Dysregulation of Amyloid-β Protein Precursor, β-Secretase, Presenilin 1 and 2 Genes in the Rat Selectively Vulnerable CA1 Subfield of Hippocampus Following Transient Global Brain Ischemia

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Abstract. The interaction between brain ischemia and Alzheimer’s disease (AD) has been intensively investigated recently. Nevertheless, we have not yet understood the nature and mechanisms of the ischemic episodes triggering the onset of AD and how they influence its slow progression. The assumed connection between brain ischemia and the accumulation of amyloid-β (Aβ) peptide awaits to be clearly explained. In our research, we employed a rat cardiac arrest model to study the changes in gene expression of amyloid-β protein precursor (AβPP) and its cleaving enzymes, β- and γ-secretases (including presenilins) in hippocampal CA1 sector, following transient 10-min global brain ischemia. The quantitative reverse-transcriptase PCR assay demonstrated that the expression of all above genes that contribute to Aβ peptide generation was dysregulated during 30 days in postischemic hippocampal CA1 area. It suggests that studied Aβ peptide generation-related genes can be involved in AβPP metabolism, following global brain ischemia and will be useful to identify the molecular mechanisms underpinning...
that cerebral ischemia might be an etiological cause of AD via dysregulation of AβPP and its cleaving enzymes, β- and γ-secretases genes, and subsequently, it may increase Aβ peptide production and promote the gradual and slow development of AD neuropathology. Our data demonstrate that brain ischemia activates delayed neuronal death in hippocampus in an AβPP-dependent manner, thus defining a new and important mode of ischemic cell death.

**Keywords**: Alzheimer’s disease, amyloid-β protein precursor, β-secretase, brain ischemia, CA1 area, dementia, γ-secretase, genes, hippocampus, presenilin 1 and 2

**INTRODUCTION**

Transient global brain ischemia and Alzheimer’s disease (AD) are two familiar neurodegenerative disorders, which at first cause progressive degeneration in rodents as well as in humans, followed by death of hippocampal populations of pyramidal neurons in the CA1 area, with the final development of dementia [1–11]. Ischemic brain injury is the second cause of dementia, the third leading cause of death in the world, and a major cause of progressing adult disability worldwide, for which there is no effective therapy so far [12]. Transient global ischemia in rodents and humans induces a continuous loss of hippocampal pyramidal neurons, beginning 48 h after the end of ischemia [13–16]. This form of neuronal loss is termed selective delayed neuronal death or the “maturation phenomenon” [13–16]. Selective delayed neuronal death of CA1 field of hippocampus is intensively studied in rodents, and it is the most sensitive area of the brain to ischemic damage [9]. A phenomenon of specific delayed neuronal death in ischemic hippocampus is defined, however, the molecular chain of events ending in death of CA1 area pyramidal neuronal population is not stated precisely. AD, the leading source of dementia worldwide, is defined neuropathologically by the loss of CA1 area neurons in hippocampus and presence of the soluble and fibrillar amyloid-β (Aβ) peptide within the brain intra- and extracellular space along with hyperphosphorylated tau protein inside neurons [10, 11]. In addition to AD, Aβ peptide and hyperphosphorylated tau protein are noted to be powerfully involved in the development of neurodegeneration following transient global brain ischemia in humans and animals [16–22]. Brain ischemia may trigger mitotic processes in the cascade of neuropathogenesis in AD onset, progression, and development finishing with full-blown cognitive deficits with Alzheimer phenotype [6–8, 23, 24]. Complete brain ischemia induces delayed neuronal death characterized by massive neuronal loss in hippocampal CA1 subfield, which starts during the 2nd day and finishes during the 7th day of recirculation [13–16].

Ischemic delayed neuronal loss in animals and humans is associated with spatial memory deficits and hyperactivity, and these alterations directly correlate with ischemia-induced hippocampal CA1 area cell death [4, 8, 25], hippocampal and brain atrophy [26], and finally result in severe dementia [8, 24]. It has been demonstrated that increased levels of different fragments of amyloid-β protein precursor (AβPP) immunoreactivity in hippocampus, starting 2 days following global brain ischemia in rat, are still present within one year [17, 26–31]. Additionally, Aβ peptide deposition was also detected in the human hippocampus following complete brain ischemia [18, 32]. In rat and human ischemic brains, different fragments of AβPP have been found, especially in neuronal cells [16–18, 32, 33]. The above data indicate that an ischemic episode induces pathological AβPP metabolism in ischemic neurons. After the transient global brain ischemia, recirculation affects AβPP processing, finally ending in the rise of Aβ peptide accumulation in the intra- and extracellular space of brain. Aβ peptide plaques in AD are produced from AβPP in a rapid and highly complex process via proteolytic cleavage by β-secretase and γ-secretase complex (including presenilins). γ-Secretase can cleave AβPP at several neighboring intramembranous sites to release Aβ peptide, a hydrophobic self-aggregating peptide, containing 39–42 amino acid residues.

