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Forum Review

Ischemia-Induced Neuronal Cell Death and Stress Response

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ABSTRACT

Neuronal cell death is a major feature of various diseases, including brain ischemia, neuronal degenerative diseases, and traumatic injury, suggesting the importance of investigating the mechanisms that mediate neuronal cell death. Although the various factors that contribute to brain ischemia have been defined and the mechanism through which each factor causes neuronal cell death has been investigated, definite strategies have not been established. In this brief review, we focus on two important mechanisms that contribute to the pathogenesis of brain ischemia. First, we discuss the glutamate theory, a proposed mechanism for the understanding of ischemia-induced neuronal cell death. Second, an accumulation of recent molecular neurobiology evidence regarding the dysfunction of a cellular organelle, the endoplasmic reticulum (ER), suggests that it plays a major role in the pathogenesis of neuronal cell death. Whereas the former theory reflects the role of neuron-specific factors in the induction of cell death, the stress response of the ER for maintenance of its function is regarded as a defense mechanism. Because hypoxia, another major factor in ischemia, results in further dysfunction of the ER, studies on the malfunction of this cellular organelle may facilitate the development of novel strategies to block ischemia-induced cell death. *Antioxid. Redox Signal.* 9, 573–587.

INTRODUCTION

THE BRAIN IS THE ONLY INTERNAL ORGAN that uniquely determines the characteristics of mankind, and it functions with the greatest expenditure of metabolic energy. Among the various internal organs that constitute the human body, the central nervous system is unique in the sense that the energy metabolism of neurons almost completely depends on aerobic glycolysis. Hence, cerebral infarction, for instance, which occurs because of an insufficient or obstructed blood supply, leads to the immediate failure of brain nutrition and oxygenation. Furthermore, in accordance with the aging of the population in many advanced countries, the incidence of this disease is currently growing rapidly.

When the supplies of oxygen and glucose are terminated, as a result of an insufficient blood supply, the phenomenon of so-called brain ischemia (although this phenomenon is not specific for the brain) results in a rapid disruption of metabolism in the brain, especially in neuronal cells. However, the brain comprises several different types of cells, and cell types other than neurons, such as astrocytes, oligodendroglias, and endothelial cells, show a phenotype that is more resistant to ischemic conditions (94).

Therefore, although all the cellular components that constitute the central nervous system are exposed to ischemic stimuli, only neuronal cells die even after a short period of brain ischemia. Moreover, it is also known that some neurons, such as neurons in hippocampus and Purkinje neurons of the cerebellum, show enhanced vulnerability to ischemia. Neuronal cell death occurs in these regions with even shorter periods of ischemia. Hence, from a perspective of susceptibility to ischemia, several different types of neuronal cells are present in the central nervous system, and the resistance of neuronal cells to ischemia differs depending on their location, despite

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the apparent similarity of neurons from the viewpoint of cell biology. This phenomenon also characterizes brain damage in stroke, as viewed from a neuroanatomy perspective.

In this review, we outline the models and conceptual ideas that have been proposed to understand the brain injury that accompanies ischemia, and we further discuss factors of importance in the stress conditions caused by ischemia. We then go on to address the stress response that occurs naturally in cells that are under such stress, which leads to a discussion of possible defense strategies for protecting neurons from ischemic damage.

NEURONAL CELL DEATH IN THE HIPPOCAMPUS (GLUTAMATE–CALCIUM THEORY)

Why are hippocampal neurons more vulnerable to ischemic stress?

As demonstrated in murine brain ischemia models, most neurons of the hippocampus, and especially those in the area referred to as CA1, exhibit marked cell death after only a short period of ischemia, whereas neuronal cells in other parts of the brain survive such stress (105). This phenomenon was explained originally by a suggested latent deficiency in blood flow to the hippocampus and because blood vessels tend to lapse under ischemic conditions. Some of the brain areas that show fragility to ischemia, such as the hippocampus, have a blood supply that is vulnerable even under normal conditions (vascular theory) (51). However, when ischemic foci are examined microscopically, the affected neurons, whose cell death and degeneration are apparent from morphologic criteria, are often intermingled with neurons that appear to be normal after an ischemic event. Therefore, it is difficult to conclude that neuronal cell death in the hippocampus is only due to an insufficient blood flow at the microcirculation level and to metabolic disparity.

At present, the glutamate–calcium theory (11) is one of the most widely accepted hypotheses that can explain the selective vulnerability of the neurons of the hippocampus. In this context, it has been shown that neurons subjected to ischemia-induced cell death are morphologically similar to those observed in the same hippocampus region after direct administration of excitatory neurotransmitter, such as glutamate.

In addition, the following observations have been reported:

1. Neuronal cells that are naturally dependent on glutamate (glutaminergic neurons) exist abundantly in the CA1 hippocampus region, and the patterns of neuronal cells that undergo cell death after an ischemic event are similar to those of the glutamate-dependent neurons (63).
2. Exudation of glutamate from glutaminergic neurons is very sensitive to the atmospheric temperature (6), and hypothermia, which suppresses the release of glutamate from glutaminergic neurons, also suppresses ischemia-induced neuronal cell death (7).
3. A glutamate-receptor antagonist that suppresses the depolarization of glutaminergic neurons also shows a protective effect against neuronal cell death. However, although this was confirmed in an *in vitro* experimental system (20, 94), the effect of this antagonist proved to be due mainly to a reduction of body temperature. Nonetheless, based on this circumstantial and indirect evidence, glutamate toxicity is regarded as being involved in the selective and enhanced vulnerability of neurons in the hippocampus after an ischemic event.

Role of NMDA receptors in glutamate-induced hippocampal neuronal cell death

The cloning of glutamate receptors and their subunits has been carried out by many investigators (67, 73), and they are now roughly divided into two categories: ion channels and metabolic-regulation receptors. Ion-channel glutamate receptors, which play a major role in the pathogenesis of ischemia-induced neuronal cell death, are divided into three subtypes, NMDA (*N*-methyl-D-aspartate) receptors, AMPA (α-amino 3-hydroxy-5-methyl-4-isoxazolepropionate) receptors, and kinate receptors, based on their pharmacologic properties. The NMDA-type receptor channel has been studied extensively in the context of Ca^{2+} permeability triggered by the excitatory amino acid, as discussed later (11). Further, whereas most AMPA-type glutamate channels are Ca^{2+} impermeable, an evolving body of evidence supports the contention that relatively unusual Ca^{2+}-permeable AMPA channels might be crucial contributors to injury in ischemia (42).

