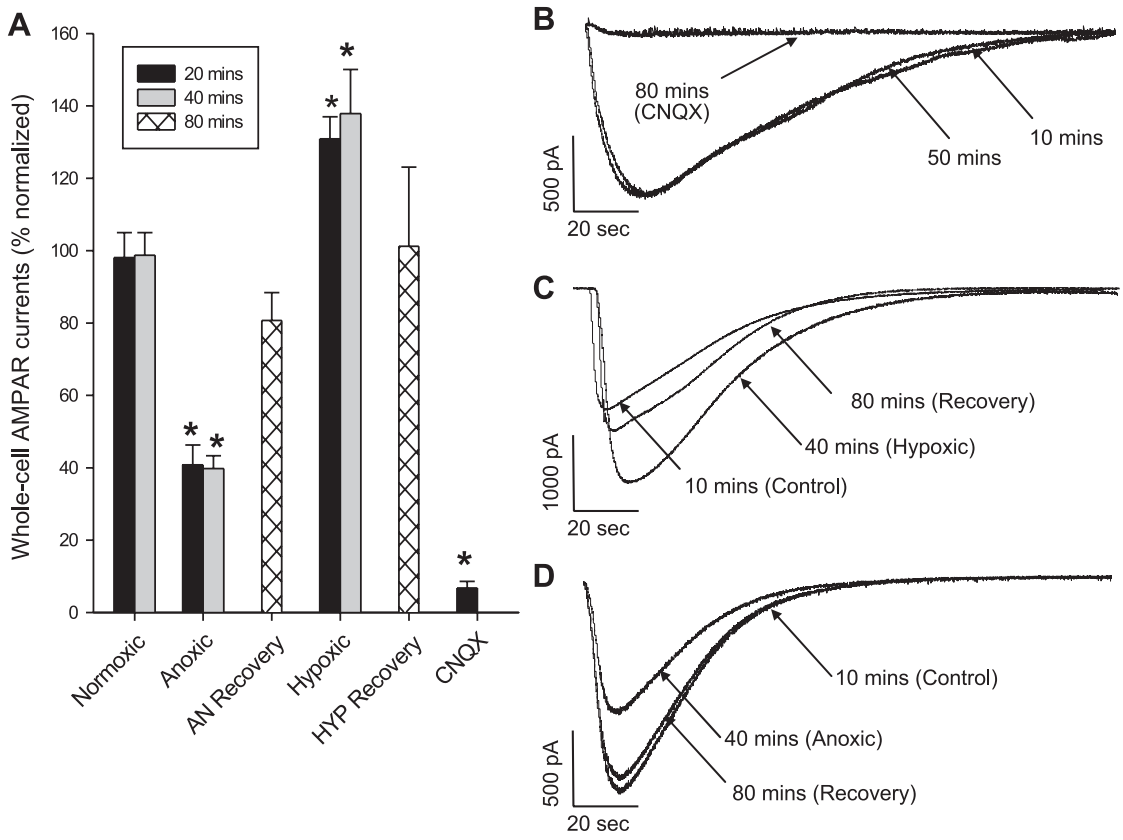

Fig. 3. *A*: summary of normalized AMPA receptor (AMPAR) whole cell currents from turtle cortical neurons undergoing various treatments. Raw whole cell AMPAR currents recorded from a single cell undergoing the following treatments: normoxic perfusion and with and without 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (*B*), normoxic to anoxic transition and recovery (*C*), and normoxic to hypoxic transition and recovery (*D*). All cells were perfused with APV to prevent currents from NMDARs. *Significant difference from corresponding normoxic values ($P < 0.05$). Data are presented as means ± SE from 7 to 14 separate experiments.

sion. AMPA currents ranged from 1,146 ± 180 pA at $t = 0$ min to 1,122 ± 193 pA at $t = 80$ mins ($n = 9$, Fig. 3*B*). Under hypoxic conditions, AMPAR currents were significantly increased in six of seven patches ($P < 0.001$). AMPAR currents increased on average 30.9 ± 6.1 and 37.9 ± 12.1% at 20 and 40 min, respectively, and returned to control levels after 40 min of reoxygenation ($n = 7$, Fig. 3*C*). Under anoxic conditions, AMPAR currents decreased significantly in all patches by an average of 59.2 ± 5.5 and 60.2 ± 3.5% at 20 and 40 min of anoxic perfusion, respectively ($P < 0.001$). After 40 min of normoxic reperfusion, AMPAR current magnitude was not significantly different from normoxic controls ($n = 11$, Fig. 3*D*). AMPA-induced currents were reduced by 93 ± 1.8% by the AMPAR-specific blocker CNQX in normoxia and anoxia ($n = 9$, Fig. 3*B*).

