© Med Sci Monit, 2004; 10(Suppl 3): 88-93

WWW.MEDSCIMONIT.COM
Supplement 3

GABA in ischemic stroke. Proton magnetic resonance study

Lidia Głodzik-Sobańska[1], Agnieszka Słowik[1], Justyna Kozub[2], Barbara Sobiecka[2], Andrzej Urbanik[2], Andrzej Szczudlik[1]

[1] Department of Neurology, Collegium Medicum, Jagiellonian University, Cracow, Poland
[2] Department of Radiology, Collegium Medicum, Jagiellonian University, Cracow, Poland

Author's address: Lidia Głodzik-Sobańska, Department of Neurology, Institute of Neurology, Botaniczna 3, 31-503 Kraków, Poland, e-mail: lsob@interia.pl

Summary

Background: *Experimental studies have suggested that ischemic stroke causes increment in extracellular level of gamma-aminobutyric acid in response to excessive glutamate concentration and function. The increased GABA concentration is followed by subsequent inhibition of GABA synthesis, thus leading to GABA-ergic dysfunction. Enhancing GABA function seems to be a way of neuroprotection after cerebral insult.*

Animal models have shown there is overactivity of excitatory neurotransmitters and decreased tone of GABA-ergic system also in the remote neocortical regions. Data concerning changes in brain areas outside the stroke lesion in humans is sparse. These regions could be possible targets for therapeutic intervention. Progress in imaging techniques enables separation of a great number of chemical compounds. Our aim was to assess GABA levels outside the ischemic lesion by means of proton magnetic resonance spectroscopy (^1H MRS).

Material and Methods: *The study compared 31 patients with first-ever ischemic stroke and 20 healthy subjects. Single voxel H^1-MRS was performed to measure GABA/Cr ratios in structurally normal prefrontal regions, distant from the stroke lesion. The amonut of the remaining metabolites (NAA, Cho, mI, Glx) was also estimated. Patients underwent the examination in the acute phase of the disease and 3 months later.*

Results: *Both early after stroke and more than 3 months later, the patients had lower GABA levels. However, during the second examination, this difference was evident only in the frontal cortex ipsilateral to the lesion.*

Conclusions: *These findings suggest that GABA function is decreased outside the infarct. Further studies are needed for confirmation of the results and elucidation of the possible role of GABA alterations in stroke recovery and therapy.*

Key words: stroke • proton magnetic resonance spectroscopy • GABA

BACKGROUND

Proton magnetic resonance studies of stroke carried out to date concerned mainly the changes present in the ischemic focus itself. Typical abnormalities include the decreased level of N-acetylaspartate (NAA) and increased concentration of lactates (Lac) [1,2]. These changes reflect neuronal deficits (NAA) and phagic activity of macrophages within the area of malacia [3,4]. They are, however, the final effect of a cascade of unfavorable events, initiated, among others, by an increase of extracellular glutamate level, activation of NMDA receptors, and calcium inflow into the cells. Compensatory increase of gamma-aminobutyric acid (GABA) concentration occurs in response to the excesive accumulation of the glutamates in the extracellular space, to inhibit their excitotoxic effect [5]. Thus, enhancing the GABA-ergic system function seems to be one of the possible ways of neuroprotection after a cerebral insult [6].

The course of stroke depends on the equilibrium between the system of excitatory and inhibitory neurotransmitters. Lower plasma concentration of gamma-aminobutyric acid and higher concentration of glutamate was associated with worsening and exacerbation of the neurological deficits in lacunar strokes [7].