The distribution of NMDA-type receptor channels within the brain shows a pattern similar to that of the neurons that show marked vulnerability to ischemic stress. Moreover, the NMDA receptor is regulated in a complex manner and may cause unrestricted calcium inflow into neurons, especially during an ischemic event, with the calcium inflow usually accompanied by a high concentration of glutamate. Because failure in energy metabolism in both neurons and astroglias results in the delay of the clearance of glutamate from the synaptic space, this situation becomes prevalent in ischemic regions (5, 19, 84).

Under quiescent conditions, with a normal membrane potential, the ion channel coupled to a glutamate receptor has a very low permeability for bivalent ions, especially for Ca^{2+}, because Mg^{2+} located in the channel serves as a "cork," which does not allow Ca^{2+} to pass through the channel. Because channel regulation by the Mg^{2+} is dependent on the membrane potential, the "cork" effect of Mg^{2+} is lost with depolarization of the membrane, caused by the binding of glutamate to a receptor (62). This series of reactions allows the influx of Ca^{2+} into glutaminergic neurons (Fig. 1; quiescent state).

In the normal course of neurotransmission, glutamate that collects in the synaptic cleft is properly collected and processed for reuse. This function is performed by another membrane protein, the glutamate transporter. Although glutamate transporters exist in both neurons and astroglias, glia-derived glutamate transporters are the most important (86). By coupling to the transmembrane Na^+/K^+ concentration gradient, both cell types actively perform glutamate reuptake. In contrast, when it becomes impossible to maintain the Na^+/K^+ concentration gradient, as observed during metabolic failure after an ischemic event, malfunction of the

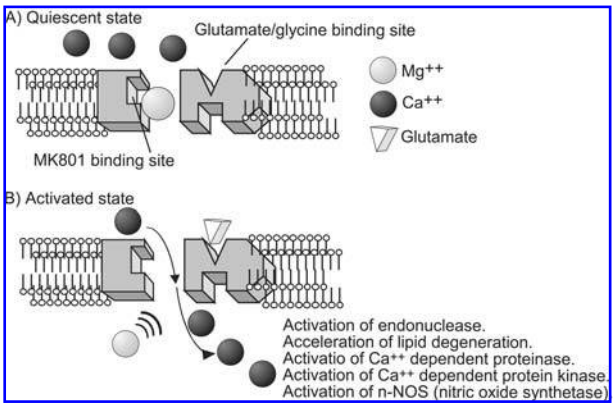

FIG. 1. Conformational change of the *N*-methyl-D-aspartate (NMDA) receptor after binding of glutamate. Under quiescent conditions, magnesium ions serve as a "cork" and not let calcium ions pass (**A**). However, because channel regulation by the magnesium ion is dependent on the membrane potential, the "cork" effect of the magnesium ion is lost with depolarization of the membrane, which is caused by glutamate binding to a receptor and inflow of calcium starts (activated phase; **B**). The increase in Ca^{2+} in the cytoplasm initiates a series of reactions that may further damage neurons (see Fig. 2).

glutamate active-transport system occurs. As a result, the energy-metabolism incompetence of the cells at the ischemic focus makes it impossible to maintain the transmembrane glutamate concentration gradient (Fig. 1; activated state).

Data obtained from an ischemia model in Mongolian gerbil, for example, suggest that a rapid elevation of the glutamate concentration occurs on a large scale in the synaptic cleft. The concentration of glutamate in the synaptic cleft reaches a level that may be 15 to 20 times higher than that measured under nonischemic conditions (58). Although it is well established that high levels of glutamate in the extracellular space appear rapidly after the onset of ischemia, a direct link between enhanced release of glutamate and neuronal injury has not been fully established (70).

Further, during prolonged ischemic conditions, a failure in cellular energy metabolism also is accompanied by a delay in membrane depolarization. Once this has happened, hypoglycemia, which also accompanies an ischemic event, further accelerates this failure by the reasons listed.

1. Glutamate-transporter function is compromised, because it is coupled to the transmembrane Na^+/K^+ concentration gradient, and this results in an inversion of glutamate transport.
2. A more-prolonged ischemic event causes exudation of a larger amount of glutamate from astrocytes cells, where more transporter exists.
3. Increase of glutamate concentration in synaptic space causes depolarization of neuronal cells through the binding of glutamate to the NMDA-type glutamate receptor, which causes further inflow of calcium ions into neurons.

Before describing the mechanism of cellular trauma that is triggered by the influx of excess Ca^{2+} into the intracellular space, we provide more-precise details regarding neuronal cell death and glutamate toxicity. For instance, MK-801 was developed as a noncompetitive inhibitor of NMDA-type glutamate channels. MK-801, which can cross the blood–brain barrier, has been described as an epoch-making agent, because it showed a neuronal protective action in experimental brain ischemia models (20). Further investigation has, however, revealed that the neuroprotective action of MK-801 also may be due to a secondary effect caused by the function of MK-801 to lower the body temperature. Therefore, it is still unclear whether MK-801 has a direct protective effect on neuronal cells under ischemic stress.

Moreover, owing to the various and severe side effects of MK-801, such as antipsychiatric action and formation of cavitative degeneration in neuronal tissue, its clinical application has become more difficult. Other NMDA-receptor antagonists have failed to show efficacy in clinical trials for stroke or brain trauma injury, although later compounds have shown less antipsychiatric action and cavitative degeneration. Glutamate may be involved in the acute neurodestructive phase that occurs immediately after traumatic or ischemic injury (excitotoxicity), but after this period, it may assume that normal physiologic functions of glutamate will be of importance, which includes promotion of neuronal survival. NMDA-receptor antagonists failed in stroke and traumatic brain injury trials in humans because blockade of synaptic transmission mediated by NMDA receptors hinders neuronal survival (30). In this context, a combination of low-affinity NMDA-receptor antagonist and β_2-adrenoceptor agonist, which suppress the antipsychiatric action of NMDA antagonist, is proposed for a possible therapeutic interaction in ischemic stroke (13).