When voltage-clamped at −70 mV, EPSCs are entirely AMPAR dependent. The average normoxic EPSC frequency was 4.12 ± 1.14 Hz, and this frequency decreased ~50% with anoxic perfusion to 2.03 ± 0.42 Hz ($n = 12$, Fig. 4, *A*, *D–E*). The average normoxic EPSC amplitude was 21.8 ± 3.4 pA, and this also decreased ~50% with anoxic perfusion to 10.5 ± 1.2 pA ($n = 6$, Fig. 4, *A–C*).

The average normoxic excitatory postsynaptic potential frequency was 1.8 ± 0.4 Hz, and this frequency was significantly decreased in all patches by 28.7 ± 6.4% with anoxic perfusion ($n = 11$, Fig. 5, *A* and *C*). The average EPSP amplitude was 4.3 ± 0.1 mV, and this also decreased significantly during anoxic perfusion in all patches by 13.2 ± 1.7%. CNQX abolished EPSP firing under normoxic conditions (99.6 ± 0.7% reduction, $n = 6$, Fig. 5*B*), suggesting EPSPs are primarily mediated by AMPARs. During anoxia, CNQX significantly depressed EPSP firing by 95.3 ± 2.4% ($n = 4$, data not shown). Perfusion of the NMDAR antagonist APV had no effect on EPSP frequency ($n = 4$, data not shown).