GABA is a primary neurotransmitter and modulator secreted by all migrating cells during the period of brain development. Precursor neuronal cells, synthesizing GABA, are then influenced by diffrentiating substances and transformed into glutaminergic, monoamin-

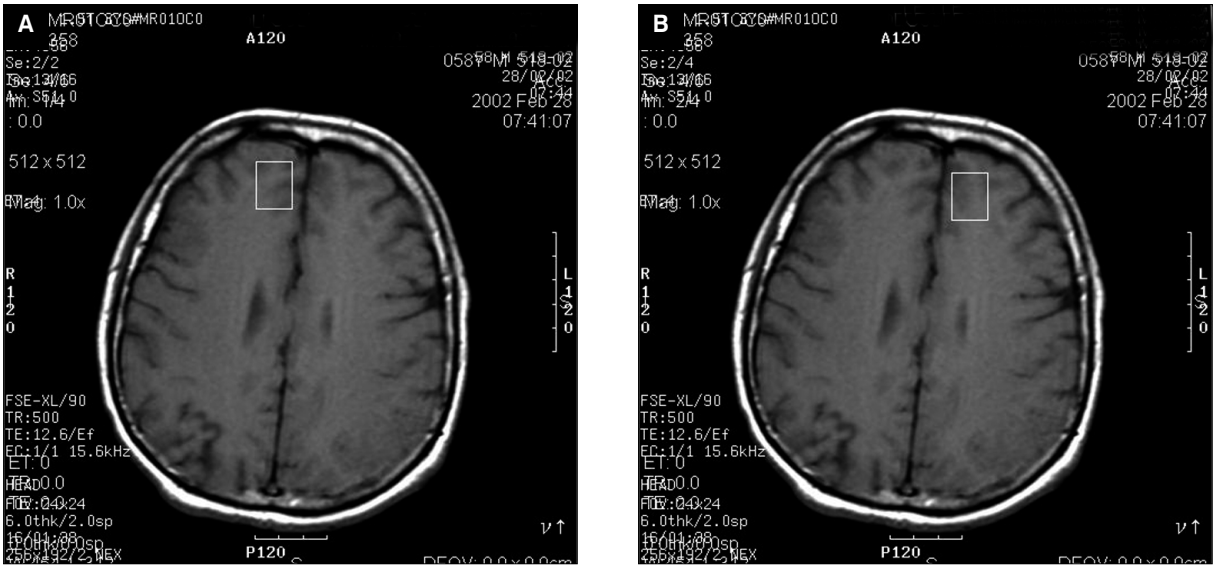


Figure 1A,B. Localization of the region of interest in T1-weighted images.

ergic, cholinergic or peptidergic neuronal phenotypes. However, even mature, differentiated neuronal or glial cell phenotypes are still characterized by measurable activity of glutamic acid decarboxylase (a GABA-synthesizing enzyme) and GABA [8]. GABA-expressing cells are common in the whole nervous system, but they are especially numerous in the telencephalic structures – particularly in the cerebral cortex [9]. They form neuronal networks influencing the function of other transmitter systems [10]. Animal model studies have demonstrated that focal ischemia, in addition to local changes, causes a disturbance of the equilibrium between the glutaminergic and GABA-ergic systems in neocortical areas remote from the ischemic focus in the hemisphere ipsi-, as well as contralateral to the lesion. This disturbance involves an increase of NMDA and decrease of GABA receptors density [11].

Little is known about the distant changes in the course of stroke in humans. Such knowledge would be important, because the unaffected cerebral tissue is the target site of pharmacological agents used in treatment. ^{1}H MRS is a technique allowing non-invasive *in vivo* assessment of biochemical changes in the particular brain region. The advances in spectroscopy (better resolution of the spectrum), have made it possible to identify more and more chemical compounds, including gamma-aminobutyric acid bands.

The aim of the study was to assess the changes of GABA levels in brain areas distant from the ischemic focus and rich in GABA-producing neurons – in the neocortex, by means of ^{1}H MRS technique. The amonut of the remaining metabolites (NAA, Cho, mI, Glx) was also estimated.

MATERIAL AND METHODS

The patients with ischemic stroke treated in the Stroke Unit, Colegium Medicum Jagielonian University Department of Neurology, in Cracow from January 2001 to February 2002 were included in the study. The inclusion criteria were as follows: the first-ever ischemic stroke, defined according to WHO criteria [12], no symptoms of cerebellum and brain stem damage or symptoms suggesting ischemia in the area supplied by the anterior cerebral artery both in neurological assessment and in CT. The following exclusion criteria were applied: signs of intracerebral hemorrhage in cranial CT, the history of previous depression, psychotic disorders, alcoholism, dementive syndromes, treatment with psychoactive drugs within one month before admission to hospital, poor clinical condition or other contraindications making it impossible to perform MRI.

