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Neuronal membrane potential is mildly depolarized in the anoxic turtle cortex

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ABSTRACT

Neuronal membrane potential (E_m) regulates the activity of excitatory voltage-sensitive channels. Anoxic insults lead to a severe loss of E_m and excitotoxic cell death (ECD) in mammalian neurons. Conversely, anoxia-tolerant freshwater turtle neurons depress energy usage during anoxia by altering ionic conductance to reduce neuronal excitability and ECD is avoided. This wholesale alteration of ion channel and pump activity likely has a significant effect on E_m. Using the whole-cell patch clamp technique we recorded changes in E_m from turtle cortical neurons during a normoxic to anoxic transition in the presence of various ion channel/pump modulators. E_m did not change with normoxic perfusion but underwent a reversible, mild depolarization of 8.1±0.2 mV following anoxic perfusion. This mild anoxic depolarization (MAD) was not prevented by the manipulation of any single ionic conductance, but was partially reduced by pre-treatment with antagonists of $GABA_A$ receptors (5.7±0.5 mV), cellular bicarbonate production (5.3±0.2 mV) or K^+ channels (6.0±0.2 mV), or by perfusion of reactive oxygen species scavengers (5.2±0.3 mV). Furthermore, all of these treatments induced depolarization in normoxic neurons. Together these data suggest that the MAD may be due to the summation of numerous altered ion conductance states during anoxia.

1. Introduction

Maintenance of resting membrane potential (E_m) during anoxic insults is critical to neuronal function since voltage-dependent ion channels and pumps largely mediate membrane permeability to excitatory ions. For example, intracellular calcium ($[Ca^{2+}]_c$) is regulated by the voltage-sensitive Na^+/Ca^{2+} exchanger and glutamatergic *N*-methyl-D-aspartate receptors (NMDARs) (Courtney et al., 1990; Hoyt et al., 1998). E_m is determined primarily by the activity of the Na^+/K^+ATPase, which uses 50–60% of the total energy consumed in the normoxic brain (Glitsch, 2001). When neurons from mammalian brain slices are deprived of oxygen they do not produce sufficient energy to maintain pump activity and neurons undergo a sudden and profound loss of E_m (Anderson et al., 2005; Lundberg and Oscarsson, 1953). This is termed anoxic depolarization (AD) and neurons that undergo AD exhibit cytotoxic increases in $[Ca^{2+}]_c$ due to voltage-sensitive channels activated by the loss of E_m (Fung et al., 1999; Xie et al., 1995). Thus, a profound loss of E_m contributes substantially to excitotoxicity in ischemic mammalian brain (Choi, 1994).

The western painted turtle (*Chrysemys picta bellii*) is a facultative anaerobe that is capable of surviving months of acute anoxic exposure at low temperature without detriment, while neuronal integrity is maintained for at least 6–9 h of anoxia at room temperature (Doll et al., 1993; Jackson and Ultsch, 1982). While it is accepted that turtle neurons do not suffer AD following anoxic exposure, there are conflicting reports as to the effect of anoxia on E_m (Doll et al., 1993; Perez-Pinzon et al., 1992a). During anoxia, turtle brain relies on glycolytic metabolism and employs a variety of mechanisms to minimize ATP consumption. Na^+/K^+ pump activity, K^+, Na^+ and Ca^{2+} leakage, and Ca^{2+} and Na^+ channel density are all decreased in the anoxic turtle brain (Bickler et al., 2000; Chih et al., 1989; Hylland et al., 1997; Pamenter et al., 2008a; Perez-Pinzon et al., 1992b). Furthermore, numerous cellular signals, acid-base state or organelle activity are up- or down-regulated during anoxia, including a mild decrease in pH, mild mitochondrial uncoupling, changes in cellular redox state and elevations of GABA and adenosine (Buck et al., 1998; Lutz and Kabler, 1997; Milton et al., 2007; Pamenter et al., 2007; Pamenter et al., 2008b). These changes may regulate the activity of membrane proteins that impact E_m. For example: GABA increases substantially with anoxia in turtle brain and activates $GABA_A$ and $GABA_B$ receptors, which increase Cl^- and HCO_3^-, and K^+ permeability, respectively (Kaila et al., 1997; Kaila et al., 1993; Nilsson et al., 1990).