Role of AMPA receptors in glutamate-induced hippocampal neuronal cell death

Despite the pessimistic outcome of MK-801 in clinical trials, MK-801 clearly shows a protective action on neuronal cells in *in vitro* experimental systems, including in cultured neurons and brain slices. Furthermore, NBQX (2,3-dehydroxy-6-nitro-7-sulfamoylbenzoquinoxaline), which has been developed as an AMPA-type glutamate channel antagonist, suppresses neuronal cell death in ischemia with comparative reproducibility (89). AMPA-type receptor antagonists have received considerable attention in recent years, and within the class of excitatory amino acid–receptor antagonists, AMPA-receptor antagonists have shown excellent neuroprotection in several models of cerebral ischemia and neuronal injury (68).

The AMPA receptor differs from the NMDA receptor in several ways (9). It is a ligand-gated cation channel, which is primarily permeable to Na^+, not to Ca^{2+}, and under physiologic conditions, the AMPA receptor functions as the principal mediator of fast excitatory neurotransmission. Recent studies involving animal models of transient forebrain ischemia and epilepsy show that gluR2 messenger RNA (mRNA), which codes for a subunit of the AMPA receptor, is downregulated in vulnerable neurons under pathologic conditions, such as a chronic state of brain ischemia. Further, AMPA receptors can be formed without the gluR2 subunit, which leads to the loss of its important characteristic, impermeability to

Ca^{2+} (82, 83). These observations suggest that downregulation of gluR2 gene expression may serve as a "molecular switch," leading to the formation of Ca^{2+}-permeable AMPA receptors and enhanced toxicity of endogenous glutamate, after a neurologic insult. This switching plays a quite important role in the pathogenesis of delayed neuronal cell death. Transient global or forebrain ischemia induced experimentally in animals can cause selective, delayed neuronal death of hippocampal CA1 pyramidal neurons. GluR2-lacking AMPA receptors result in the delayed death of CA1 neurons. Blockade of calcium-permeable AMPA receptors protects hippocampal neurons from the delayed type of cell death, suggesting a role of AMPA receptor in the chronic stage of brain ischemia (72).

Further, the AMPA receptor is found in the grey and white matter, where it is expressed by oligodendrocytes. Therefore, this AMPA-type receptor is thought to participate in demyelinization, which occurs through cell death of oligodendrocytes, as present in human pathologic stroke specimens. Phase I clinical trial data indicate that some AMPA-receptor antagonists can be safely administered to young and elderly subjects, although sedation and other CNS-associated adverse events become problematic with infusion times exceeding 24 h. Phase II studies for acute ischemic stroke also are ongoing (1).

Role of body temperature in ischemia-induced neuronal cell death

From the viewpoint of experimental pharmacology, MNDA and AMPA antagonists have been proven to suppress Ca^{2+} influx by blocking the action of glutamate receptors, thereby shedding considerable light on the important role of glutamate receptors in the pathogenesis of neuronal cell death. Accordingly, when glutamate binds to a non–NMDA-type receptor, depolarization of hippocampal neuronal cells occurs, followed by opening of other calcium channels, which leads to a massive inflow of Ca^{2+} into the cell. Therefore, despite MK-801 not being clinically useful, the experimental facts associated with MK-801 do not contradict the concept that neuronal cell death is triggered by glutamate in the hippocampus. Consistent with this, in homozygote mice carrying a truncated Glu-R6 gene, which codes for a subunit of the glutamate receptors in the mouse hippocampus, neuronal cell death caused by kainate, a glutamate agonist, is markedly suppressed, because activity of two types of glutamate receptors (AMPA and kinate type receptors) are suppressed (64).

Temperature is one of several environmental factors to play an important role in neuronal cell death in the hippocampus. Procedures that reduce the temperature of the internal organs can often control cell death caused by ischemia–reperfusion. This has been observed not only in brain ischemia, but also in other systemic organs, including the heart, liver, and kidney (96, 97). This has been thought to be a secondary result that is due to mitigation of the load on the energy-metabolism system caused by the decrease in temperature. However, the protective effect of low temperature against ischemic damage in hippocampal neurons appears to be more notable, compared with other internal organs. For example, in the gerbil hippocampus CA1 region, temporal ischemia at normal temperature (37ºC) causes the death of 90% or more of the neuronal cells, but reducing the body temperature by about 4ºC results in the survival of almost all neurons in this region.

Consistent with this result, glutamate release from neuronal cells under ischemic conditions is markedly suppressed by low temperatures (7, 74). In a gerbil model of unilateral occlusion of the common carotid artery, a decrease in temperature resulted in the suppression of glutamate to as little as 25% of that at a normal temperature. The details of the mechanism through which hypothermia reduces glutamate release in the hippocampus after an ischemic insult remain unknown (8, 59). However, many of the agents reported to have a neuroprotective function also cause a decrease in body temperature, thus raising a question of whether the neuroprotective action of these agents is due to an indirect or secondary effect caused by the low temperature. It has also become clear that the effects of MK-801 *in vivo,* which initially suggested it to be an effective antagonist of the NMDA-type glutamate receptor, resulted from its pharmacologic activity of reducing the brain temperature, as previously discussed.

Since 1990, 26 reports confirming the brain-protecting effect of hypothermia in rat focal cerebral ischemia models have been published. In these models, effective reduction of the infarct volume requires hypothermia to be started during ischemia or within no more than 1 h from the beginning of reperfusion. However, it is not clear whether this neuroprotective effect of hypothermia can also be observed in the chronic stage, such as several months later (61). Despite its obvious therapeutic potential, the clinical applicability of hypothermia as a form of neuroprotective therapy for human stroke has been investigated in only a few small studies. Therapeutic hypothermia is feasible in acute stroke, but owing to serious side effects, such as hypotension, cardiac arrhythmia, and pneumonia, it is still thought of as experimental, and evidence of efficacy from clinical trials is needed.