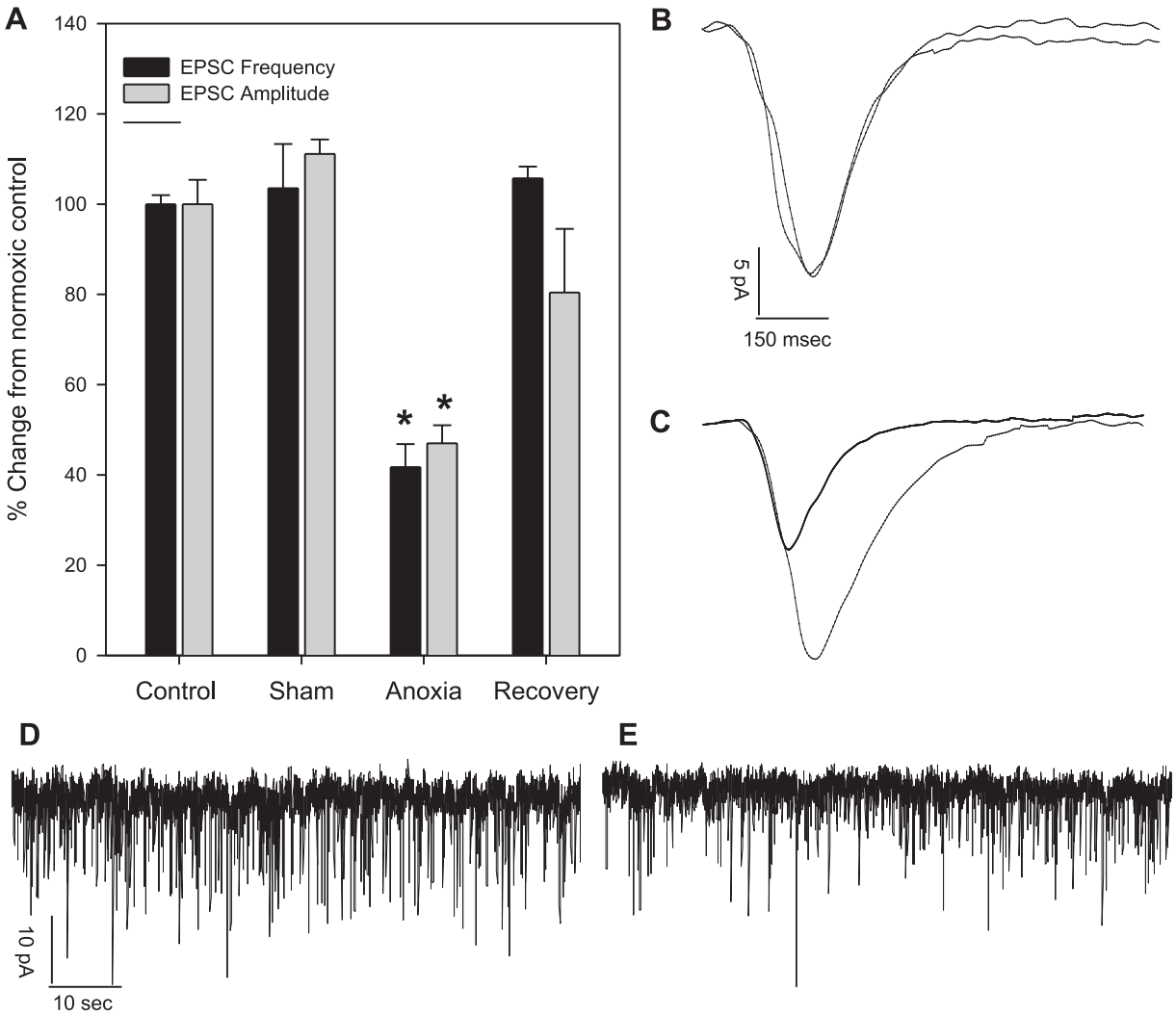

Fig. 4. *A*: summary of normalized spontaneous AMPA-mediated EPSC frequency and amplitude from cortical neurons undergoing normoxic to anoxic transitions. *B* and *C*: composite excitatory postsynaptic currents (EPSC) averages (50 events each) during sham normoxic to normoxic (*B*) and normoxic to anoxic (*C*) transitions in the same cell. *D* and *E*: sample raw EPSC activity from the same neuron under normoxia (*D*) and anoxia (*E*). *Significant difference from corresponding normoxic values ($P < 0.05$). Data are presented as means ± SE from seven separate experiments.

