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Blood-mediated control of the Physiology and Pathology of Glutamate in brain

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The work in our laboratory focuses mainly on the problem of how to reduce the neurotoxic levels of the main excitatory neurotransmitter Glutamate (Glu) that are found in brain fluids in a large number of acute and chronic neurological conditions. We aim ultimately at providing acute neuroprotection in cases such as brain stroke, perinatal brain damage, traumatic brain injury, bacterial meningitis, hemorrhagic shock, and to hamper the chronic neurodegenerative process taking place in amyotrophic lateral sclerosis, experimental allergic encephalitis and multiple sclerosis.

We are also interested in the properties of Glu receptors and how these can be modulated by external agents such as antibodies and by internal proteins such as spectrin or neurofilament.

1) Boosting brain autoprotective mechanisms in neurodegenerative diseases

Brain functions depend entirely on the steady supply of blood-borne nutrients reaching every single cell in the brain via the extremely dense network on blood capillaries. Crossing the capillary endothelial cells that form the blood-brain barrier, the nutrients diffuse and are taken up by neuronal and glial cells to enable their various activities. However, brain metabolic and synaptic activities are also accompanied by the formation of potentially neurotoxic products that ought to be eliminated in order to allow the safeguarding and continuation of normal brain functions. The autoprotective mechanisms preventing the brain self poisoning are still poorly studied although it is recognized that the brain-blood barrier plays an important role.

Our laboratory is focusing on the study of the brain defense mechanisms against Glutamate which are based on sets of Glu transporters present not only on neurons and glia but also on the brain side of the capillary endothelial cells that form the blood brain barrier.

We have shown that the Glu transporters present on the brain blood capillaries exert an autodefense mechanism since the injection into brain of radioactive Glu in the lateral ventricles cause a rapid appearance of radioactivity in blood. This brain-to-blood Glu efflux is the basis of several of our studies which include whole animal investigations, the use of in vitro models of the blood brain barrier and of in silico theoretical models. We have asked whether it might be possible to boost the natural brain-to-blood Glu efflux and provide thereby a novel approach to the treatment of those neurodegenerative diseases such as stroke and head trauma that are characterized by elevated and neurotoxic levels of Glu in brain. We surmised that an increased brain-to-blood Glu efflux could result from lowering the Glu levels in plasma increasing thereby the driving force for Glu fluxes from brain interstitial or cerebrospinal fluids into blood.

To test this idea, we studied the *in vitro* and *in vivo* conditions allowing a decrease of plasma Glu. We achieved this objective by causing the activation of two blood resident enzymes Glu-pyruvate transaminase and Glu-oxaloacetate transaminase (GOT) upon administration into blood of the Glu co-substrates, pyruvate (Pyr) and oxaloacetate (OxAc). We established that the intravenous administration of Pyr and OxAc produces not only a decrease of blood Glu levels but causes also an increased brain -to-blood Glu efflux and a concomitant decrease of Glu levels in brain interstitial and cerebrospinal fluids (see Fig.1).

So far, we have obtained evidence that intravenous blood Glu scavengers OxAc and Pyr provide neuroprotection in a rat model of an acute brain injury resulting from closed head trauma. In this case, rats were injected intravenously, 1 hour after the head injury, with solutions of Pyr, OxAc, with or

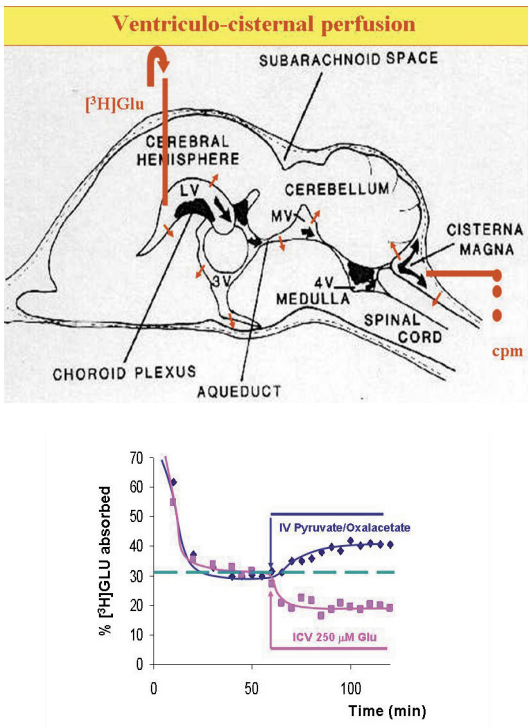

Fig. 1 *Evidence of an increased brain-to-Glu efflux by blood Glu scavenging. Upon its perfusion through the lateral ventricule (LV) to the cisterna magna, [3H] Glu is absorbed into brain tissue (small red arrows upper figure) until a steady absorption level is reached (Green line –lower figure). The extent of [3H] Glu absorption by brain can be increased by the intravenous injection of blood Glu scavengers (blue arrow), or decreased when the Glu transporters are saturated following the perfusion of [3H] Glu with high doses of Glu (purple arrow).*

without Glu, and a neurological severity score was determined 48 hours later.

We observed that the intravenous administration of OxAc but not that of OxAc + Glu, reduced significantly the neurological severity score, establishing the therapeutic activity of OxAc. The observation that Glu counteracts the neuroprotective effects of OxAc and Pyr argues in favor of the concept that the beneficial effects of OxAc and Pyr observed in this acute neurodegenerative condition are indeed exerted via blood Glu scavenging.

To optimize the process of blood Glu scavenging, we are now developing by *in vitro* evolution GOT mutants that are then selected for improved enzymatic properties. We hope that the intravenous administration of one such mutant together with OxAc will provide more effective neuroprotection in all emergency conditions involving excess brain Glu.

2) Interaction of the NMDA-Receptor cytoplasmic domain with subsynaptic proteins

It is well established that dendritic spines that carry Glu receptors undergo morphological changes upon exposure to Glu . This observation suggests that the activation of the dendritic Glu receptors by Glu is transduced within the dendritic spine to a contractile element within the cytoskeleton. Investigating the possible protein partners of the NMDA receptor subtype of Glu receptors, we have found that neurofilament-light binds selectively to the C-terminal cytoplasmic domains of the NMDA receptor and regulates its surface expression by preventing its ubiquitinylation.

Selected Publications

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Gottlieb M. and Wang, Y. Teichberg V.I, (2003) Blood-mediated scavenging of cerebrospinal fluid Glutamate . J. Neurochem 87, 119-126.

Wang Y., Gottlieb M and Teichberg V.I (2004) An evaluation of erythrocytes as plasma Glu scavengers. Neurochem.Res 29, 755-760

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