Here we investigated whether AβPP signaling pathway participates in ischemia-induced delayed neuronal death in CA1 area of hippocampus. In this study, we have presented for the first time the time course of AβPP, β-secretase and presenilin 1 and 2 gene dysregulation by quantitative reverse-transcriptase PCR assay protocol, all of which are implicated in AD, in the selectively vulnerable CA1 sector of rat hippocampus subjected to transient global brain ischemia due to cardiac arrest with survival times of 2, 7, and 30 days. Our data demonstrate that brain ischemia activates delayed neuronal death in hippocampus in an AβPP-dependent manner, thus defining a new and important mode of ischemic cell death.
MATERIALS AND METHODS

Animals and global brain ischemia

Two-month-old female Wistar rats ($n = 54$, $160–180$ g) were submitted to 10-min global brain ischemia due to cardiac arrest [34]. The rats were maintained in pairs per cage in a room temperature of $24 \pm 2^\circ$C, with $55 \pm 5\%$ humidity, and with a $12$-h light-dark cycle. All animals had free access to commercial laboratory chow and tap water 

ad libitum. All experimental procedures were performed during the light phase, between $13:00$ and $17:00$ under identical conditions. The rats used for experiments were treated in strict accordance with the NIH Guide for Care and Use of Laboratory Animals (1985) and European Communities Council Directive 142 (86/609/EEC), as well as with the approval of the local Ethical Committee. All efforts were made to minimize animal suffering and to reduce the number of rats used.

Our animal model of global brain ischemia in rats clinically represents reversible cardiac arrest. Global brain ischemia was performed by cardiac arrest of 10-min duration [34]. The animals were allowed to survive2 ($n = 11$), 7 ($n = 18$) days, and 1 month ($n = 25$) after recirculation. Sham-operated rats ($n = 54$) were exposed to the same procedures as ischemic animals but without induced cardiac arrest and thus served as controls.

Determination of gene expression

Prior to sampling, the brains were perfused with cold $0.9\%$ NaCl via the left ventricle in order to flush blood vessels. After removing the brain from the skull, the brain was put on an ice chilled Petri dish. The brains were cut in the coronal plane through the intersection of the optic nerves. The ischemic and control pooled samples circa $1$ mm$^3$ volume of hippocampal left and right CA1 area were taken with a narrow scalpel. Pooled left and right CA1 areas were immediately placed in RNALater solution (Life Technologies, USA). Gene expression of amyloid-$\beta$ protein precursor (App), $\beta$-secretase (Bace 1), presenlin 1 (Psen 1), and presenilin 2 (Psen 2) was assessed by reverse-transcription quantitative PCR (RT-qPCR) method. Genes were quantitatively assessed in each sample taken from the ischemic group and referred to gene expression determined in the corresponding samples in the control group – 1:1.

Isolation of total cellular RNA

Total cellular RNA isolation was performed according to the method described by Chomczynski and Sacchi [35] with modification, using TRI-Reagent Solution (Ambion, USA). During this process, the samples of hippocampal CA1 area were homogenized in $1$ ml TRI-Reagent buffer (Ambion, USA) to obtain homogenous suspension. The suspensions were incubated for $5$ min at room temperature until complete dissociation. At the next stage, $200 \mu$l chloroform (Sigma-Aldrich, USA) per $1$ ml of TRI-Reagent buffer was added to the sample and shaken for $15$ s to completely mix the phases. Next, the samples were left for $15$ min to incubate at room temperature after which they were centrifuged for $15$ min at $14,000$ rpm at $4^\circ$C in $5415$ R Eppendorf centrifuges. After the separation, the aqueous phase was placed in a new tube and $500 \mu$l 2-propanol (Sigma-Aldrich, USA) was added per $1$ ml of TRI-Reagent buffer. The samples were thoroughly mixed and incubated for $20$ min at room temperature. Following that, the mixtures were centrifuged for $20$ min at $14,000$ rpm at $4^\circ$C in $5415$ R Eppendorf centrifuges. Aqueous phase containing RNA was removed from the above precipitate. The RNA phase was washed in cool $80\%$ ethanol and next, obtained RNA samples were stored in $80\%$ ethanol at $-20^\circ$C for further analysis.