The control group consisted of 20 sex- and age-matched healthy volunteers.

Magnetic resonance and proton spectroscopy

The patients were subjected to MR and ^{1}H MRS in the acute phase of the disease and after a few months using Signa GE Horizon 1.5T equipment, Dept. of Radiology, CMUJ. Transverse section T1- (TR-500 ms, TE-12, 6 ms) and T2-weighted (TR-5000 ms, TE- 100 ms) images were acquired. The slice thickness was 6 mm, the gap between slices 2 mm. ^{1}H MRS spectra were obtained using single voxel localized spectroscopy method.

The localization of the region of interest (ROI) was determined on the basis of T1-weighted MR images. Each time, ROI was placed subsequently in the right, and then in the left hemisphere, in the anterior part of the frontal lobe, in the prefrontal area, near the midline, in apparently normal area unaffected by ischemia. It was localized in the image, in which the central or upper part of the lateral ventricle was still visible (Figure 1). The region of interest, of ca. 6 cm^{3} mean volume, included the cortex of the frontal lobe and the subcortical white matter. The spectrum was recorded using point resolved spectroscopy (PRESS) impulse sequence (TR=1500 ms, TE=35 ms).

Table 1. Comparison of particular metabolite levels during the first spectroscopy, in the prefrontal region ipsi- and contralateral to the ischemic focus, in stroke patients and control subjects (40 measurements).

	Stroke patients	Control group	Statistical significance
$NAA/Cr_{ipsilateral}$	1.7981±0.4938	1.6890±0.2770	n.s.
$NAA/Cr_{contralateral}$	1.6256±0.3876	1.6890±0.2770	n.s.
$Cho/Cr_{ipsilateral}$	0.9275±0.2287	0.9657±0.1836	n.s.
$Cho/Cr_{contralateral}$	0.8838±0.1889	0.9657±0.1836	n.s.
$mI/Cr_{ipsilateral}$	0.9946±0.4624	0.8656±0.1886	n.s.
$mI/Cr_{contralateral}$	0.8479±0.3026	0.8656±0.1886	n.s.
$GABA/Cr_{ipsilateral}$	0.4612±0.2506	0.5373±0.1874	$p<0.03$
$GABA/Cr_{contralateral}$	0.4134±0.1367	0.5373±0.1874	$p<0.004$
$Glx/Cr_{ipsilateral}$	0.4711±0.2228	0.4655±0.1855	n.s.
$Glx/Cr_{contralateral}$	0.4296±0.1335	0.4655±0.1855	n.s.

Three months after the onset of the disease, stroke patients were still characterized by lower GABA/Cr values in both prefrontal regions (both in the hemisphere ipsilateral to the ischemic focus and in the contralateral one); however, the difference reached statistical significance for the ipsilateral area only (Table 2). The values of NAA/Cr, Cho/Cr, mI/Cr and Glx/Cr did not differ significantly on both examinations

Table 2. Comparison of particular metabolite levels during the second spectroscopy, in the prefrontal region ipsi- and contralateral to the ischemic focus, in stroke patients and control subjects (40 measurements).

	Stroke patients	Control group	Statistical significance
$NAA/Cr_{ipsilateral}$	1.5700±0.2962	1.6890±0.2770	n.s.
$NAA/Cr_{contralateral}$	1.6959±0.3791	1.6890±0.2770	n.s.
$Cho/Cr_{ipsilateral}$	0.9031±0.1564	0.9657±0.1836	n.s.
$Cho/Cr_{contralateral}$	0.9169±0.2135	0.9657±0.1836	n.s.
$mI/Cr_{ipsilateral}$	0.9033±0.2798	0.8656±0.1886	n.s.
$mI/Cr_{contralateral}$	0.8494±0.2662	0.8656±0.1886	n.s.
$GABA/Cr_{ipsilateral}$	0.4072±0.1898	0.5373±0.1874	$p<0.004$
$GABA/Cr_{contralateral}$	0.4744±0.1722	0.5373±0.1874	n.s.
$Glx/Cr_{ipsilateral}$	0.4305±0.1682	0.4655±0.1855	n.s.
$Glx/Cr_{contralateral}$	0.4339±0.1169	0.4655±0.1855	n.s.

