

## GABA<sub>A</sub> RECEPTOR STRUCTURE AND FUNCTION IN NT2-N CELLS AFTER HYPOXIA

### Hypoxia Alters GABA<sub>A</sub> Receptor Function and Subunit Expression in NT2-N Neurons

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Hypoxia causes dysfunction of excitatory and inhibitory neurotransmission, often resulting in encephalopathy, seizures, or myoclonus. We evaluated the effects of hypoxia on  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> receptor (GABA<sub>A</sub>R) function and expression in an in vitro model of neuronal hypoxia. NT2-N cells, derived from the human NT2 teratocarcinoma cell line, were exposed to  $\leq 1\%$  O<sub>2</sub> for 8 hours and then used immediately for experiments or allowed to recover under normoxic conditions (95% air/5% CO<sub>2</sub>) for 24, 48, or 96 hours. Hypoxic treatment did not cause obvious morphologic changes or cell death. In whole-cell patch-clamp recordings, the GABA current median effective concentration (EC<sub>50</sub>) was unchanged; however, maximal GABA-evoked currents changed in a biphasic manner. Maximal GABA currents were significantly increased immediately after hypoxia, but were significantly reduced after 48-hour normoxic recovery, and then returned to baseline after 96-hour recovery. Maximal potentiation of 10  $\mu$ M GABA currents by diazepam was increased 48 hours after hypoxia, but potentiation by zolpidem was decreased. Barbiturate enhancement and zinc inhibition of GABA currents were unchanged. Semiquantitative reverse transcriptase (RT)-polymerase chain reaction (PCR) showed decreased  $\alpha_1$ ,  $\alpha_5$ ,  $\beta_2$ , and  $\gamma_2$  subunit mRNA after hypoxia. Hypoxic exposure altered GABA<sub>A</sub>R physiology and subunit mRNA expression, which may correlate with symptoms observed after hypoxia in vivo.

animal, brain-slice preparations, primary co-cultures, and acutely dissociated neuronal preparations. These studies have yielded a diverse yet complementary set of findings associated with the physiologic and pathologic changes in neurons caused by hypoxia. Adding creatively to this mix, Gao et al. studied the effects of hypoxia on the structure and function of GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) expressed in NT2-N cells. NTera2 cells, the progenitors of NT2-N cells, are derived from human teratocarcinoma and undergo terminal differentiation to NT2-N cells after treatment with retinoic acid. NT2-N cells are characterized by dendritic and axonal processes and express neuronal markers and functional neurotransmitter receptors, including human GABA<sub>A</sub>Rs. Importantly, these cells do not form synapses and therefore allow studies that assess receptor pharmacology and kinetics as well as the regulatory mechanisms of receptor and receptor subunit expression devoid of presynaptic influences.

In their studies, Gao et al. exposed NT2-N cells to 8 hours of hypoxia, which resulted in little to no cell death or significant alteration of cellular morphology. By pharmacologically assessing GABA-evoked chloride currents in individual cells and performing RT-PCR of GABA<sub>A</sub>R subunit mRNA expression in populations of cells up to 4 days after hypoxia, the authors were able to correlate various structure–function relations at time points reflecting the acute and resolving effects of the hypoxic conditions. In general, their findings indicated that several aspects of GABA<sub>A</sub>R structure and function changed dramatically after hypoxia, if transiently, whereas other properties were unchanged. A notable exception to these findings was the persistently reduced expression of  $\alpha_1$  subunit mRNA.

A point of interest in these studies is that the O<sub>2</sub> tensions ( $\leq 1\%$ ) used by the authors likely approximate those found in penumbral areas during periods of experimental ischemia. Thus this experimental paradigm raises several issues regarding the extent, time dependence, and reversibility of alterations in neuronal gene expression, physiologic functioning, and potentially permanent injury induced by other models using hypoxic or ischemic conditions or both. Some of the changes to GABA<sub>A</sub>R composition and function seen in this study may contribute to the molecular substrate that transitions compromised tissue to epileptogenicity, but it is often unclear whether these changes are cause and effect, correlative, or compensatory in nature. A simple question prompted by the study's straightforward

### COMMENTARY

The effects of hypoxia on neuronal function have been studied in various experimental systems, including whole

experimental design is whether transient changes in neuronal receptor function after insult can contribute to the latent period of epileptogenesis in contrast to more permanent changes; the latter is more readily considered a potential earmark of epileptogenesis. Some of these issues have been broached in other models of hypoxia (1), ischemia (2), and temporal lobe epilepsy (3), and await thorough elucidation.

Combining patch clamping with the use of single-cell RT-PCR and amplified RNA techniques applied to NT2-N cells would permit the present studies to be extended to obtain more specific information regarding GABA<sub>A</sub>R subunit mRNA expression in individual human cells after varied conditions of hypoxia and, ultimately, to be correlated with determinations of subunit protein expression. Taken together, these techniques can address basic questions of cellular physiology and GABA<sub>A</sub>R structure and function, including (a) why do some properties of GABA<sub>A</sub>Rs remain invariant after lesioning; (b) why do certain aspects of GABA<sub>A</sub>R function undergo significant change, only to revert to baseline condition; and (c) why do some GABA<sub>A</sub>R properties, once altered, appear destined to remain altered permanently. Admittedly, this system has significant constraints as a result of the lack of synapse formation and functional cel-

lular networks, and extrapolations of these findings must be guarded. However, the authors have provided an imaginative in vitro system to complement other experimental approaches to understand more fully the potential relations between hypoxic injury, human GABA<sub>A</sub>Rs, and epileptogenesis—an extremely important area for translation to clinical studies and therapy.

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## References

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