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$GABA_B$ receptor expression and cellular localization in gerbil hippocampus after transient global ischemia

F. Vollenweider [a,*], K. Bendfeldt [a], W. Maetzler [a,1], U. Otten [b], C. Nitsch [a]

[a] *Functional Neuroanatomy, Institute of Anatomy, Basel University, Pestalozzistrasse 20, CH-4056 Basel, Switzerland*
[b] *Institute of Physiology, Basel University, c/o Institute of Anatomy, Pestalozzistrasse 20, CH-4056 Basel, Switzerland*

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Abstract

Using in situ hybridization, the expression of the GABA receptor subtype B subunit 1 ($GABA_B$ R1) and subunit 2 ($GABA_B$ R2) following transient global ischemia in the gerbil hippocampus was investigated. In sham-operated animals, mRNAs of both subunits were mainly detected in hippocampal pyramidal cells and interneurons with lower expression levels of the $GABA_B$ R2 in the CA1 field. Four days after transient cerebral ischemia, neuronal message decreased in conjunction with neuronal death and both receptor subunits disappeared from the pyramidal cell layer. However, $GABA_B$ R1 and $GABA_B$ R2 were still expressed in a few cells. In situ hybridization of the GABA synthesizing enzyme glutamic acid decarboxylase 67 (GAD67) remained unchanged after the ischemic insult. Double-labeling experiments revealed that in the postischemic hippocampus $GABA_B$ R1 and $GABA_B$ R2 were not present in GFAP-reactive astrocytes, but that the surviving parvalbumin-containing interneurons possessed $GABA_B$ R1 and $GABA_B$ R2 mRNA.

Keywords: Stroke; CA1; Neurodegeneration; Neuroprotection; GAD67; Parvalbumin

Cerebral ischemia leads to nerve cell death in vulnerable regions of the brain. Excessive release of the neurotransmitter glutamate has been so far identified as one of the major reason leading to neurodegeneration. However, no clinically acceptable glutamate antagonists have emerged because of their toxicity and numerous side effects. Since many neurotransmitters play a role in the degeneration of neurons produced by ischemia, and because many GABAergic drugs have demonstrated neuroprotective effects in animal model of cerebral ischemia, studying GABA neurotransmission may be of particular interest.

In transient cerebral ischemia, hippocampal GABAergic neurons survive the ischemic insult while the CA1 principal neurons degenerate. In several instances, it has been shown that GABA interneurons may be more resistant to cell death in comparison to pyramidal neurons [9,24,25]. However, some studies demonstrated that interneurons expressing the GABA-synthesizing enzyme GAD in the gerbil CA1 field can be very susceptible to ischemia [17]. In addition, GABAergic neurons could be very sensitive to ischemia in the dentate gyrus, degenerating 12 h after ischemia [7]. Finally, in vitro studies demonstrated that GABAergic neurons could be sensitive to oxygen deprivation [13].

GABA is the brain's main inhibitory neurotransmitter and acts on two different types of target receptors, the $GABA_A$ receptor and the $GABA_B$ receptor. The $GABA_A$ receptor belongs to the broad class of multi-subunit proteins that form a multimeric ligand-gated Cl^- ion channel, whereas the $GABA_B$ receptor is a heterodimeric G-protein-coupled receptor, composed of two subunits, B1 and B2 ($GABA_B$ R1 and $GABA_B$ R2) and is expressed post- and presynaptically [1]. Activation of the $GABA_A$ receptors results in a rapid chloride-dependant conductance changes whereas the actions of the $GABA_B$ receptors tend to last much longer.

Few investigators have focused on the ischemia induced alterations in inhibitory neurotransmission: GABA accumulates

Abbreviations: GABA, γ-aminobutyric acid; $GABA_B$ R, GABA receptor subtype B; $GABA_B$ R1, GABA receptor subtype B subunit 1; $GABA_B$ R2, GABA receptor subtype B subunit 2; GFAP, glial fibrillary acidic protein; PV, parvalbumin; NeuN, neuronal nuclei; GAD67, glutamic acid decarboxylase 67; CA, cornu amonis; RT, room temperature; mRNA, message ribonucleic acid; PBS, phosphate-buffered saline; PFA, paraformaldehyde; NGS, normal goat serum; ABC, avidin–biotin complex; ER, endoplasmic reticulum

* Corresponding author. Tel.: +41 61 2672729; fax: +41 61 2673959.
E-mail address: Florence.Vollenweider@unibas.ch (F. Vollenweider).
[1] Present address: Department of Neurodegenerative Diseases and Hertie-Institute for Clinical Brain Research, University of Tuebingen, Germany.

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in the extracellular space during ischemia, and in the case of transient ischemia, it returns to normal levels within 1 h of reperfusion [12,16,26]. Alteration in the GABA-gated channel following transient forebrain ischemia has been demonstrated in Mongolian gerbils [22] and in rats after MCA-occlusion [27]. Different measures have been used to assess the effects of ischemia in vivo and in vitro on $GABA_A$ receptor function including electrophysiology and optical imaging of changes in Cl^- flux. In addition to the reduction of GABA neurotransmission observed early after ischemia in vivo, there are also long-lasting effects [20]. A decrease in $GABA_B$ receptor expression (mRNA) but not functionality (determined by the inhibitory effect of its agonist baclofen on f-EPSP) in CA1 and CA3 was observed 24 h after ischemia, a time when the ischemia-induced delay in neuronal death had not yet taken place [10,18,28].