Quantitative and qualitative analysis of RNA

The RNA concentration and purity were measured by spectrophotometry on NanoDrop2000 (ThermoScientific, USA). Precipitate of RNA in $80\%$ ethanol was taken out of

−20°C and next centrifuged for $15$ min at $14,000$ rpm at $4^\circ$C in $5415$ R Eppendorf centrifuges. The liquid part was removed, and RNA pellets were left to dry completely at room temperature. Subsequently, the precipitate was dissolved in DNase-, RNase-, and protease-free water (Sigma-Aldrich, USA) at $4^\circ$C, the volume depending on RNA concentration.

The cDNA synthesis

The cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kit, according to manufacturer’s instructions (Applied Biosystems, USA). Each reactive mixture contained the following set of reagents: $1 \times$ RT buffer, $20$ U RNase inhibitor, $50$ U reverse transcriptase (Muti scribe Reverse Transcriptase), $1 \times$ RT Random Primers, $4$ mM of each deoxynucleotide: dATP, dGTP, dTTP, and dCTP plus
examined 1 μg RNA in DNase-, RNase-, and protease-
free water (Sigma-Aldrich, USA) to complete the
volume required for reaction. Final volume of reactive
mixture was 20 μl. Afterwards, the reactive compo-

The qPCR protocol

The cDNA, which was obtained by reverse transcrip-
tion (RT) procedure, was amplified by real-time gene
expression analysis (qPCR) on 7900HT Real-Time Fast
System (Applied Biosystems, USA), using the man-
ufacturer’s SDS software. Triplicate qPCR reactions
were conducted for each sample. To exclude reagent
contamination by foreign DNA, a blind trial was always
performed without DNA target. Reaction components
included: 1.25 μl mixture of probe and oligonucleotide
starters specific for genes examined, 12.5 μl buffer Taq-
Man Universal PCR Master Mix, 1 μl cDNA, DNase-,
RNase-, and protease-free water (Sigma Aldrich,
USA) to complete the required reactive volume. The
reaction was performed on epitic 25-μl well reaction
plate, using probe sets of TaqMan Gene Expression
Assays (Applied Biosystems, USA) with FAM-NFQ
markers and oligonucleotide starters for rat genes:
App, Bace 1, Psen 1, Psen 2, and the housekeeping
markers and oligonucleotide starters for rat genes:

RESULTS

Changes in gene expression of presenilin 1,
presenilin 2, β-secretase, and AβPP in rats two
days after total cerebral ischemia

Presenilin 1 gene expression increased to a maxi-
mum of 2.203 - fold, presenilin 2 - to 3.208 - fold, β-
secretase - to 3.916 - fold, and the expression of
AβPP gene decreased to a minimum -0.542. The mean
expression levels of genes were as follows: presenilin 1
5.08 ± 0.298, presenilin 2 2.449 ± 0.323, β-secretase
2.642 ± 0.414, and AβPP -0.075 ± 0.101 (Figs. 1–4).

Changes in gene expression of presenilin 1,
presenilin 2, β-secretase, and AβPP in rats 7 days
after total cerebral ischemia

Presenilin 1 gene expression increased to a maxi-
mum of 0.874, presenilin 2 - to 0.709, β-secretase - to
0.952, and the AβPP gene - to 0.746. The mean expres-
sion levels of genes were the following: presenilin 1
0.175 ± 0.061, presenilin 2 0.129 ± 0.064, β-secretase
0.121 ± 0.113, and AβPP 0.477 ± 0.042 (Figs. 1–4).
Fig. 1. The mean gene expression levels of presenilin 1 in the hippocampus CA1 area in rats 2, 7, and 30 days after 10-min of global brain ischemia. Marked SEM, standard error of the mean. Indicated statistically significant differences in levels of gene expression between 2 and 30 and between 7 and 30 days after 10-min of global brain ischemia (Mann-Whitney U test). \( \ast p \leq 0.005, \ast\ast p \leq 0.0001 \).

