

**The major contribution of brain GABAergic function to anoxic survival.**

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## **Introduction.**

The paper by Ellefsen et al (3) is a fascinating large scale analysis of genes associated with GABA neurotransmission in an extremely important model of anoxia tolerance, the crucian carp, *Carassius carassius*. Under conditions of anoxia the crucian carp brain increases its extracellular GABA levels in conjunction with elevating GABA activity. In the mammalian brain GABA is known to play an inhibitory role and this neurotransmitter may function as part of a defense system against the effects of the massive release of excitatory neurotransmitters that occurs during ischemia/anoxia (10). In the ischemic mammalian brain however GABAergic activity is decreased (18), defense systems are overwhelmed and the net effect is catastrophic cell death within minutes of the onset of anoxia. Understanding the mechanisms underlying brain survival in anoxia tolerant organisms can contribute important information on the key pathways that may contribute to neuro-protection in the face of anoxia and hypoxia/ischemia.

The current study reports quantification of 22 genes involved in GABA neurotransmission by Real Time RT-PCR including GABA-A receptor subunits, GABA-B receptor subunits, enzymes involved in GABA synthesis and metabolism, GABA transporters, GABA-A receptor associated proteins and the KCL co-transporter. Amongst GABA-A receptor subunits alpha 4, alpha 6 and delta were the predominant forms in crucian carp brain while the major GABA transporters expressed were GAT2a, GAT2b and GAT3. At one day anoxia the GABA-A receptor alpha 3 subunit decreased significantly compared to controls and at day seven the alpha 3 and alpha 6 and delta subunits were decreased. After one day anoxia the GABA transporters GAT2a and GAT3 were significantly reduced whereas at 7 day anoxia GAT2a, GAT2b and GAT3 expression were decreased. The key finding that levels of GATs were decreased would be consistent with the observed increase in extracellular GABA as well as elevated GABAergic function in the brain of the anoxic crucian carp (9).

The GABA synthesizing enzyme GAD65 was also decreased at seven days of anoxia. In contrast to the aforementioned alterations, expression levels of GABA-B receptor subunits were unchanged during anoxia. Importantly there was no change in expression of the potassium chloride cotransporter KCC2 which plays an essential role in maintaining GABAergic activity by restoring chloride homeostasis following opening of the GABA-A receptor channel. KCC2 is decreased in mammals after an excitotoxic insult (16,17).

## **Strategies for anoxic brain survival.**

The crucian carp employs unique mechanisms for anoxic brain survival. This organism is capable of up-regulating key glycolytic enzymes and enhancing anaerobic glycolysis while maintaining much neural activity for continued motor and sensory functions. Unlike all other vertebrates the genus *Carassius* employs ethanol as the major end product of glycolysis instead of lactate and thus the crucian carp can maintain high level glycolysis without experiencing the detrimental consequences that would otherwise ensue from lactate buildup (12). In its anoxic survival strategy the crucian carp has been contrasted with the freshwater turtle in which the anoxic brain demonstrates a profoundly

decreased energy consumption associated with a comatose-like state. The turtle achieves its anoxia tolerance through a tight regulation of excitatory neurotransmission, elevated levels of inhibitory neurotransmission and diminished ATP utilisation through decreased ion pumping (termed “channel arrest”) and through decreases in other ATP consuming processes including protein synthesis (8,10).