A role of $GABA_B$ receptors in seizure disorders has been demonstrated, as $GABA_B$ receptors antagonists could alter absence-type seizures in genetic animal models [14,19]. However, little is known about their role under other pathological conditions, in particular those implying neuronal cell death like ischemia. Changes in either receptor activity or quantity could alter the normal level of synaptic inhibition present in the hippocampus and therefore modify the efficacy of excitatory neurotransmission. It has been demonstrated that $GABA_A$ receptor agonists (benzodiazepines) can exert neuroprotective effects against ischemia by increasing endogenous GABA levels via activation of both $GABA_A$ and $GABA_B$ receptors [6]. To maintain inhibitory neurotransmission at a critical time after an ischemic event, GABAergic neurotransmission may help to promote recovery from early signals related to cell injury [29].

The present study was undertaken to clarify $GABA_B$ R1 and $GABA_B$ R2 expression and distribution in surviving neurons of the hippocampus after an induced transient forebrain ischemia in gerbils. In particular, we determined which types of cells express the proteins and correlated the expression levels with the changes provoked by the insult.

Two to 6-month-old male Mongolian gerbils (50–70 g) were subjected either to bilateral forebrain ischemia for 7 min or to a sham operation. At 2 and 4 days after recirculation, animals were deeply anaesthetized and transcardially perfused with 4% paraformaldehyde in 0.1 M PBS (PFA). At least three animals per time point were used. All animal experimentation was performed with permission of the local animal care committee and according to present Swiss law.

The brains were post-fixed in PFA, cryoprotected in a 30% sucrose/PBS buffer overnight, and then frozen at −80 °C in isopentane. Serial sagittal 40 μm sections were cut with a cryostat. One section series per animal was stained with cresyl violet to obtain a general estimate of the status of the tissue preservation.

Immunolabeling of glial fibrillary acidic protein (GFAP; 1:1000; Dako), parvalbumin (PV; 1:2500; Swant), and neuronal nuclei (NeuN; 1:1000; Chemicon) was carried out as previously described [25], by using the avidin–biotin detection system (Vectastain ABC kit) and diaminobenzidine for visualization. Labelled cells were studied by light microscopy and photographed using a digital imaging camera (Zeiss Axio Vision 3.1).

In situ hybridization was performed as described [30]. Briefly, $GABA_B$ R1 and $GABA_B$ R2 were transcribed in vitro from pBS SK vector containing the rat GABA receptor subtype B subunit 1a/b and subunit 2 and were kindly given by Dr. B. Bettler (Pharmacenter, Basel, Switzerland). The rat glutamic acid decarboxylase GAD67 (generously given by Dr. Tobin; University of California, Los Angeles, USA) was transcribed in vitro from a pBluescript SK vector [8]. The riboprobes were labeled with digoxigenin-UTP according to the manufacturer's recommendation (Roche Molecular Biochemicals). After the hybridization and washing procedures, messenger RNA was detected with a digoxigenin antibody conjugated to alkaline phosphatase according to the manufacturer's protocol (Roche Molecular Biochemicals). A sense probe, which did not bind to mRNA was used as control cell staining. Sections were mounted on Superfrost slides and coverslipped with Kaiser gelatin. A double-labeling technique was used to further characterize the localization of the $GABA_B$ receptors. First, in situ hybridization was carried out. Sections were then incubated either with a monoclonal antibody against PV or GFAP. Antibody complexes were finally detected using ABC.

Transient forebrain ischemia in the Mongolian gerbil is a reliable model for cerebral ischemia. Due to its incomplete circle of Willis, bilateral transient occlusion of the common carotid arteries produces a highly reproducible forebrain ischemia, resulting in delayed cell death in CA1 principal neurons. The CA1 pyramidal cells start dying 2–3 days after the restoration of circulation. After 4 days widespread regressive change was observed in the CA1 field and most pyramidal neurons have disappeared, as shown in the neuronal NeuN staining (compare Fig. 1A and B). In some cases a number of CA2/CA3 pyramidal cells could also die. However, a certain amount of neurons in CA1 appeared to be resistant to the insult and had been shown by immunocytochemistry to be GABAergic interneurons [9,24]. In this study we used in situ hybridization to demonstrate that four days after ischemia GAD67 mRNA expression remained unchanged (compare Fig. 1C and D). There was no change in the overall number of cells expressing GAD67. In parallel, PV labeling, which stains a subgroup of inhibitory GABA interneurons, did not change 4 day after the ischemic insult (not shown). This demonstrated that in gerbils, GABAergic neurons in CA1 labeled by a GAD67 riboprobe were resistant against neurodegeneration produced by transient ischemia.

In gerbil, localization of the $GABA_B$ R1 mRNA, detected by in situ hybridization was restricted to the perikaryon and was intense in the hippocampal formation, cortex, thalamus, striatum, basal forebrain, superior and inferior colliculus, as well as in the substantia nigra and cerebellum. The $GABA_B$ R2 subunit mRNA presented a staining with a more restrictive pattern than the B1 subunit: mostly in hippocampus, cortex, superior and inferior colliculus, thalamus and cerebellum (data not shown). In hippocampus, $GABA_B$ R1 message was strong in the principal cell layers of CA1, CA2, CA3 and dentate gyrus. Interneurons in the stratum radiatum and lacunosum moleculare were also stained (Fig. 2A). $GABA_B$ R2 message