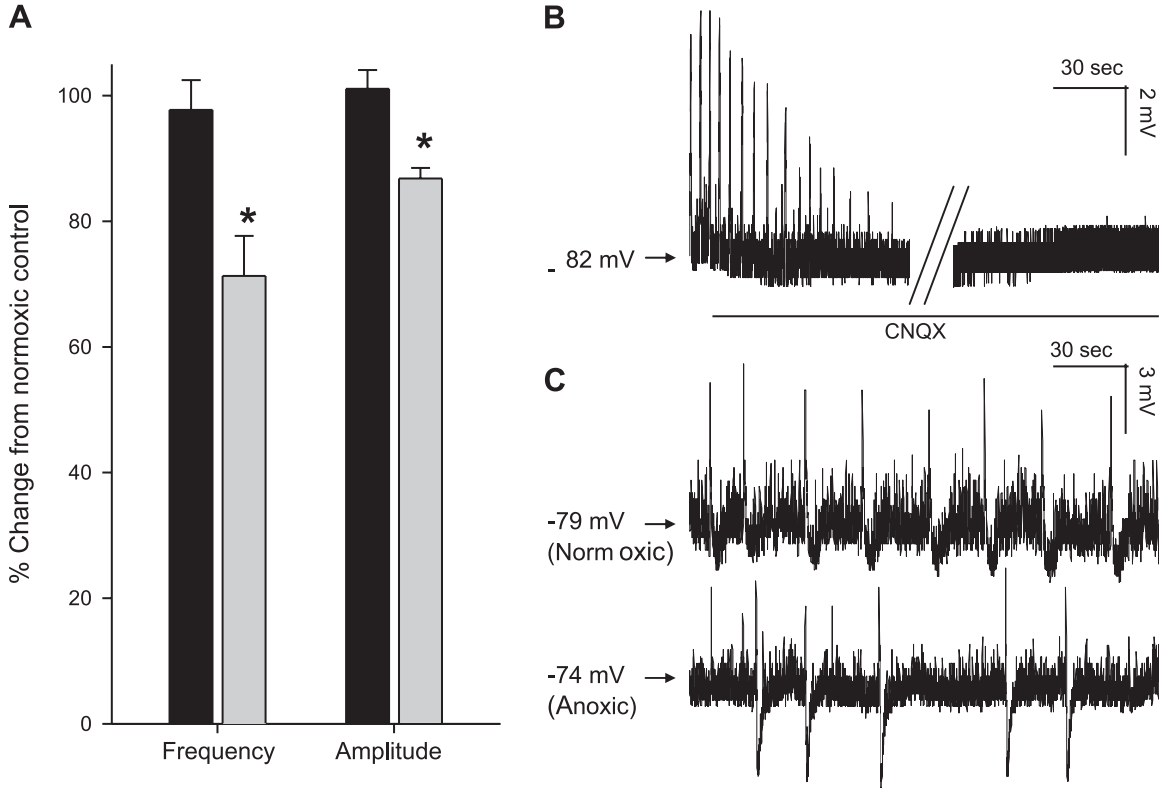

Fig. 5. *A*: summary of normalized excitatory postsynaptic potentials (EPSP) frequencies and amplitudes from cortical neurons 30 min following sham normoxic to normoxic (solid bars) or normoxic to anoxic (gray bars) transitions. *B*: CNQX abolishes spontaneous EPSP activity (solid line represents duration of CNQX exposure). Note: break represents 5 min of CNQX perfusion. *C*: raw spontaneous EPSP activity from a single cell during a normoxic to anoxic transition. *Significant difference from corresponding normoxic values ($P < 0.05$). Data are presented as means ± SE from 5 to 11 separate experiments.

Normoxic and anoxic whole cell NMDAR activity. Summary data of whole cell NMDAR currents are shown in Fig. 6*A*. NMDAR currents did not change during 80 min of normoxia, ranging from 1,853.7 ± 696 to 1,894.5 ± 856 pA at $t = 0$ and 80 min, respectively ($n = 11$, Fig. 6*B*). The anoxic depression in NMDAR activity is well documented (3, 7, 8, 39, 40), but for the purpose of statistical comparisons, it was repeated for this paper. NMDAR currents decreased significantly in all patches by an average of 48.6 ± 4.4% and 54.0 ± 4.3% following 20 and 40 min of anoxic perfusion, respectively ($P < 0.001$, $n = 5$, Fig. 6*C*). Currents recovered to control levels following 40 min of reoxygenation. NMDAR currents were abolished by APV ($n = 5$, Fig. 6*B*). The anoxic decrease in NMDAR currents was unaffected by AMPAR blockade: NMDAR currents were significantly decreased in all patches by an average of 49.8 ± 7.9 and 48.8 ± 6% at 20 and 40 min of anoxic perfusion when both the normoxic and anoxic aCSF contained CNQX throughout the experiment ($P < 0.03$, $n = 5$, Fig. 6*D*).

DISCUSSION

We demonstrate that in the hypoxic turtle cortex, AMPAR currents are significantly potentiated. This potentiation is completely reversed by reoxygenation. Similar responses to hypoxia have been reported in rat hippocampal AMPA receptors. AMPAR currents isolated from NMDAR-mediated contributions (2 mM Mg^{2+}) have been shown to potentiate 25–80% during short-term hypoxia in rat neurons (29, 35). However, during prolonged hypoxia, potentiation of AMPAR currents is not observed, suggesting AMPA activity may become suppressed during prolonged oxygen deprivation in mammals (1).

Increased AMPAR activity during hypoxia contributes to the hypoxic reorganization of synapses, including the appearance of AMPAR-mediated events at previously silent synapses and increased synthesis of excitatory receptor subunits (23, 35). However, synaptogenesis in this context is not associated with the normal "healthy" function of mammalian neurons during hypoxia and may permanently lower seizure thresholds. Neonatal rats are moderately tolerant to hypoxia compared with adult rats and survive brief periods of hypoxia without cell death (22). However, in neonatal rats exposed to hypoxia, seizures occur, and following the hypoxic episode, susceptibility to seizures is permanently increased. Furthermore, cell death occurs following subsequent, previously sublethal hypoxic insults (21, 27). Blockade of AMPA receptors, but not NMDA receptors, prior to the hypoxic insult prevented seizures and the long-term increase in seizure susceptibility (22). If enhanced AMPAR activity leads to formation of new synapses during hypoxia, and mammalian AMPAR blockade prevents permanent hypoxia-mediated decreases in seizure thresholds, then it is logical that the synaptic connections formed during hypoxia may underlie the permanent reorganization toward a state of increased seizure susceptibility following hypoxic insult in rat brain.