Role of Ca^{2+} in ischemia-induced neuronal cell death in the hippocampus

Unrestricted release of calcium ions into the intracellular space is a general cause of cell death, and this phenomenon is not restricted to neuronal cells (56). Under normal conditions, cytosolic Ca^{2+} combines with calcium-binding proteins, such as calmodulin, or exists in storage in cellular organelles, such as the endoplasmic reticulum (ER) (10) and the mitochondrion (17). Furthermore, free Ca^{2+} released into the intracellular space is actively excreted by the Na^{+}/Ca^{2+} exchange system, which is coupled to ATPase, and the concentration is adjusted to 1/10,000, or less than that outside the cell. It is understood that the Ca^{2+} concentration difference is established to maintain the S/N (signal/noise) ratio as rigorously as possible. Ca^{2+} plays a central role in signal transduction in the cell, which in turn controls the main cellular activities, including differentiation and proliferation. Glutamate binding to a glutamate receptor causes inflow of Ca^{2+} ions into the cell, and thus plays a major role in the pathogenesis of ischemia-induced cell death. When forced overexpression of the NMDA-type glutamate receptor is carried out in cell lines other than neurons, this can also cause inflow of Ca^{2+} ions, as well as subsequent cell

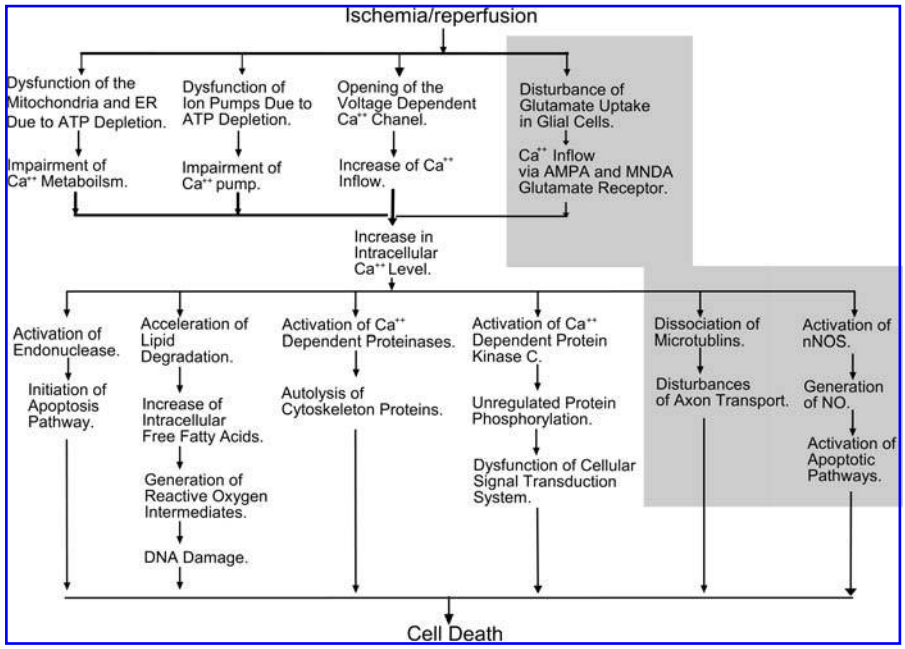

FIG. 2. Pathways to neuronal cell death in ischemia–reperfusion via the elevation of intracellular Ca^{2+}. Many pathways related to elevated Ca^{2+} level have been proposed to explain the mechanism of neuronal cell death after ischemia–reperfusion. However, only the pathways highlighted in the shaded area are specific to neurons. The other pathways are commonly observed in other cell types or tissues. Thus, to explain the vulnerability of neurons under stress, it is reasonable to hypothesize that the neuronal response to ischemia–reperfusion differs from those observed in other cell types.

death (2). Conversely, Ca^{2+} chelating agents prevent neuronal cell death in the hippocampus.

The influx of Ca^{2+} through the NMDA-type glutamate receptor is further accelerated by delayed depolarization, which is triggered mainly by the impairment of the calcium-exchange system. This impairment is much enhanced by energy starvation under ischemia. Furthermore, energy-metabolism impairment also paralyzes the intracellular storage of calcium, which results in an unregulated increase of intracellular Ca^{2+}. The influence of the disengagement of Ca^{2+} regulation on the cellular organelles is summarized in Fig. 2.

Because the Ca^{2+}-dependent pathways participate in important cellular activities, such as replication and differentiation, these pathways are strictly regulated under normal conditions. However, they are unrestrictedly activated by the delayed elevation of free Ca^{2+} under ischemic conditions, which eventually results in proteolysis of the proteins in the cell and subsequent cell death (44). Inflow of calcium into neurons starts with activation of glutamate receptors, but the pathways that are activated by the Ca^{2+} inflow and lead to cell death are by no means specific to neuronal cells.

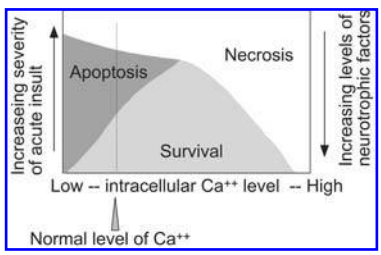

FIG. 3. Role of intracellular calcium in neuronal cell death. Although elevation of the Ca^{2+} concentration plays a very important role in cell death under ischemic conditions, as shown in Fig. 2, neurotrophic factors, differentiation of the neuron, and stress proteins all simultaneously determine the fate of the neuron.

Although elevation of the Ca^{2+} concentration in the cell plays a very important role in ischemia-induced cell death, the existence of neurotrophic factors, the extent of differentiation of the neuron, and the expression of stress proteins all simultaneously determine the fate of the neuron (Fig. 3).