The number of acquisitions was 256. Prior to spectrum recording, an automatic procedure increasing the field homogeneity within the patient's head and the selected region of interest was applied. The signal of water was suppressed using chemical shift selective sequence (CHESS). The whole procedure of the examination was automatic and resulted in the total examination time of 3 min 42 sec. The spectrum was processed using SAGE software. The bands corresponding to glutamates Glx (2.1–2.5 ppm), GABA (2.3 ppm), creatine Cr (3.03 ppm), N-acetylaspartate (NAA) – 2.02 ppm, choline (Cho) – 3.2 ppm and myoinositol (mI) 3.5–3.65 ppm were assessed in the spectrum.

The proportions of NAA, Cho, mI, Glx and GABA to creatine were analyzed. The level of creatine was adopted as a stable internal standard.

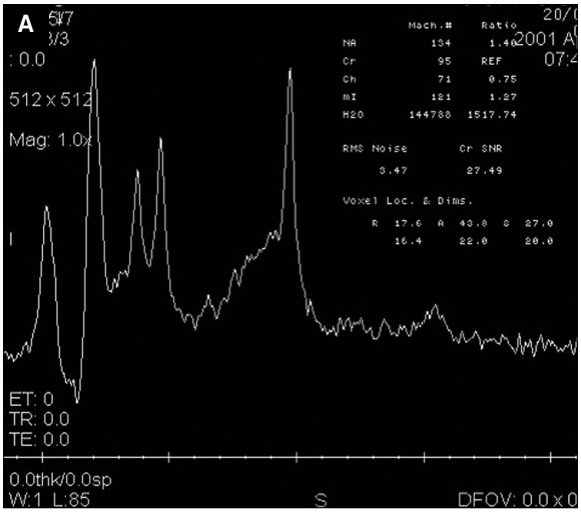
Statistical analysis

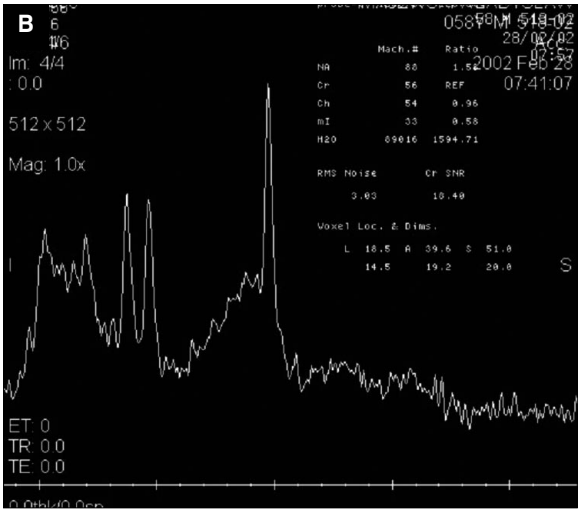
Because of asymmetrical distribution of most analyzed parameters, Mann-Whitney U test was used. The analysis was carried out using STATISTICA 5, 97 software package. The values are expressed in the tables as means ±standard deviation. The analysis of metabolite levels in the prefrontal region of the hemisphere ipsi- and contralateral to the infarct was carried out comparing the values obtained in the patient group with measurements from both hemispheres obtained in control subjects (40 measurements). Earlier comparison of the values of metabolite to creatine ratios for particular metabolites in the right and left hemispheres in the control group demonstrated no significant differences (Wilcoxon test).

Results

The first examination in the acute phase of stroke (9±2 days from the onset of the disease) was performed in 31 patients, including 15 women and 16 men aged from 45 to 80 years; mean age: 62.9 (±9) years. The next examination was performed after about 110 (±30) days in 27 patients, (15 women and 12 men); aged from 45 to 80 years; mean age: 63 (±9) years.