Fig. 2. The mean gene expression levels of presenilin 2 in the hippocampus CA1 area in rats 2, 7, and 30 days after 10-min of global brain ischemia. Marked SEM, standard error of the mean. Indicated statistically significant differences in levels of gene expression between 2 and 30 and between 2 and 7 and between 7 and 30 days after 10-min of global brain ischemia (Mann-Whitney U test). \( \ast p \leq 0.005, \ast\ast p \leq 0.0001 \).

Changes in gene expression of presenilin 1, presenilin 2, \( \beta \)-secretase, and A\( \beta \)PP in rats 30 days after total cerebral ischemia

Presenilin 1 gene expression decreased to a minimum of \(-1.093\), presenilin 2 to \(-0.728\), \( \beta \)-secretase to \(-0.731\), and the A\( \beta \)PP gene expression increased to \(0.623\). The mean expression levels of genes were as follows: presenilin 1 \(-0.343 \pm 0.059\), presenilin 2 \(-0.131 \pm 0.052\), \( \beta \)-secretase \(-0.335 \pm 0.064\), and A\( \beta \)PP \(0.151 \pm 0.051\) (Figs. 1–4).

Summary of the mean levels of gene expression in hippocampal CA1 area in rats after 2, 7, and 30 days following the resumption of circulation after total brain ischemia

Figures 1–4 show changes in the mean expression levels of genes with statistical significance: presenilin
1 (Fig. 1), presenilin 2 (Fig. 2), β-secretase (Fig. 3), and the AβPP (Fig. 4) after 10-min total cerebral ischemia in rats with survival of 2, 7, and 30 days. Significant correlations between the expression of genes in the rat hippocampal CA1 area postischemia (a minimum observed downregulation in AβPP – 0.542-fold). This may be associated with the onset of necrotic neurodegenerative processes of pyramidal neurons in the hippocampal CA1 subfield [33, 50]. The level of expression of AβPP investigated gene did not coincide with the earlier data concerning staining of different parts of AβPP [17]. Our results show that there is a discrepancy between both AβPP and secretase gene expression in the ischemic hippocampal CA1 region at 2 days postischemia. Currently, it has become clear that at this time necrotic neuronal cell death is associated with ischemia-reperfusion hippocampal CA1 area neurodegeneration [51–60]. Now, it has become obvious that at this time necrotic neuronal brain pathology is associated with the abnormal deposition of AβPP [33]. In the hippocampus, granular deposits of AβPP immunolabeling appear in the damaged CA1 area [33]. Lysosomal leakage appears at days 2 and 3 postischemia in hippocampal CA1 area neurons [53] which are a homogenous neuronal population [60]. Morphology-based studies have found that the vast majority of ischemic CA1 neurons display necrosis-like features and only a minority show morphologic changes similar to apoptosis [60]. During necrotic neuronal death, increased cytoplasm Ca++ concentration [61] leads to mitochondrial dysfunction, activation of phospholipases, and proteases initiate lipid and DNA damage, and finally, the loss of membranes’ integrity/plasma membrane rupture [52, 54, 57, 62]. AβPP is present in large quantities in all cell membranes [63] and in the above mentioned situation, there is an excess of secretase substrate which is the AβPP. The loss of membrane integrity and release of intracellular content, grant necrotic cells the ability to induce a pathological AβPP metabolism in a vicious cycle [38, 63]. This assumption is apparently supported by AβPP immunolabeling the remnants of necrotic CA1 neurons [33].

Our data indicate that in the 7 consecutive days, the β-secretase (maximal increase of 0.952-fold), presenilin 1 and 2 gene (maximal increase of 0.874- and 0.709-fold, respectively) expression dropped subsequently, but it was still above the control values. At that time, overexpression of AβPP gene was noted (maximum 0.746-fold). The data demonstrate an increase in AβPP gene expression in the selectively vulnerable CA1 sector of hippocampus after 10-min complete brain ischemia at the 7th day postischemia. These results are complementary to the increase noted in different parts of AβPP immunostaining after 7 days of recirculation [17]. But after 30 days, gene expression of secretases was below the control level (maximal β-secretase –0.731-fold, presenilin 1 –1.093-fold, and

