Gene transcription of brain voltage-gated potassium channels is reversibly regulated by oxygen supply

Howard M. Prentice,1 Sarah L. Milton,2 Daniela Scheurle,1 and Peter L. Lutz2

1Department of Biomedical Sciences, Florida Atlantic University, Boca Raton 33431, and 2Department of Biological Sciences, Florida Atlantic University, Boca Raton, Florida 33431

Submitted 12 May 2003; accepted in final form 28 July 2003

Prentice, Howard M., Sarah L. Milton, Daniela Scheurle, and Peter L. Lutz. Gene transcription of brain voltage-gated potassium channels is reversibly regulated by oxygen supply. Am J Physiol Regul Integr Comp Physiol 285: R1317–R1321, 2003; 10.1152/ajpregu.00261.2003.—Voltage-dependent potassium channels (Kv channels) are important determinants of brain electrical activity. Hypoxia may be an important modifier, because several voltage-gated K+ channels are reversibly blocked by acute hypoxia and are thought to act as oxygen sensors. Here we show, using the anoxia-tolerant turtle brain (Trachemys scripta) as a model, that brain Kv1 channel transcription is reversibly regulated by oxygen supply. We found that in turtle brains exposed to 4-h anoxia Kv1 transcripts were reduced to 18.5% of normoxic levels. Kv1 channel mRNA levels were restored to normal within 4 h of subsequent reoxygenation. Our results provide clear evidence that brain Kv channel expression is sensitive to oxygen supply and indicate an important mechanism that matches brain activity to oxygen supply.

Trachemys scripta; turtle; anoxia; Kv1 channel

UNABLE TO COMPROMISE on their intense energy consumption, most vertebrate brains go into energy failure within minutes of being deprived of oxygen, resulting in a loss of ion gradients with consequent depolarization and eventual neuronal death (20a). The freshwater turtle (Trachemys scripta) is one of the few exceptions, having a brain that can survive at least 48 h of anoxia at 25°C (19), allowing it to be described as the archetype of an anoxia-tolerant brain against which the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

which would enhance excitability by decreasing the action potential period. Most ischemia/anoxia-induced changes in the brain, however, are probably pathological, many coinciding with the onset of tissue damage caused, in part, by the release of oxygen free radicals (17).

The aim of this research was to determine whether the turtle reduces Kv channel transcription as part of its long-term anoxia defense strategy of reducing brain metabolic demands. It should be pointed out that, although in the mammal it is often difficult to distinguish between adaptive (functional) and pathological responses to such destructive insults as brain anoxia/ischemia and subsequent reperfusion, the turtle brain responses can be presumed to be adaptive as it survives and fully recovers from many hours of anoxia and subsequent reoxygenation (2, 20a). As the gene responses to hypoxia are highly conserved, the turtle can provide a useful insight into mechanisms behind mammalian failure and survival (13).

**MATERIALS AND METHODS**

**Tissue preparation.** Freshwater turtles (*Trachemys scripta*) obtained from commercial suppliers (W. H. Lemberger, Oshkosh, WI) weighing 300–500 g were individually placed in sealed 2-liter plastic chambers at room temperature (25°C). Three experimental sets of *n* = 5 included normoxic controls, anoxic animals exposed to 4 h 99.99% N₂ (positive pressure flow-through, County Welding, Pompano Beach, FL), and a third group of 4-h anoxia/4-h normoxic recovery. Animals were killed by cervical separation, and the brains were removed into liquid nitrogen in <2 min.

![Fig. 1. Expression levels of Kv1 and HIF-1α transcripts in the turtle brain.](image-url)
OXYGEN REGULATES POTASSIUM CHANNEL GENE TRANSCRIPTION

For semiquantitative assessment of Kv1 transcript levels and HIF-1 transcript levels, respectively, RT-PCR signal intensities were expressed as a ratio of levels of PCR products amplified from turtle actin cDNAs. All experiments were conducted with the approval of Florida Atlantic University Institutional Animal Care and Use Committee.