The patients who were not examined the second time were three men with the infarct located in the right hemisphere and one man with the infarction in the left hemisphere. The reason for failure to perform the examination was deterioration of the patients' condition providing contraindication for transport in two cases and resignation from participation in the study in the remaining two.





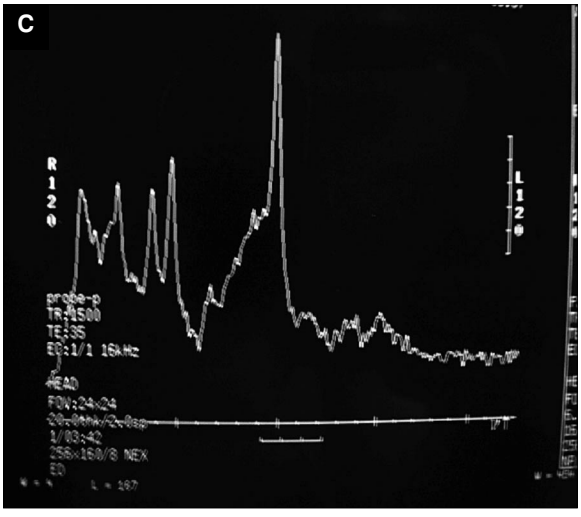


Figure 2A–C. Spectrum obtained with PRESS sequence.

The control group consisted of 20 healthy volunteers (9 women and 11 men). The age ranged from 49 to 79 years, mean 59.6 ((9.3) years.

The infarct was located in the right hemisphere in 15, and in the left in 16 cases. It involved only subcortical areas in 17 patients, only cortical areas in 3, and in 9 both the cortex and the subcortical areas.

At the time of the first examinations, the stroke patients had lower GABA/Cr values in both prefrontal regions (both ipsi- and contralateral to the ischemic focus) (Table 1).

Three months after the onset of the disease, stroke patients were still characterized by lower GABA/Cr values in both prefrontal regions (both in the hemisphere ipsilateral to the ischemic focus and in the contralateral one); however, the difference reached statistical significance for the ipsilateral area only (Table 2). The values of NAA/Cr, Cho/Cr, mI/Cr and Glx/Cr did not differ significantly on both examinations.

Example of the obtained spectrum is presented in Figure 2. (A,B – patients group, C – control group.

DISCUSSION

Considering the results obtained with magnetic resonance spectroscopy method, it should be emphasized that there are certain limitations. The region of interest included the cortex of the frontal lobe, and the mean measurement volume was ca. 6 cm^3. Such volume was dictated by the intention to include predominantly grey matter in the investigated area and to ensure the best possible field homogeneity in the analyzed region. However, reduction of the volume of the analyzed area is associated with a decrease of the signal to noise ratio. Thus, it is possible that reduction of the area of interest affected the spectrum quality, especially in view of the fact that because of automatic measurement procedure and the patients' condition, which often required shortening of the examination time, the possible signal loss could not be compensated by prolonged time of the examination.

Whereas the determination of resonance lines rather does not raise any doubt in case of creatine, N-acetylaspartate, myoinositol and choline, the analysis of glutamate, glutamine and gamma-aminobutyric acid signals is much less reliable. The signal intensity of these compounds is relatively low, which results in low signal to noise ratios. Additionally, these substances are visualized as complex multiplets [13], which makes the assignment of resonance lines difficult. The GABA signal was assigned at 2.3 ppm. The signal of gamma-aminobutyric acid C4 functional group is localized at 3.03 ppm [15–17]; however, it is not visible there because of the overlapping, of much stronger creatine signal. As the method of GABA measurement described by Goddard et al. and Sanacora et al. [16,17], using J-editing impulse sequence, was not available in the present study, only the signal intensity for CH^2 GABA group localized in the spectrum at 2.3 ppm was determined. Glutamate and glutamine are difficult to separate in spectrum obtained using 1.5 T field intensity and therefore marked as a common Glx peak. Antuono et al. investigated Glx resonance signal in 0.5 T field, with

Glx β and γ singlet best visible at 2.35 ppm [14]. The present study utilized a standard 1.5 T magnet, in which the Glx β and a signal is not visualized as such a distinct peak as obtained with the technique proposed by the Wisconsin team. Although in proton spectroscopy spectrum the resonance line for glutamate/glutamine is also localized in the 3.75–3.8 area [15], in order to simplify the comparisons, one resonance line corresponding to Glx β – localized at 2.1–2.5 ppm (ca. 2.2 ppm) was analyzed in the present study [15]. Considering all the above limitations, it should be emphasized that the obtained results can be treated as preliminary and require confirmation with more accurate techniques.