Since numerous ions and receptors that contribute to E_m exhibit activity changes during anoxia, it is unlikely that E_m remains static. Therefore a comprehensive examination of the contribution of various ion conductance states to E_m is warranted. The purpose of this paper was to examine changes is turtle neuronal E_m during anoxic exposure and the potential ion channels/receptors that may underlie any anoxic change. We recorded changes in E_m from cortical pyramidal neurons undergoing a normoxic to anoxic transition in the presence of pharmacological modifiers targeting individual ionic conductance or

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second messenger systems. Specifically, we manipulated membrane conductance to K^+, Cl^-, Ca^{2+}, Na^+, or HCO_3^- ions, or blocked GABA or adenosine receptors to abrogate any potential contribution of anoxic elevations of GABA or adenosine to E_m. In addition, the roles of decreased pH, ROS reductions or mild mitochondrial uncoupling were examined.

2. Materials and methods

2.1. Dissection and whole-cell patch-clamp recordings

This study was approved by the University of Toronto Animal Care committee and conforms to the Guide to the Care and Use of Experimental Animals, Volume 2 as determined by the Canadian Council on Animal Care. Adult turtles were obtained from Niles Biological Inc. (Sacramento, CA, USA). All experiments were conducted at a room temperature of 22 °C. Turtles were decapitated and whole brains were rapidly excised from the cranium. Cortical sheets were isolated as described elsewhere (Blanton et al., 1989), 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). To maintain osmalarity, some compounds were ion-substituted as appropriate. For 0 $[Ca^{2+}]_e$ experiments, $Ca^{2+}$was excluded from the aCSF and 5 mM glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) was added. For TEA experiments, 40 mM TEA-Cl replaced an equimolar concentration of NaCl.

Cortical sheets were placed in a RC-26 chamber with a P1 platform (Warner Instruments, CT, USA), which was gravity perfused with aCSF 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, the perfusion tubes from the IV bottle was double jacketed and the outer jacket gassed 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 gassed with 95%N_2/5%CO_2. Throughout the entire anoxic experiment, aCSF was constantly gassed with this N_2/CO_2 mixture. The partial pressure of oxygen (PO_2) in the recording chamber decreased from approximately 610 mmHg PO_2 to 0.5 mmHg PO_2 (anoxia) within 4 min, which is the limit of detection for the PO_2 electrode and not different from that in the N_2/CO_2 bubbled reservoir. PO_2 levels were maintained at this level for the duration of anoxic experiments.

Whole-cell recordings were performed using 2–6 MΩ electrodes containing the following (in mM: 8 NaCl, 0.0001 $CaCl_2$, 10 NaHepes, 110 K-gluconate, 1 $MgCl_2$, 0.3 NaGTP, 2 NaATP, adjusted to pH 7.4). For experiments with varied $[Cl^-]_c$, changes in Cl^- concentration were compensated for with equimolar replacement of NaCl with Na-gluconate to maintain osmolarity. Cell-attached 5–20 GΩ seals were obtained using the blind-patch technique (Blanton et al., 1989). Whole-cell patches were obtained by applying a brief suction and E_m was recorded for up to 2 h from cortical pyramidal neurons at 2 kHz using an Axopatch-1D amplifier, a CV-4 headstage, a digidata 1200 interface and Clampex 7 software (Axon Instruments, CA, USA). E_m was recorded for 10 min from cortical sheets in normoxic aCSF. Cortical sheets were then exposed to treatment aCSF for 40 min. Finally, cells were reperfused with control normoxic saline for 20 min. Access resistance (R_a) was 10–30 MΩ. R_a was determined at regular intervals and patches were discarded if R_a changed by more than 20% during the course of an experiment. The liquid junction potential was assessed at 10 mV and all data have been corrected for this value (raw data traces are unaltered).