Statistical analysis. Results are expressed as means ± SE. Statistical significance was evaluated using ANOVA. A value of P < 0.05 was used to denote statistical significance.

RESULTS

To determine if brain Kv1 channel transcription is influenced by oxygen supply, we measured messenger RNA levels for Kv1 subunits in cerebral tissue from normoxic turtles, turtles subjected to 4-h anoxia, and turtles subjected to 4-h anoxia followed by 4-h normoxic recovery. Levels of mRNA expression were normalized relative to actin mRNA control levels. HIF-1 transcript was also measured.

The anoxic brains showed a substantial downregulation in Kv1 channel transcription to 18.5% of normoxic levels (Fig. 1, A and B). In the recovery set, transcript levels for Kv1 rose 5.6-fold relative to the anoxic brain, returning to original normoxic levels (Fig. 1, A and B). Some studies point to a two-phase response to anoxia in the turtle brain: an initial transitory phase during the first 1 to 2 h anoxia in which there is a coordinated downregulation of energy demanding processes followed by a long-term (h/days) maintenance of the deep hypometabolic state (20a). The purpose of this study was, therefore, to see if a downregulation of Kv was evidenced in the hypometabolic brain and a subsequent restoration of Kv transcription on reoxygenation. No changes were observed for actin or HIF-1 transcripts during anoxia and subsequent recovery, indicating the absence of a generalized transcriptional response to anoxia (Fig. 1, C–E).

RT-PCR. Total RNA was extracted using the TRIzol reagent (Life Technologies, Grand Island, NY) according to the manufacturer’s protocol, and RNA was subjected to treatment with DNase I to eliminate DNA contamination. Complementary DNA was synthesized from total RNA using manufacturers recommended conditions. The PCR reaction using Taq polymerase comprised denaturation for 7 min, 94°C, PCR: 40 cycles (Kv1) or 30 cycles (actin) (1 min, 94°C; 2 min, 57°C; 3 min, 72°C) followed by elongation: 10 min, 72°C. For HIF-1, PCR was conducted for 40 cycles (1 min, 94°C; 2 min, 57°C; 3 min, 72°C). The primers employed for PCR reactions were the following: Kv primers: 5'-TGGTCTATYTVATCTCBBATHTCA-3' (forward) and 5'-ACBACNGCCCACCARAAVGCATC-3' (reverse); actin primers: 5'-CACCAGTGGACGATGGG-3' (forward) and 5'-GTGCGAAGACTGCTGAGTGCCTCCT-3' (reverse). Controls in which RNA or RT were omitted from the RT reaction were carried out to confirm the absence of residual genomic DNA. After electrophoresis of PCR products, gels were stained with ethidium bromide and photographed using a digital camera for quantification using National Institutes of Health Image 1.60 software.

Table 1. Oligonucleotide primer sequences employed for RT-PCR

<table>
<thead>
<tr>
<th>Target</th>
<th>Predicted Size, bp</th>
<th>Sense/Antisense Location, Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1</td>
<td>587</td>
<td>5'-TGGTCTATYTVATCTCBBATHTCA-3' 508-532</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-ACBACNGCCCACCARAAVGCATC-3' 1072-1094</td>
</tr>
<tr>
<td>Actin</td>
<td>505</td>
<td>5'-CACCAGTGGACGATGGG-3' 228-247</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GTGCGAAGACTGCTGAGTGCCTCCT-3' 713-732</td>
</tr>
<tr>
<td>HIF-1s</td>
<td>393</td>
<td>5'-TGGTCAAATAGAGAATTGGAGA-3' 422-444</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GTCTCCCTGTGCTAGCAGACATAGG-3' 791-814</td>
</tr>
</tbody>
</table>