Stroke patients had lower GABA/Cr values in both prefrontal regions (both ipsi- and contralateral to the ischemic focus). Also at the time of the second examination, the patient group was characterized by parameters indicating decreased GABA levels. No differences in the ratios of the remaining metabolites to creatine were observed.

The studies carried out to date have demonstrated that an ischemic event leads to pathologic increase of excitotoxic amino acid activity, which initiates a cascade of unfavorable events leading to cell death [5]. This sequence of unfavorable phenomena has been suggested to be dependent on the equilibrium between the excitatory and inhibitory mechanisms [18]. Gamma-aminobutyric acid, antagonizing the effect of glutamates, is the main inhibitory neurotransmitter [19]. It has been demonstrated that stimulation of GABA-ergic transmission with clomethiazole or its analogs acting directly on $GABA_A$ receptor complex chloride channels exerts a neuroprotective effect and inhibits glutamate release caused by ischemia [20]. Tiagabine, a selective GABA uptake inhibitor, exerts a similar effect [21]. Animal model experiments have demonstrated that accumulation of GABA in extracellular space occurs in response to cerebral tissue ischemia [5,19], probably in order to reduce the effect of excitotoxic amino acid activity. Nevertheless, the consequence of this rapid GABA accumulation is a decrease of production and release of this neurotransmitter [5], and, consequently, reduction of GABA-ergic transmission.

The processes described above take place at the ischemic site. Experiments with focal ischemia in mice have demonstrated that persistent disturbances of the equilibrium between the excitatory (increase of glutaminergic activity) and inhibitory system (decrease of GABA-ergic activity) are observed also in the regions remote from the ischemic focus [11].

In this context, the decreased level of GABA in stroke patients seems to be a consistent finding. The first examinations were performed on the average 10 days after the stroke, i.e. after the period of rapid increase of gamma-aminobutyric acid concentration in extracellular space. The obtained results may reflect the GABA synthesis inhibition phase and disturbances of GABA-ergic transmission also outside the ischemic focus. However, as long as it is possible to explain low GABA levels in the acute phase of stroke, it is unclear whether the persistence of such low values several months after the onset of the disease should be attributed to sustained suppression of the synthesis. No increase of glutamate level, as it could be expected, was observed. As the fluctuations of glutamate levels precede the changes of GABA concentrations, the selected time of the examination might already not have allowed to demonstrate such fluctuations.

The mechanisms of post-stroke function restoration involve, among others, the reorganization of spared neuronal circuits, which leads to extended activation and recruitment of additional cortical areas during the motor task performance [22]. The presented study has demonstrated that changes which may be important for remission or persistence of the neurological deficits take place even in the regions regarded as unaffected on the basis of morphological images. It has been suggested that the predominance of the glutaminergic system (imbalance between GABA and glutamates) observed after focal ischemia may be associated with hyperexcitability and occurrence of focal epileptic activity [11]. Further studies are needed to answer the questions whether the abnormalities observed outside the ischemic focus affect the process of post-stroke rehabilitation, as well as whether, and to what extent, they can become a target of pharmacological interventions.

CONCLUSIONS

Stroke patients demonstrate, in comparison to the control group, decreased levels of gamma-aminobutyric acid in the cerebral areas outside the infarct. This may reflect the impact of the ischemic focus on the remote brain regions, probably resulting in the inhibition of GABA production. Further studies are needed to confirm these results.