2.2. Pharmacology

For whole-cell experiments, neurons were perfused with pharmacological modifiers in the bulk perfusate as specified in the results section. Potassium channels were blocked with TEA (40 μM). pH-sensitive ion channels were activated by intracellular recording solution pH-adjusted to 6.8 or by proprionate (10 mM) to mimic the

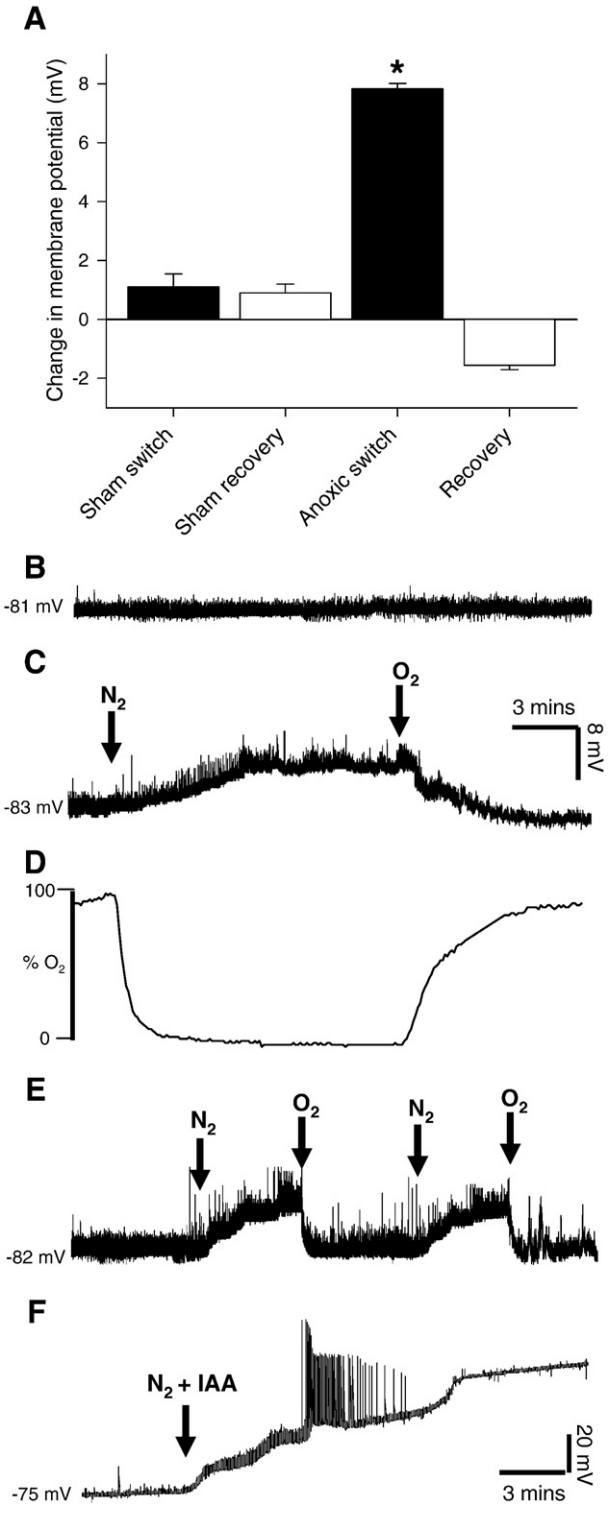

Fig. 1. Anoxia-mediated regulation of neuronal resting membrane potential. (A) Summary of resting membrane potential (E_m) changes during two hours of normoxic or normoxic to anoxic recordings at one hour (black bars) and two hours (recovery, grey bars). (*) different from control values. Data are presented as mean±SEM. (B–C and E) Raw data traces of E_m changes during a normoxic recording (B) a normoxic to anoxic transition and recovery, or (E) multiple normoxic–anoxic switches. Arrows indicate switches between normoxic and anoxic saline. (D) Raw data trace of recording chamber oxygen levels during the experiment in C. (F) Raw data trace of E_m changes during a normoxic to anoxic switch in the presence of the glycolytic inhibitor iodoacetate (IAA).