STRESS RESPONSES IN THE CENTRAL NERVOUS SYSTEM

Neuronal cell death caused by ischemia tends to be viewed as irreversible cell death resulting from energy failure caused by rapid loss of blood supply, or by the activation of Ca^{2+}-dependent pathways, as discussed earlier. However, the expression of stress proteins (heat-shock proteins; HSPs) is observed in neurons under ischemic conditions (16, 48, 88). It has also been reported that tolerance for ischemia can be introduced by a preceding nonfatal ischemic stress (36). Therefore, it should be noted that the organ damage observed in ischemia is the result of a series of responses to compound stress. Here, we consider the role of stress proteins in brain ischemia and further discuss the function of stress proteins in cellular organelles.

What are the components of ischemia?

Brain ischemia is represented by an occlusion or insufficient blood supply in the central nervous system, which thereby initiates various types of environmental stresses (92, 93). These stress conditions include a decline in the oxygen concentration (hypoxia), glucose deprivation, lactic acid accumulation caused by a shift in anerobic metabolism, and reactive oxygen intermediates generated in the course of reperfusion. In the later phase, various immunologic responses threaten neuronal survival at the ischemic focus

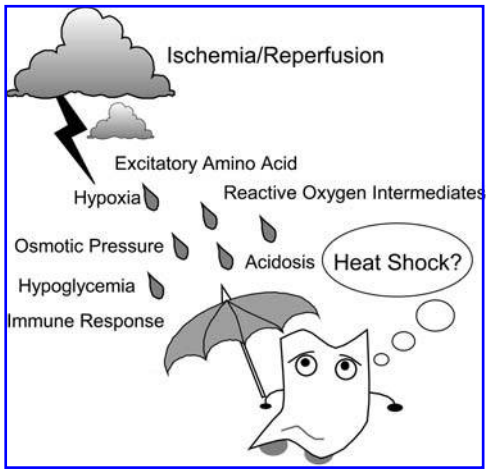

FIG. 4. Brain ischemia *in vivo*. It has been reported that unique stresses are imposed on neurons during ischemia–reperfusion *in vivo*. These stresses are caused by environmental changes, including exposure to excitatory amino acids and reactive oxygen intermediates, deprivation of oxygen and glucose, elevation of osmotic pressure and [H^+], and the initiation of a host immune response. Cells exposed to these stresses respond by expressing a specific set of proteins, which are referred to as stress proteins. The stress response of neurons in ischemia–reperfusion was first identified through recognition that the 70-kDa heat-shock protein (HSP70) is expressed in neurons in ischemic brain. Since this discovery, the expression of proteins in the HSP family has been extensively studied. The mechanism of expression of HSPs, which are commonly induced by elevated temperature, is still under investigation, but it appears that HSPs expressed in the brain play a role in neuronal protection during ischemia–reperfusion.

(Fig. 4). However, since the report of the expression of an HSP in brain ischemia, stress proteins, as represented by HSPs, have attracted considerable attention among the investigators as a mechanism that may play a protective role in ischemia (34, 87).

HSPs are induced when cells are exposed to hyperthermic conditions (43 to 46ºC) and are believed to have a protective function for cellular metabolism at elevated temperatures. HSPs are molecular chaperones; proteins that recognize and bind to improperly folded proteins and prevent their irreversible degeneration. This function is especially important because cellular proteins are easily denatured under hyperthermic conditions. Consistently, HSPs are indispensable for the maintenance of cellular metabolism in hyperthermia, and cells that lack HSPs cannot survive at an elevated temperature (81, 104). Given these properties, one might ask why such HSPs are expressed under conditions of ischemic stress, which has no apparent biologic relation to hyperthermia?

Stress proteins expressed in ischemia/reperfusion

Before answering this question, it should be noted that the chemical stress that constitutes ischemia *in vivo* targets different cellular organelles. During the course of a response against an environmental challenge, cells express a special apparatus, referred to as stress proteins, in each organelle (Fig. 5). For example, the generation of oxygen radicals, which are observed mainly during reperfusion, results in DNA damage, whereas reduction of cellular ATP denatures cytoplasmic proteins and impairs their function, as is discussed later. Furthermore, a decrease in the glucose or oxygen concentrations causes serious functional disorder in the ER. At the cellular level, various stress proteins are expressed in each cellular organelle under ischemic stress, suggesting that each intracellular component responds differently to extracellular stresses. The HSPs, which form a large family that is represented by the HSP of molecular mass 70 kDa (3, 104), and other stress proteins, which have been reported to be expressed in brain ischemia, are summarized, along with their localization in the cell, in Fig. 5. HSP70 (32) and RA301 (52) have been identified as major stress proteins in reoxygenated cultures. These proteins are translocated to the nucleus under stress. HSP110 (106), HSP72 (an inducible

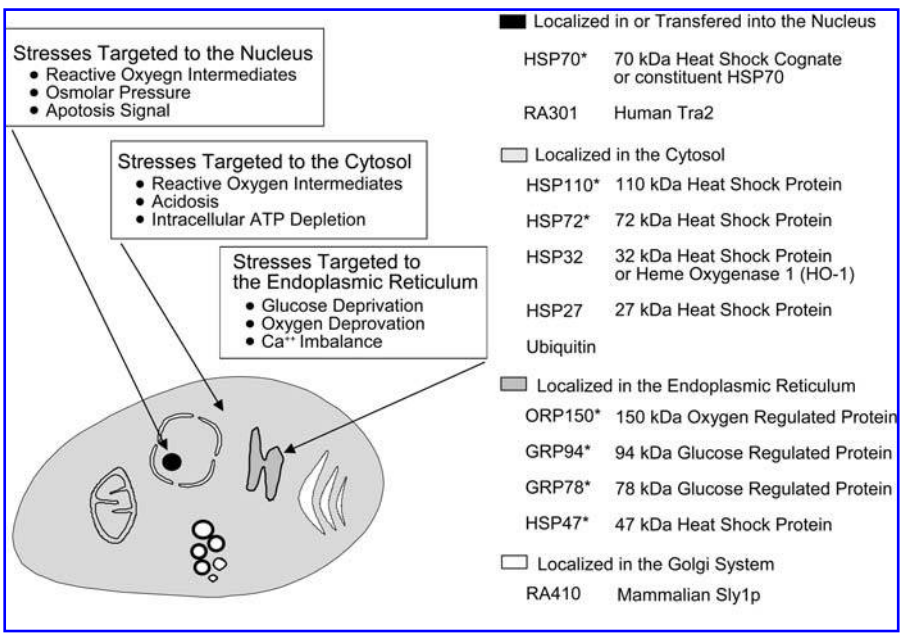

FIG. 5. Cellular organelles targeted by chemical stimuli and stress proteins. The chemical stimuli that cause stress during ischemia–reperfusion target different cellular organelles, and different types of stress proteins are induced to protect the function of each organelle.