REFERENCES:

1. Mathews VP, Barker PB, Blackband SJ et al: Cerebral metabolites in patients with acute and subacute strokes: concentrations determined by quantitative proton MR spectroscopy. Am J Radiology, 1995; 165: 633-38
2. Petroff OAC, Graham GD, Blamire AM: Spectroscopic imaging of stroke in humans: histopathology correlates of spectral changes. Neurology, 1992; 42: 1349-54
3. Douglas LA, Matthews PM: Practical aspects of clinical applications of MRS in the brain. In: Young IR, Charles VH (eds.): MR spectroscopy, clinical applications and techniques. Martin Dunitz, London, 1996, pp. 139-59
4. Walecki J: Choroby naczyniowe ośrodkowego układu nerwowego. In: Walecki J (ed): Neuroradiologia, Upowszechnianie Nauki – Oświata, Warszawa, 2000, pp. 303-20
5. Green AR, Hainsworth AH, Jackson DM: GABA potentiation: a logical pharmacological approach for the treatment of acute ischemic stroke. Neuropharmacology, 2000; 39: 1483
6. Gladstone DJ, Black SE, Hakim AM: Toward wisdom from failure. Lessons from neuroprotective stroke trials and new therapeutic directions. Stroke, 2002; 33: 2123-33
7. Serena J, Leira R, Castillo J et al: Neurological deterioration in acute lacunar infarctions. Stroke, 2001; 32: 1154-59
8. Marczyński TJ: GABAergic deafferentiation hypothesis of brain aging and Alzheimer's disease revisited. Brain Research Bull, 1998; 45: 341-79
9. Jones EG: GABA-peptide neurons in primate cerebral cortex. J Mind Behav, 1987; 8: 519-36
10. Benes FM, Berretta S: GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology, 2001; 25: 1-27

11. Qu M, Mittmann T, Luhmann HJ et al: Long-term changes of ionotropic glutamate and GABA receptors after unilateral permanent focal cerebral ischemia in the mouse brain. Neuroscience, 1998; 85: 29-43
12. Bamford J, Sandercock P, Dennis M et al: Classification and natural history of clinically identifiable subtypes of cerebral infarction. Lancet, 1991; 337: 1521-26
13. Soher BJ, Vermathen P, Schuff N et al: Short TE *in vivo* H MR spectroscopic imaging at 1, 5 T: acquisition and automated spectral analysis. Magnetic Resonance Imaging, 2000; 18: 1159-65
14. Antuono PG, Jones JL, Wang Y et al: Decreased glutamate+glutamine in Alzheimer's disease detected *in vivo* with 1H-MRS at 0.5. Neurology, 2001; 56: 737-42
15. Sokół M: *In vivo* H MR spectra analysis by means of second derivative method. Magnetic Resonance Materials in Physics, Biology and Medicine, 2001; 12: 177-83
16. Goddard AW, Mason GF, Almai A et al: Reductions in occipital cortex GABA levels in panic disorder detected with H1-magnetic resonance spectroscopy. Arch Gen Psychiatry, 2001; 58: 556-61
17. Sanacora G, Masin GF, Rothman DL et al: Reduced cortical (gamma)-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. Arch Gen Psychiatry, 1999; 56: 1043-47
18. Meldrum B: Protection against ischemic neuronal damage by drugs acting on excitatory neurotransmission. Cerebrovascular and Brain Metabolism Reviews, 1990; 2: 27-57
19. Schwartz-Bloom RD, Sah R: a-aminobutyric acid. A neurotransmission and cerebral ischemia. J Neurochemistry, 2001; 77: 353-71
20. Nelson RM, Hainsworth AH, Lambert DG et al: Neuroprotecxtive efficacy of AR-A008055, a clomethiazole analogue, in a global model of acute ischaemic stroke and its effect on ischaemia-induced glutamate and GABA efflux *in vitro*. Neuropoharmacology, 2001; 1: 159-71
21. Yi Y, Qiu L, Chen-Xu W et al: Dose-dependent neuroprotection with tiagabine in a focal cerebral ischemia mosel in rat. Neuroreport, 2000; 11: 2307
22. Seitz RJ, Azari NP, Knorr U et al: The role of diaschisis in stroke recovery. Stroke, 1999; 30: 1844-50