For Kv1 RT-PCR products, predicted size and location of oligonucleotide binding sites are based on coding sequences of the rat Kv1 family members Kv1.1–Kv1.6 (29a). For HIF-1 and actin RT-PCR products, predicted size and location of oligonucleotide binding sites are based on the trout HIF-1a coding sequence (accession no. AF304864) and the salmon β-actin coding sequence (accession no. AF309819), respectively. Degenerate primers were employed in each case. Primers for HIF-1 were homologous to both the trout and mouse coding sequences and those for β-actin were homologous to both the salmon and human coding sequences. Nomenclature for degenerate primers: N = A+C+G+T, B = G+C+T, V = G+A+C, H = A+T+C, Y = C+T, R = A+G.
DISCUSSION

This is the first report of the regulation of Kv transcription by oxygen supply in neuronal tissue. The downregulation of Kv1 transcripts within 4 h would likely result in a decrease in the corresponding channel protein as Kv channels are known to have a rapid turnover. In rats, for example, Kv1.5 channel protein and mRNA have half-lives of 4 and 0.5 h, respectively (33). In the turtle, a reduction in Kv channel gene expression may be a critical component in the orchestrated reduction in brain energy demand, the key response for brain anoxia survival (29), because it would reduce excitability and ion flux and therefore decrease the cost of ion pumping.

Alterations in Kv channel activity may also have other protective functions in anoxia and recovery. Inhibition of Kv channel expression could contribute to the defense against apoptosis by helping to maintain intracellular K+ concentrations during prolonged anoxia. Physiological levels of potassium inhibit caspase activation by abrogating oligomerization of Apaf-1, a critical process in apoptosome formation (4). Moreover, there is evidence in vascular smooth muscle that part of the anti-apoptotic effect mediated by Bcl-2 is due to an inhibition of Kv channel activity (10).

The downregulation of Kv expression could be mediated by hypoxia-related events, either directly through an O2 sensor-initiated process and/or indirectly through hypoxia-enhanced metabolites, neurotransmitters, or growth factors (Fig. 2) (30). In the anoxic turtle brain, for example, the downregulation of ATP-sensitive K+ channels is in part regulated through the activation of adenosine receptors (27). Antiapoptotic components such as Bcl-2 may also play a role in regulating Kv channel transcription (4). In the turtle, Bcl-2 mRNA levels are increased in the anoxic brain with maximal levels of expression observed at 6 h of anoxia in hindbrain and forebrain (22). In the mitochondria, the in situ oxygen affinity of cytochrome aa3 is lower in the turtle brain compared with the rat (31), suggesting that mitochondrial redox changes may occur earlier and thus be detected by the hypoxic turtle brain sooner than in mammals.

Potential signals or mediators for the upregulation of Kv transcription when oxygen supply is restored include activated O2 sensors, release of reactive oxygen species (ROS) and/or changes in mitochondrial redox status (34, 18). We previously found mRNA expression in turtle brain for the hypoxia sensor and transcriptional regulator HIF-1α (22). In the turtle brain, switching from normoxia to anoxia produced changes in a DNA binding activity that is specific to an HIF-1 promoter consensus site and in levels of the redox-regulated transcription factor NF-κB (22). In addition to its remarkable ability to survive anoxia, the turtle brain has an exceptional capacity to tolerate a massive increase in ROS on reoxygenation. The turtle has enhanced mechanisms that protect against the formation of ROS and mechanisms to protect against the damaging effects of ROS (23).

In conclusion, our observations of a decrease in turtle brain Kv1 mRNA in anoxia and a subsequent increase on reoxygenation demonstrate that brain Kv channel expression is sensitive to oxygen supply, either directly or indirectly, and indicate an important mechanism that matches brain activity to oxygen availability.

DISCLOSURES

This work was supported by the Florida Atlantic University Foundation and the American Heart Association (Florida Affiliate).

REFERENCES


AJP-Regul Integr Comp Physiol • VOL 285 • DECEMBER 2003 • www.ajpregu.org