form of HSP70; 32), HSP32 (also known as heme oxygenase-1; 28), HSP27 (also known as small-molecular-weight HSP) (14, 46), and ubiquitin (102) are all induced in the cytosol, whereas other groups of stress proteins are induced in the ER. ORP150 (150-kDa oxygen-regulated protein) (38), GRP94 (94-kDa glucose-regulated protein) (28), GRP78/Bip (B-cell immunoglobulin-binding protein) (28), and HSP47 (27) are known to be induced in both hypoxic astrocytes and brain ischemia. RA410 (52) is induced in the trans-Golgi system.

Proteins marked with an asterisk (*) in Fig. 5 are members of the HSP family, and it has been suggested that this family plays an important role under ischemic conditions. The roles of these HSPs are discussed in the following section.

Physiologic function of heat shock proteins

Because the stress caused by hyperthermia can be reproduced comparatively simply in a cultured cell, the roles of HSPs under conditions of elevated temperature have been well characterized (Fig. 6). Intracellular environmental changes caused by the elevated temperature produce a critical situation for metabolism in the cell, including protein denaturation and aggregation (through which the protein function is totally abolished), and further protein denaturation that leads to cell death. HSPs, by binding to denatured proteins, prevent them from being further denatured, unless the environmental alteration is lethal. After the environmental stress is removed, HSPs are able to reform the denatured proteins by using energy from ATP hydrolysis. Furthermore, under conditions of severe stress, HSPs can discriminate between severely denatured and recoverable proteins and then combine the fatally denatured proteins with ubiquitin, a low-molecular-weight polypeptide. When denatured proteins are polyubiquitinated, they are directed into the proteasomal protein-degradation pathway. HSPs, therefore, act as a defense factor against the impairment of cellular metabolism produced by protein denaturation (3) and thus play a pivotal role in organ protection in disease (47). In addition, more-direct action of HSPs is known; such as direct suppression of proapoptotic signaling events and stabilization of mitochondrial outer membrane permeabilization (4).

Consistent with this, tolerance against heat shock is acquired in cells that express HSPs under hyperthermic conditions, suggesting that HSPs plays a central role in maintaining metabolism under elevated temperatures. More surprisingly, this phenomenon is observed not only at a cellular level, but also on an organ level. Consistent with the expression of HSPs at ischemic foci, a protective effect against ischemia or trauma in neurons can be obtained in animals by exposing them to high temperature before the ischemic insult (37). Furthermore, an effect known as the "ischemia tolerance phenomenon" has been identified, in which neurons are protected from subsequent fatal ischemic stress by treatment with an earlier nonfatal and transient ischemic stress (36). The expression of HSP70 has been reported under experimental conditions that can cause this phenomenon. Moreover, it has also been shown that ischemic stress can activate heat-shock factor-1 (HSF1) in cultured cells. The rapid decline of ATP content under ischemic conditions induces aggregation of intracellular proteins, including HSF1, eventually leading to expression of HSPs (Fig. 7) (32). With the availability of transgenic animals and gene-transfer technology, it has become increasingly clear that such HSPs do indeed protect cells from injury. Several reports have now shown that selective overexpression of HSP70 leads to protection in several different models of nervous system injury (107).

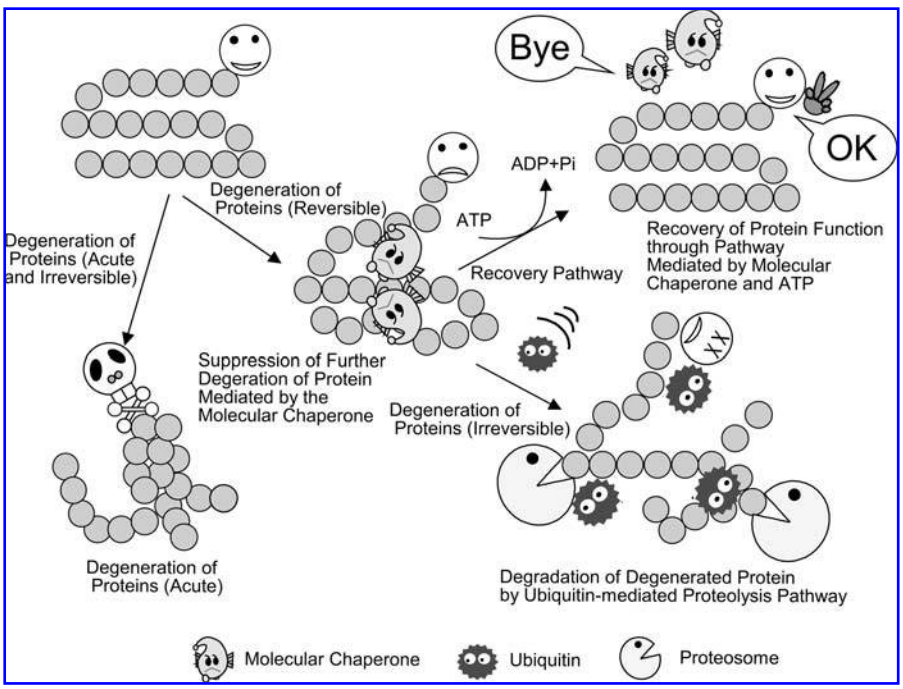

FIG. 6. Role of molecular chaperones in protein degradation. Heat-shock proteins (HSPs) function as molecular chaperones to maintain cellular metabolism. Because temporarily and irreversibly degraded proteins are toxic to maintenance of cellular homeostasis, molecular chaperones and ubiquitins participate in the removal of such proteins. Degenerated proteins are temporarily neutralized by the binding of molecular chaperones. When the protein is only mildly damaged (misfolded), proteins are refolded by HSPs, which use ATP in the recovery process. If the degeneration of the protein is irreversible, it is ubiquitinated and degraded through the ubiquitin-mediated proteolysis pathway.