### **Comparisons between anoxia tolerant organisms and mammals.**

After 6 hours of anoxia the crucian carp brain demonstrates a doubling of extracellular GABA levels and in prolonged anoxia a 5 fold increase is observed (13,9). In turtle brain there is a large and sustained increase in extracellular GABA levels rising from 0.3 to 27 micromolar over 2-3 hours of anoxia and increases in GABA-A receptor density are found which continue over at least 24h of anoxia (14,10). These elevations in GABAergic activity in the crucian carp and turtle brains appear to contribute to a reduction in ATP use in anoxia. In mammalian models GABA may also contribute a protective function. In rats subjected to excitotoxic seizures the GABA synthesizing enzymes GAD65 and GAD67 are increased in expression which would be consistent with an enhanced GABAergic activity (5). In studies on ischemic preconditioning in mammalian models an enhancement of GABA release and an increase in GABA-A receptor number is found in conjunction with a decrease in glutamate release (7). Neuroprotection through enhanced GABAergic function has been demonstrated in mammalian models either by preventing GABA reuptake and metabolism or through increasing GABA-A receptor activity (18). Recent gene therapy interventions for Parkinson’s disease also point to a key neuroprotective role for GABAergic neurotransmission and experiments involving viral mediated GAD over-expression are very promising in this regard (6).

### **Constitutive preconditioning in the brain.**

Ischemic preconditioning is a phenomenon described in mammals whereby a mild brief ischemic episode can result in a later protection against a more severe ischemic episode. Preconditioning has been reported in a variety of tissues including brain, heart and kidney and may either result from ischemia or other stimuli such as exercise, or a slight increase in temperature. In the crucian carp brain the subunit composition of the GABA-A receptors reported in the current paper may represent an example of constitutive preconditioning. As discussed by Ellefsen et al. (3) GABA-A receptors in crucian carp comprise delta and alpha 4/alpha 6 subunits which are extra-synaptic in mammals and it is reported that delta subunit containing receptors do not desensitize in response to long term binding of GABA (1). Hence delta subunit containing receptors may be continuously functional and may not require to be induced in the transition to anoxia. In the turtle brain evidence of constitutive preconditioning has also been proposed in relation to the heat shock proteins Hsp72 and Hsp73 which are significantly expressed in normoxia and which may represent a state of preparedness before Hsp72 and Hsp73 levels are further elevated in anoxia (15).

## **Excitatory neurotransmitters in anoxia.**

The current paper complements another recent publication by the same group (4) analyzing expression of genes involved in excitatory neurotransmission in the anoxic crucian carp. In that publication the NMDA receptor subunits NR2B and NR2D dominated the expression of NR2 subunits as they do in neonatal rats which are themselves hypoxia tolerant thus confirming the suggestion that NR2 subunit composition is a determining factor in hypoxic survival. Importantly NR1, NR2C and NR3A expression decreased in anoxia in the crucian carp brain. The decrease in NR2C was potentially part of a strategy to decrease calcium influx and thus save energy otherwise expended on ion pumping. Thus while the crucian carp remains active in anoxia though at a reduced level and does not appear to demonstrate a major degree of “channel arrest” there may be specific examples of decreased ion pumping related to NMDA receptor subunits in the crucian carp brain.

The crucian carp to a large extent maintains its neuronal function in anoxia unlike the freshwater turtle which can also tolerate complete anoxia but which renders itself essentially comatose in the face of total oxygen deprivation. The turtle brain demonstrates tightly regulated control over glutamate neurotransmission as part of a survival strategy whereby glutamate release is decreased and glutamate reuptake mechanisms continue to operate (11). In the turtle therefore glutamate neurotransmission is inhibited but is still active and functional albeit at a low level. A similar effect on brain protection has been reported in the rat brain after percussion injury where stimulation of NMDA receptors by NMDA 24 and 48 hours after closed head injury attenuated neurological deficits (2). A number of clinical trials using NMDA blockers after head injury had failed to show efficacy. Thus in mammals prolonged blockade of NMDAR may potentially be harmful in head trauma or after ischemia.

## **Concluding remarks.**

In conclusion the paper by Ellefsen et al. (3; this issue) presents an important large scale analysis of genes associated with GABAergic neurotransmission in the brain of a unique model of anoxia tolerance the crucian carp. As the nature of the GABA-A receptor subunit composition in the brain of crucian carp points to a constitutive GABAergic mediated protection and as GAT transporters are decreased this paper provides evidence for a major contribution of control over GABAergic neurotransmission in the mechanisms of anoxia tolerance. Recent approaches in mammalian models to enhancing GABAergic function point to potential clinical applications for treatment of ischemic stroke and neurodegenerative disease.

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