mild anoxic acidification observed in anoxic cortex (Bickler, 1992). GABA receptors were blocked by SR-95531 (gabazine: $GABA_A$) and CGP55845 ($GABA_B$). Bicarbonate production was blocked by ethoxyzolamide (EZA: 50 μM). Glutamatergic AMPA and NMDA receptors were blocked by CNQX (30 μM) and APV (60 μM), respectively. Voltage-gated Na^+ channels were blocked with tetrodotoxin (TTX: 50 μM). To prevent mild mitochondrial uncoupling during anoxia, mitochondrial ATP-sensitive K^+ channels (mK_{ATP}) were blocked with glibenclamide (80 μM) or the mK_{ATP}-specific antagonist 5-hydroxydecanoic acid (5HD: 100 μM). Adenosine A_1 receptors were blocked by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 nM). To chelate Ca^{2+}, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA, 5 mM) was included in the recording electrode solution. All chemicals were obtained from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada).

2.3. Statistical analysis

For statistical purposes, the baseline E_m recorded for 10 min prior to treatment perfusion was compared to E_m recorded for a ten-minute period after stabilization of E_m following anoxic perfusion. Significance was determined using a one-way repeated ANOVA followed by Tukey's post hoc test. Significance was determined at $P<0.05$, and all data are expressed as the mean±SEM (standard error of mean).

3. Results

During normoxia, E_m was unchanged throughout 2-hours of recordings, from −81.4±1.5 mV at the start of the experiment to −80.0±1.8 mV at the conclusion of the 2-hour period (Fig. 1A and B). Conversely, anoxic perfusion resulted in a rapid mild anoxic depolarization (MAD) of 8.1±0.2 mV within minutes (Fig. 1A and C, Table 1). This change was reversed following reoxygenation. The recording chamber became anoxic (0% O_2) within 3–4 min following the switch to anoxic perfusion and MAD was initiated at very hypoxic oxygen levels, reaching a new depolarized steady state at ~10 min (Fig. 1C and D). Oxygen tensions were measured in experiments where the recording electrode was replaced with an O_2 electrode (Fig. 1D). All cells exposed to anoxia underwent MAD and the individual changes in E_m all fell within the range of 7.0 to 10.5 mV. This change was rapid, reversible and repeatable with multiple switches between normoxic and anoxic perfusion (Fig. 1E). Anoxic neurons treated with iodoacetate to block glycolysis underwent a rapid and severe AD similar to AD in mammalian neurons (Fig. 1F).

We examined the respective roles of the two GABA receptors by antagonizing them individually during normoxic to anoxic transitions. Blockade of either receptor during anoxia resulted in excitatory activity, so we co-treated neurons with TTX to prevent excitatory events. Blockade of $GABA_B$ receptors during anoxia had no significant effect on the reversible depolarization, however $GABA_A$ antagonism during anoxia resulted in a depolarization of 5.7±0.5 mV, significantly smaller than the control anoxic depolarization (Table 1). $GABA_A$ receptors are permeable to Cl^- and bicarbonate (HCO_3^-) ions. To examine the role of Cl^- in the anoxic depolarization we recorded anoxic changes of E_m in cells dialyzed with a wide range of intracellular Cl^-($[Cl^-]_c$) (1–30 mM). These concentrations provide a range of Cl^- reversal potentials (E_{Cl}) from ~−60 mV (at 30 mM) to −120 mV (at 1 mM). Since E_m in these neurons is ~−80 mV, MAD should reverse and become a mild hyperpolarization in cells patched with 1 mM $[Cl^-]_c$ if the MAD is Cl^- dependent. In cells clamped with 1 mM $[Cl^-]_c$, the anoxic depolarization was not different from controls (10 mM $[Cl^-]_c$), however in cells clamped with 30 mM $[Cl^-]_c$ MAD was enhanced to 11.9±1.7 mV (Table 1). Finally, to examined the role of HCO_3^- in MAD we pre-treated cells with the carbonic anhydrase inhibitor ethoxyzolamide (EZA), which abolishes neuronal HCO_3^- production. As in cells treated with gabazine, the anoxic depolarization was significantly reduced to 5.3±0.2 mV with EZA treatment (Table 1).