Contrary to mammals, our observation that potentiation of turtle AMPAR currents during hypoxia was not suppressed is

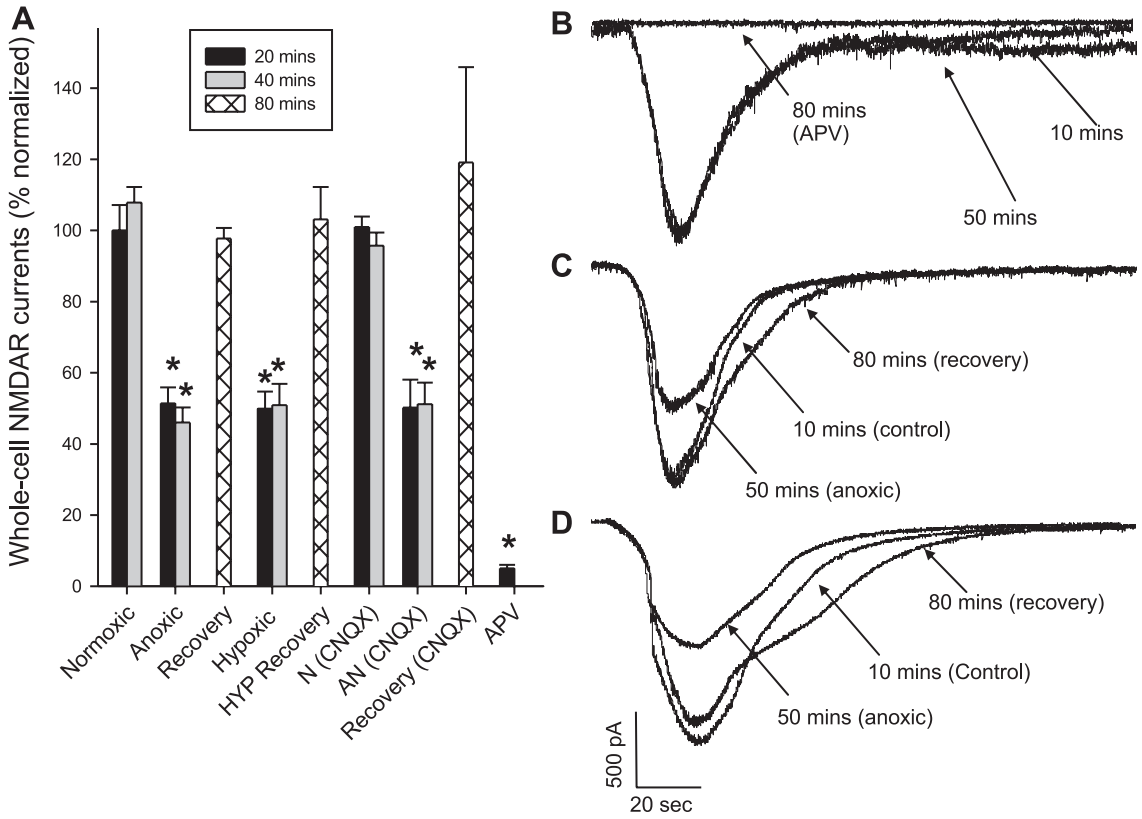

Fig. 6. The effect of AMPAR blockade on NMDAR currents was also examined. *A*: summary of normalized NMDA receptor whole-cell currents from turtle cortical neurons undergoing various treatments. Raw whole cell NMDAR currents recorded from a single cell undergoing the following treatments: normoxic perfusion and with APV (*B*), normoxic to anoxic transition (*C*), normoxic to anoxic transition with CNQX perfusion throughout the experiment (*D*). *Significant difference from corresponding normoxic values ($P < 0.05$). Data are presented as means ± SE from 4 to 12 separate experiments.

intriguing. The turtle is an oxygen conformer, that is, it adapts its metabolic rate in a graded fashion to match available oxygen concentrations and does not simply switch cellular functions on and off (9). Behaviorally, turtles are frequently submerged in normoxic water for prolonged periods in their natural environment. At the tissue level, hypoxic exposure mimics prolonged submergence of the animal in normoxic water during foraging, feeding, and to escape predation. Indeed, turtles are able to extract oxygen from water while they are submerged, and thus during prolonged dives or while overwintering, they likely undergo long periods of falling oxygen levels as the oxygen content of ice-covered ponds slowly dissipates (43).