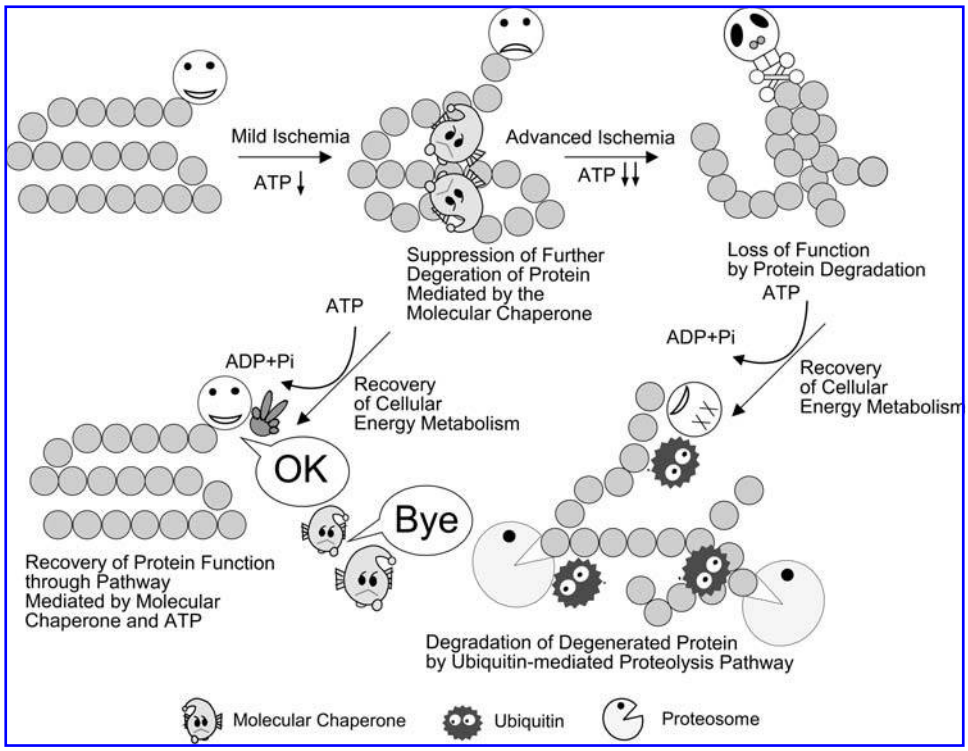

FIG. 7. Role of molecular chaperones in ischemia. The role of cytosolic molecular chaperones, such as HSPs, can be understood by analogy to their role in response to an elevated temperature. Several aspects of ischemia–reperfusion (decline in pH and generation of reactive oxygen intermediates) cause degeneration of proteins in the cytosol. HSPs protect these proteins from irreversible degradation and assist in refolding of mildly degenerated proteins, after removal of the stress. Proteins that are irreversibly degraded are ubiquitinated and processed via the proteolysis pathway.

Expression of stress proteins in cultured cells under ischemic conditions

Various stresses, such as glucose deprivation caused by a shift of energy metabolism to anaerobic glycolysis, generation of reactive oxygen intermediates during reperfusion, and changes in osmotic pressure, are believed to influence neurons (see Fig. 4). Among the environmental factors that constitute ischemia *in vivo*, a decrease in oxygen concentration causes the most serious stress to the cellular metabolism. When the oxygen concentration decreases in the circumference around the cell, the energy supply is maintained by shifting energy metabolism from aerobic to anerobic glycolysis. At the same time, exposure of cells to hypoxic conditions results in the expression of a group of stress proteins, which are referred to as oxygen-regulated proteins (ORPs) (25). This suggests that hypoxia is perceived by the cell as a stress condition, and that hypoxia causes induction of a specific set of stress proteins. Hence, such a response to hypoxia is analogous to the stress response to elevated temperature, in which a specific set of stress proteins also is expressed.

The expression of ORPs was first observed by radiobiologists in tumor cells (25). From a radiobiology viewpoint, it is well known that solid tumors show tolerance to radiotherapy. Solid tumors with reduced vascularization often have a very low oxygen concentration inside the tumor, and especially in the tumor core. By analogy with the role of HSPs in heat stress, it is likely that tumor cells show tolerance to hypoxic conditions through the expression of ORPs. Conversely, another category of stress proteins, whose expression is greatly enhanced by the deprivation of glucose from the culture medium or by the exposure of cells to a calcium ionophore, has been identified (65, 100). These proteins are referred to as glucose-regulated proteins (GRPS). Subsequent research has demonstrated that the amino acid sequence of ORPs of molecular masses 94 and 78 kDa are identical to those of GRPs of similar molecular masses. Moreover, the cloning of the 150-kDa ORP (ORP150) was reported, and its amino acid sequence is identical to that of GRP170 (170-kDa GRPs) (18, 45). These data suggest that oxygen deprivation and low glucose both result in stress that targets the same cellular organelle, the ER. Hence, these stresses are referred to as ER stress. ER stress is characterized by the accumulation of immature or unfolded proteins inside the ER (91), whereas heat stress is characterized by the accumulation of denatured proteins in the cytosol.