ROS production inherently requires oxygen, thus with anoxic perfusion, oxygen availability reaches nil and ROS production is abolished in the anoxic turtle cortex (Milton et al., 2007; Pamenter et al., 2007). To mimic this environment we treated normoxic cortical sheets with the free radical scavengers 2-mercaptoproionyl glycine (MPG) or N-acetyl cysteine (NAC). Perfusion of either scavenger resulted in a large depolarization of E_m of 14.4±1.4 and 26.9±3.4 mV, respectively. In cells pre-treated with scavengers and then exposed to anoxia, a further depolarization of 6.0±0.4 and 5.2±0.3 mV were observed with MPG and NAC, respectively (Table 1). Finally, blockade of K^+ channels during normoxia with TEA resulted in a large membrane potential depolarization; however, a further 6.0±0.2 mV depolarization was observed with subsequent anoxic perfusion that was reversed by reoxygenation (Table 1).

The remainder of the treatments had no effect on the MAD. Blockade of adenosine receptors or Na^+ or Ca^{2+} conductance had no effect on the MAD, nor did chelation of intracellular Ca^{2+} with BAPTA or removal of extracellular Ca^{2+} in the presence of EGTA. Furthermore, modification of pH or mitochondrial respiration did not alter the MAD. In all these experiments, anoxic perfusion induced a significant and reversible MAD in every neuron recorded from. The mean MAD for

Table 1
The mild anoxic depolarization is the summation of multiple altered ionic conductance states

Experimental treatment	Normoxic E_m (mV) 0–10 min	Anoxic E_m (mV) 40–50 min	Recovery E_m (mV) 80–90 min	Anoxic ΔE_m (mV)	Range of ΔE_m (mV)
Anoxia (40)	−84.4±1.0	−76.6±0.9*	−87.2±0.7	8.1±0.2†	7.0 to 10.5
GABAergic channels					
CGP55845 (5)	−81.2±1.7	−72.3±1.1*	−83.2±1.4	8.9±1.7†	8.0 to 12.5
Gabazine (10)	−81.2±1.6	−75.5±0.7*	−80.0±1.8	5.7±0.5†‡	5.1 to 6.2
1 mM $[Cl^-]_c$ (6)	−82.6±0.7	−74.7±1.0*	−81.2±1.1	7.9±0.4†	7.1 to 8.3
30 mM $[Cl^-]_c$ (6)	−82.5±1.1	−70.6±1.7*	−80.2±2.4	11.9±1.7†‡	9 to 13.5
EZA (9)	−81.6±2.1	−76.3±1.9*	−80.0±2.9	5.3±0.2†‡	4.6 to 6.1
ROS scavenging					
MPG (5)[a]	−68.3±0.5*	−62.2±0.4*	−69.0±1.1	6.0±0.4†‡	5.1 to 6.7
NAC (5)[a]	−55.0±3.4*	−49.9±1.1*	−54.1±0.7	5.2±0.3†‡	4.2 to 6.0
K^+channels					
TEA (6)[a]	−47.8±2.5*	−41.8±1.0*	−49.1±1.2*	6.0±0.2†‡	5.2 to 6.5

Summary of E_m changes from pyramidal neurons undergoing normoxic to anoxic transitions alone or co-treated with pharmacological agents. (*) Different from control normoxic values. (†) ΔE_m is different from control normoxic ΔE_m change. (‡) ΔE_m is different from the control anoxic ΔE_m change. Average E_m was assessed in the last 10 min of normoxic, anoxic, or recovery perfusion ($P<0.05$). Data are presented as mean±SEM. Parentheses indicates sample sizes.