A number of the protective systems used by the turtle brain to survive anoxia are also upregulated or downregulated during hypoxia to a different degree, including elevations in the rate of glycolysis and the putative O_2 sensor neuroglobin and decreases in Ca^{2+} uptake and metabolic rate (4, 13, 16, 18, 25, 31). This suggests the turtle is able to respond rapidly and appropriately to various oxygen tensions, and unlike most mammals, it is able to match its energy demand to supply under metabolically compromising hypoxic conditions. For the turtle, prolonged submergence is likely a very common situation, and tolerance of intermittent hypoxia may not require deep depression of neural functions compared with the metabolically challenging anoxic environment. Therefore, it is possible that the continued potentiation of turtle AMPAR activity during hypoxia serves a specific signaling mechanism to activate systems that will later be protective against anoxia and that this potentiation is sustainable without detriment to the turtle brain.

During anoxia, it is beneficial to reduce energy demands to a low level. Therefore, it is logical that turtle AMPAR activity is reduced during anoxia to decrease general electrical excitability and energetically expensive protein synthesis associated with synaptogenesis. Our experiments support this hypothesis. Anoxia decreased evoked peak AMPAR currents and spontaneous AMPA-mediated EPSC amplitude significantly, and these currents recovered to control levels following reoxygenation. Spontaneous AMPAR-mediated EPSP activity was also depressed by anoxia. Decreases in the frequency and amplitude of EPSPs and EPSCs reduce the overall excitability of a neuron; therefore, reduced EPSP activity and magnitude due to decreased AMPAR currents may contribute to electrical depression, or "spike arrest," in the anoxic turtle cortex (34). Channel arrest of AMPARs and subsequent electrical depression preserve cellular energy stores as they reduce ion leakage across the membrane and thus reduce the workload of energetically expensive ion pumps. It is not surprising that AMPA receptors would undergo channel arrest in the anoxic turtle cortex, as numerous studies have identified incidences of channel arrest in this organism, including NMDA receptors, K^+ channels, and the Na^+/K^+ ATPase, whose activity decreases 31–34% in the anoxic turtle brain (7, 10, 19).

AMPAR activity may also decrease the activity of NMDARs in the anoxic turtle cortex. There is some evidence to suggest that

NMDARs and AMPARs communicate via a mechanism separate from the voltage-based removal of the NMDAR Mg^{2+} block. In rat hippocampal slices, modulation of AMPARs results in inverse changes in NMDAR currents via a mechanism that is voltage and calcium independent (2). These authors suggested that since both receptors are stimulated by the same endogenous ligand (glutamate), it is beneficial for the receptors to regulate each other's activity such that a large potentiation of AMPAR currents, as occurs under hypoxic conditions, subsequently decreases NMDAR currents or vice versa. In the hypoxic turtle brain, where we observe enhanced AMPAR activity, such a mechanism might initially depress NMDAR currents until broader second messenger-based systems are initiated. To determine whether AMPA receptors mediate the previously reported depression of NMDAR activity we exposed cells to a normoxic to anoxic transition under constant CNQX application. NMDAR currents were reversibly depressed by anoxia, and the magnitude of this depression was not different from that observed in anoxic experiments without CNQX. Although decreased AMPAR activity does not appear to directly regulate NMDAR excitability, depressed AMPAR currents would nonetheless reduce NMDAR activity. Because AMPAR-mediated depolarization removes the Mg^{2+} block from the pore of the NMDAR, a reduction in AMPAR current, EPSP frequency ($EPSP_f$) and amplitude would reduce NMDAR activity in the anoxic turtle cortex. NMDAR activity is reduced by up to 65% following 20 min of anoxic perfusion (8), and 60% of the receptors are reversibly removed from the cell membrane during weeks of anoxia (3). Therefore, under prolonged anoxia, NMDAR activity may be reduced by >85%. A reduction in the AMPAR-mediated excitation of neuronal membranes upstream of NMDAR activation would likely enhance the turtle's already substantial suppression of NMDA receptors and subsequent avoidance of glutamate receptor-mediated ECD during anoxia.

Perspectives and Significance

Our data indicate that turtle AMPA receptors undergo channel arrest during anoxic episodes. Other than the NMDA receptor, this in the only channel in which channel arrest has been measured directly. Decreased AMPAergic excitability reduces NMDAR excitability and may help to prevent ECD in the cortex of the anoxia-tolerant freshwater turtle, as well as in the anoxia-intolerant mammal. Therefore, understanding how the turtle cortex is able to regulate AMPARs during anoxia may provide insight into neuroprotective mechanisms of AMPAR regulation in mammalian models of stroke.

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