**DISCUSSION**

In this paper, we studied alterations in the expression of AβPP, β-secretase, presenilin 1, and 2 Alzheimer-related genes in rats subjected to a brief (10-min) complete brain ischemia. For the first time, we show here the time course of AβPP, β-secretase, and presenilin 1 and 2 genes dysregulation in the CA1 sector of hippocampus following complete brain ischemia. Our data provide the first known in vivo role of the dysregulation of Alzheimer-related genes in neuronal death postischemia. Additionally, our data demonstrate that brain ischemia activates delayed neuronal death in hippocampus in an AβPP-dependent manner, thus defining a new and important mode of regulation of ischemic cell death. Following complete brain ischemia, some areas of the brain become more susceptible to neuronal damage/death than others, a phenomenon known as selective delayed neuronal death and/or selective vulnerability [13–16, 39]. The nature of molecular events involved in selective vulnerability of CA1 subfield of hippocampus has been disappointingly limited. To the best of our knowledge, data presented by us constitute the first report of its kind linking ischemia with acute, quantitative expression selected Alzheimer-related genes (AβPP, β- and γ-secretase). These data might support the hypothesis that the Aβ peptide generated by ischemia could be involved in the ischemia-related AD [20, 24, 40–49].

In the present study, we have demonstrated that 2 days of recirculation postischemia resulted in a quick and huge overexpression of AβPP, protease genes such as β-secretase and γ-secretase (presenilin 1 or presenilin 2) associated with Aβ peptide generation and development of AD. The investigated genes were impressively changed at 2 days postischemia; a maximum expression of β-secretase gene increased 3.916-fold, that of presenilin 1 – 2.203-fold, and that of presenilin 2 – 3.208-fold. Interestingly, at that time we observed downregulation in AβPP gene expression in the hippocampal CA1 area postischemia (a minimum of -0.542-fold). This may be associated with the onset of necrotic neurodegenerative processes of pyramidal neurons in the hippocampal CA1 subfield [33, 50]. The level of expression of AβPP investigated gene did not coincide with the earlier data concerning staining of different parts of AβPP [17]. Our results show that there is a discrepancy between both AβPP and secretase gene expression in the ischemic hippocampal CA1 region at 2 days postischemia. Currently, it has become clear that at this time necrotic neuronal cell death is associated with ischemia-reperfusion hippocampal CA1 area neurodegeneration [51–60]. Now, it has become obvious that at this time necrotic neuronal brain pathology is associated with the abnormal deposition of AβPP [33]. In the hippocampus, granular deposits of AβPP immunolabeling appear in the damaged CA1 area [33]. Lysosomal leakage appears at days 2 and 3 postischemia in hippocampal CA1 area neurons [53] which are a homogenous neuronal population [60]. Morphology-based studies have found that the vast majority of ischemic CA1 neurons display necrosis-like features and only a minority show morphologic changes similar to apoptosis [60]. During necrotic neuronal death, increased cytoplasm Ca++ concentration [61] leads to mitochondrial dysfunction, activation of phospholipases, and proteases initiate lipid and DNA damage, and finally, the loss of membranes’ integrity/plasma membrane rupture [52, 54, 57, 62]. AβPP is present in large quantities in all cell membranes [63] and in the above mentioned situation, there is an excess of secretase substrate which is the AβPP. The loss of membrane integrity and release of intracellular content, grant necrotic cells the ability to induce a pathological AβPP metabolism in a vicious cycle [38, 63]. This assumption is apparently supported by AβPP immunolabeling the remnants of necrotic CA1 neurons [33].
presenilin 2 (−0.728-fold) and AβPP was overexpressed (maximal 0.623-fold). During 30 days postischemia, there was almost complete loss of CA1 area neuronal population of hippocampus and, therefore, AβPP gene expression was increased at that time. The expression of all studied genes at this time negatively correlates with intra- and extracellular immunostaining of gene products [17, 27, 28, 30, 31, 33]. Intra- and extracellular Aβ peptide immunoreactivity in hippocampal CA1 area increases for as long as a year after injury, indicating that Aβ peptide accumulation may continue long after β-secretase and presenilin gene expression goes below the control value [30, 64]. Presumably, local and global disturbances in AβPP processing can be distinguished. This phenomenon is probably related to movement of AβPP/Aβ peptide from blood into brain tissue [22, 65]. It was also shown that RAGE protein enhanced ischemic vessel wall permeability [66, 67].