Table 2
Summary of treatments that did not abolish the mild anoxic depolarization of E_m

Experimental treatment	Normoxic E_m (mV) 0–10 min	Anoxic E_m (mV) 40–50 min	Recovery E_m (mV) 80–90 min	Anoxic ΔE_m (mV)	Range of ΔE_m (mV)
Na^+/Ca^{2+} channels					
TTX (6)	−86.6±1.1	−78.9±1.0*	−88.5±2.3	7.7±0.4†	6.5 to 9.6
CNQX (4)	−83.5±5.3	−74.6±6.3*	−85.9±4.3	8.8±1.1†	6.0 to 11.5
APV (4)	−86.9±1.2	−76.2±1.3*	−85.8±1.7	9.9±1.7†	8.2 to 11.9
BAPTA (4)	−86.7±4.4	−76.8±6.1*	−88.7±3.4	9.8±1.7†	7.5 to 14.1
0 $[Ca^{2+}]_e$+EGTA (5)	−82.1±1.1	−74.5±1.0*	−83.2±2.1	7.6±0.4†	7.1 to 8.2
(−) Mitochondrial uncoupling					
Glyb (4)	−86.5±2.2	−79.8±1.6*	−86.0±6.0	7.7±0.6†	6.6 to 8.1
5HD (5)	−81.6±3.3	−72.8±3.4*	−84.5±3.4	8.8±0.6†	6.1 to 9.5
Cellular acidification					
pH 6.8 (5)	−84.3±1.5	−78.1±2.0*	−83.7±4.8	7.2±0.8†	6.4 to 9.0
Propionate (5)	−83.1±0.8	−74.5±1.1*	−82.2±2.3	8.1±0.4†	7.2 to 9.5
Adenosine receptors					
DPCPX (5)	−82.1±2.1	−74.8±0.9*	−82.7±1.2	7.5±1.0†	7.0 to 9.1

Summary of E_m changes from pyramidal neurons undergoing normoxic to anoxic transitions alone or co-treated with pharmacological agents. (*) Different from control normoxic values. (†) ΔE_m is different from control normoxic ΔE_m change. (‡) ΔE_m is different from the control anoxic ΔE_m change. Average E_m was assessed in the last 10 min of normoxic, anoxic, or recovery perfusion ($P<0.05$). Data are presented as mean±SEM. Parentheses indicates sample sizes.

individual treatment groups fell within the absolute range of 7.2±0.8 to 9.9±1.7 mV. These data are summarized in Table 2.

4. Discussion

We report that turtle cortical neurons undergo a controlled mild anoxic depolarization of ~7–11 mV that is rapidly reversed by reoxygenation. Compared to AD in ischemic mammalian neurons the depolarization observed in our experiments is minor and unlike in mammalian neurons, did not contribute to excitotoxic events (Anderson et al., 2005). E_m remained below −70 mV after the MAD, well above the activation threshold of excitatory voltage-sensitive channels and receptors. Turtles are ectothermic and the avoidance of severe anoxic depolarization in their cortex may be partially temperature-related. Mammalian neurons exposed to oxygen-glucose deprivation (OGD) suffer AD within minutes of OGD onset, however this effect is ameliorated by temperature reductions, and at 22–24 °C rapid AD is not observed in ischemic mammalian neurons (Joshi and Andrew, 2001). In our experiments turtle neurons survived >1 h of anoxia at 22 °C and 10 mM [glucose] without AD, while others have reported similar results in neurons exposed to 3 h of anoxia or NaCN exposure at 25 °C and 10 mM [glucose] (Doll et al., 1991). Conversely, mammalian neurons exposed to anoxia or NaCN perfusion at 25 °C and 11 mM [glucose] maintained membrane potential for 30–60 min before the onset of AD. Although this survival period was significantly longer than mammalian neurons recorded from at 35 °C, which avoided AD onset for only a few minutes of either anoxia or NaCN perfusion, turtle neurons survived 5–6 fold longer than mammalian neurons at 25 °C without apparent detriment. Furthermore, turtle neurons survive significantly longer than mammalian neurons when exposed to iodoacetate during anoxia or complete metabolic arrest (Doll et al., 1991). Thus, although temperature reductions significantly delay the onset of AD in mammals, the ability of turtle neurons to avoid AD for hours longer than mammalian neurons in the same conditions indicate that turtle neurons are significantly more tolerant to anoxia or ischemia then mammalian neurons.

Although severe AD was not observed in anoxic turtle cortex, the mild repeatable depolarization we report is of interest since it is highly sensitive to oxygen and may underlie, or result from, oxygen-sensing mechanisms in turtle neurons. MAD was not abolished by the antagonism of any individual channel or receptor. Instead, modifications of $GABA_A$ receptors, HCO_3^- production, K^+ channels or cellular redox state each partially reduced the MAD. These data suggest that the MAD is the summation of E_m changes due to altered conductance states of numerous ions during anoxia. Anoxic elevations of GABA activate $GABA_A$ receptor-mediated membrane permeability to Cl^- and HCO_3^- ions (Kaila et al., 1993; Nilsson et al., 1990). The anoxic depolarization was reduced by a $GABA_A$ receptor antagonist and by inhibition of HCO_3^- production, but not by altered $[Cl^-]_c$, suggesting anoxic efflux of cellular HCO_3^- through $GABA_A$ receptors contributes to the MAD. Inhibition of HCO_3^- production may also result in intracellular acidification, but since clamping intracellular pH at 6.8 had no effect on the MAD in our experiments, this effect likely does not underlie the impact of HCO_3^- flux on the MAD.

In addition to $GABA_A$ receptors, GABA also activates a K^+ conductance (G_K) via $GABA_B$ receptors, however whole cell G_K decreases ~50% in the anoxic turtle brain, suggesting most K^+ channels are inhibited by anoxia (Chih et al., 1989). E_m is largely determined by G_K and perfusion of the general K^+ channel antagonist TEA during normoxia resulted in a large membrane potential depolarization, and reduced the MAD following subsequent anoxic perfusion. These data support a limited role for K^+ in MAD; however, many K^+ channels have been identified for which there are no known inhibitors or which are not inhibited by TEA. Therefore, the contribution of G_K to MAD may be underestimated in our experiments due to the limited information available on the variety of K^+ channels.

The remainder of the MAD may be due to reduced Na^+/K^+ ATPase activity during anoxia (Hylland et al., 1997). Although this reduction in activity is matched by a 40–50% reduction in G_K and Na^+ channel density compared to normoxic values (Chih et al., 1989; Perez-Pinzon et al., 1992b), an unequal reduction in the rate of K^+ and Na^+ pumping versus passive leakage could contribute to altered E_m. Indeed, our observation that ROS scavengers reduced the MAD may be due to redox regulation of Na^+/K^+ ATPase activity (Lehotsky et al., 2002).

Previous measurements of turtle E_m during transitions to anoxia have yielded inconsistent results. One study reported a significant change in anoxic transmembrane potential (Perez-Pinzon et al., 1992a). A second study reported an ~3.8 mV depolarization in E_m with anoxia that did not reach significance (Buck and Bickler, 1998). Finally, another study reported no change in E_m with anoxia (Doll et al., 1993). In our experiments the depolarization was observed in all 40 neurons exposed to anoxia and also persisted in almost 100 neurons in which pharmacological treatment failed to abolish the anoxic depolarization. The depolarization was initiated late in the hypoxic period following the switch from normoxic to anoxic perfusate and was maintained throughout the anoxic exposure and rapidly reversed following O_2 reperfusion. This high sensitivity to small amounts of oxygen suggests the depolarization may be the

result of the summation of several oxygen-conforming mechanisms that are only initiated at very low O_2 levels. The extreme O_2-sensitivity of MAD may underlie these conflicting observations if some experiments were hypoxic and not anoxic.

In conclusion, we demonstrate a mild anoxic depolarization of turtle neuronal membrane potential that is reversed by reoxygenation. This depolarization is partially due to $GABA_A$-mediated bicarbonate flux following anoxic GABA release, and also partially due to altered G_K and to depressed ROS production. The remainder of the depolarization may be due to alterations in G_K through unidentified K^+ channels. In addition, we rule out the involvement of other major excitatory and inhibitory mechanisms commonly affected by anoxia in the freshwater turtle. Since the depolarization is mild, it does not contribute to excitotoxic events, as in mammalian neurons, and may be a byproduct of the rearrangement of ionic homeostasis in the anoxic turtle cortex, rather than a designed response to anoxic insults.

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