The available evidence indicates that the selective vulnerability of neuronal cells to global brain ischemia is determined by both ischemic episode and reperfusion. At present, there are at least three factors considered to be important in irreversible damage of vulnerable sectors of ischemic brain during short-term survival, which are independent of both the duration of ischemia and recirculation. They include: glutamate neurotoxicity, intraneuronal loading of calcium, and tissue acidosis [26, 61]. Ischemia-induced AβPP, β-secretase, and γ-secretase (presenilins) dysregulation may provide excess soluble form of Aβ peptide, which in turn could act as an additional neurotoxin which disrupts neuronal function and increases cell death through multiple mechanisms, including additionally impaired calcium homeostasis, initiation of neuroinflammatory processes [58], loss of neurovascular integrity [66, 67], and reactivity with blood-brain barrier permeability [23, 26–30, 43, 68, 69]. Several lines of evidence suggest that production of Aβ peptide reduces neuroplasticity and contributes to increased neuronal vulnerability to ischemia [9, 43, 49, 69, 70]. On the other hand, overexpression of Alzheimer-related genes during recirculation may constitute a self-sustaining vicious cycle that leads to progressive neurodegeneration beginning in hippocampus [71, 72]. A pathological hallmark of AD, the most prevalent neurodegenerative disease, is the accumulation of plaques formed by Aβ peptide, commencing in the hippocampus, and spreading progressively throughout the brain [69, 71, 72]. It is likely that the accumulation is caused by both increased production and impaired clearance of Aβ peptide and/or movement of Aβ peptide and other parts of AβPP from blood into CA1 area of hippocampus [22, 65]. It was also noted that RAGE may additionally contribute to delayed neuronal death in hippocampus by enhancing ischemic vascular wall injury [66, 67]. These data corroborate earlier immunohistochemical observation from animal models of complete brain ischemia and from brains after cardiac arrest in humans, which all suggest a direct relationship between ischemia and increased amyloidogenic AβPP processing [17, 18, 32]. These findings are consistent with previous studies in semiquantitative analysis of secretases in local brain ischemia models and further confirm the participation of β-secretase [73] and presenilin 1 and 2 in ischemia-induced Aβ peptide generation [74, 75]. Combined all together, upregulation of genes found in our study suggests that β-secretase, presenilin 1 and 2 activation persist for 2–7 days after ischemia in hippocampal CA1 area. These data indicate that, in ischemic pyramidal neurons, AβPP, β-secretase, and γ-secretase conspire to promote an additional pathological sequence resulting in cell death. β-secretase activation can then cause further apoptotic signaling inducing caspase activation and DNA fragmentation, both events being observed in AD brains [76]. These findings will make it possible to understand the gradual postischemic damage in the structure of brain cells, delayed Aβ peptide deposition, and long-term ischemic pathogenesis of AD. The present data may partly help to define the molecular mechanism of higher occurrence of neuronal death in ischemic hippocampal CA1 area.

The induction of three AβPP metabolism-related genes, β-secretase, presenilin 1 and 2, was quick during 2–7 days postischemia. In the following 30 days, the β-secretase, presenilin 1 and 2 gene expression was downregulated. The above changes are associated with the loss of hippocampal CA1 area neurons, hippocampal and brain atrophy [26], and finally result in Alzheimer-type dementia [7–9, 24, 62]. These findings suggest that both abnormal expression of Alzheimer-related genes and accumulation of their products induced by ischemia/reperfusion might play a critical role in nectrotic neurodegeneration of CA1 area pyramidal neurons [24, 30, 33, 47, 62]. This also suggests that the above processes may be directly associated with neuronal death in ischemia. Our study indicates that transcription processes positively correlate with the condition of ischemic neurons. The expression of investigated genes was decreased at late stages (30 days) after ischemia. This may be reflected by the decrease and finally complete disappearance of pyramidal neurons in CA1 area of the hippocampus. According to our data and the findings reported by other authors, it can be summarized that focal and/or
global ischemia and reperfusion dysregulate AβPP at both gene and protein levels and lead to Aβ peptide accumulation. Lessons learned from investigation of ischemic hippocampal CA1 area genes that contribute to neuronal death and Aβ peptide generation will be important for the elaboration of therapeutic targets to treat Alzheimer-type dementia. Further studies on this topic are obviously needed. Finally, the rat model employed in the present study seems to be a useful experimental approach for delineation of the role of gene dysregulation in neurodegenerative disorders such as sporadic AD.

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