Response of astrocytes to hypoxic stress

Among the cell population that constitutes the central nervous system, astrocytes are resistant to ischemia and are strategically located to protect neurons. In addition, they play a central role in the reorganization and restoration of brain tissue after ischemia (26). Furthermore, astrocytes display

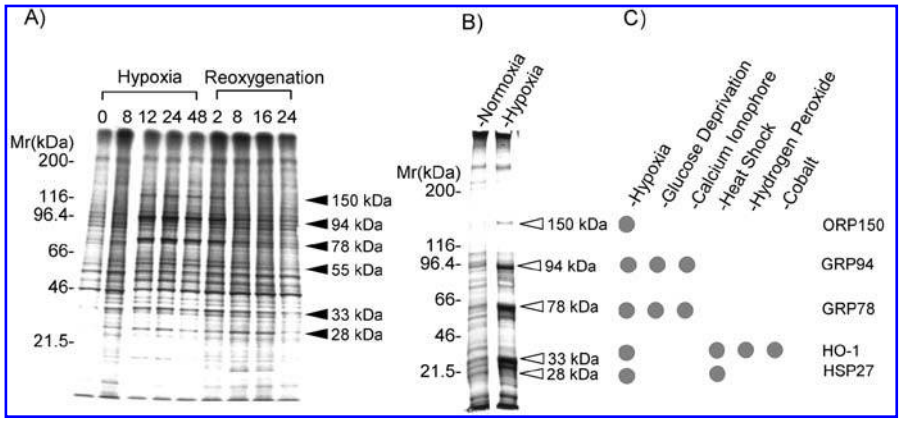

FIG. 8. Expression of ORPs in cultured astrocytes. (**A**) Cultured astrocytes were exposed to hypoxia for 48 h, followed by reoxygenation. At each time point, cells were metabolically labeled with ^{35}S-methionine for 3 h, and protein extracts were separated using SDS-PAGE. The autoradiogram of the gel showed induction of several bands (proteins) in astrocytes. The migration of molecular-weight markers is shown on the left side of the gel. The induction of oxygen-regulated proteins in astrocytes (molecular masses: 150, 94, 78, 55, 33, and 28 kDa) is indicated by solid arrowheads. (**B**) Protein extracts prepared from astrocytes maintained under either normoxic or hypoxic conditions for 48 h. Several proteins are still visible by using Coumassie staining, indicating the abundant expression of these stress proteins (indicated by the open arrowheads). The migration of molecular-mass markers is shown on the left side of the gel. (**C**) A summary of the expression of stress proteins induced by chemical stimuli. Astrocytes were exposed to either hypoxia (48 h), glucose-free conditions (glucose deprivation), a calcium ionophore, heat stress, hydrogen peroxide, or cobalt chloride (see details in Hori et al., 1996). Note that ORP150 is expressed predominantly on exposure to hypoxia. Amino acid sequence analysis of the proteins proved them to be ORP150 (150 kDa), GRP94 (94 kDa), GRP78/BiP (78 kDa), calreticulin (55 kDa), heme oxygenase-1 (33 kDa), and HSP27 (28 kDa).

neurotrophic properties, even under stressful conditions (49, 50). As shown in Fig. 8A, exposure of astrocytes to hypoxic stress results in the expression of a set of stress proteins, and autoradiography after SDS-PAGE of the protein lysate of the cells shows that these stress proteins are metabolically labeled (28). Such a marked stress response is not seen when other cell types in the central nervous system, such as microglias, brain endothelial cells, and neurons, are exposed to the same hypoxic conditions. The stress proteins expressed in astrocytes include those of molecular mass 150, 94, 78, 33, and 28 kDa and, based on both their amino acid sequence and their induction patterns under chemical stress, these proteins are presumed to be ORP150, GRP94, GRP78 (also known as B-cell immunoglobulin-binding protein; BiP), heme oxygenase-1 (HO-1), and HSP27, respectively. Surprisingly, the expression of these stress proteins is so remarkable that many of them can be recognized as a band that is detectable even by Coumassie blue staining of the gel (Fig. 8B). As shown in Fig. 8C, the stress protein of molecular mass 78 kDa is confirmed to be GRP78/BiP, and that of 33 kDa to be HO-1, by Western blot by using specific antibodies for these proteins (28). These data suggest that astrocytes have an extremely rich response to hypoxic stress. As predicted by Sutherland et al. (25), the phenotype of astrocyte resistance to ischemic stress is supported by the abundant expression of these stress proteins, each of which protects the cellular metabolism from the environmental changes caused by oxygen deprivation.

ORP150 and astrocytes

ORP150 (150-kDa ORP) was first cloned as a novel stress protein induced when a primary culture of astrocytes is maintained under deep hypoxia ($Po_2 \approx 10$ torr in the medium, for 24 hours). The ORP150 cDNA sequence shows that the protein belongs to the molecular chaperone family. Immunocytochemical analysis shows that ORP150 is localized in the ER, similar to GRP78 and GRP94 (43). Although ORP150 is induced mainly by hypoxia in cultured astrocytes (Fig. 8C), and was initially purified and cloned in these cells, its expression has now been confirmed in a murine model of stroke using occlusion of the middle cerebral artery (54), and also in human brain after a cerebrovascular accident (98). Furthermore, the expression of ORP150 is known to occur in human arteriosclerotic lesions, which are believed to be exposed to chronically low perfusion and hypoxia (101). In human embryonic kidney cells, the permanent expression of an antisense sequence against ORP150 results in an ORP150 deficit, and in these cells, hypoxia resulted in the ultrastructural changes in the ER and nucleus (Fig. 9). Consistently, cells deficient in ORP150 showed vulnerability to hypoxia, whereas resistance to chemical stresses, such as oxygen radicals, and to heat remains unchanged.

ORP150 is expressed in the liver, the pancreas, and the thyroid gland, where protein neogenesis and secretion are significant. GRP78 and GRP94 show the same distribution pattern (29). In these internal organs, the ER is well developed, reflecting the significant protein secretion, and increased protein trafficking through the ER can cause ER stress. Conversely, exposure of tumor cells, and also macrophages and astrocytes, to hypoxic conditions causes expression of ORP150, as well as expression of GRP78 and GRP94. This can be interpreted as a protective response through which cells protect their ER during hypoxia. Under hypoxic conditions, it is assumed that immature proteins accumulate in the ER because of the shortage of energy (ATP) caused by intracellular glucose starvation. Further, retardation of protein transportation causes a relative increase in protein traffic through the ER, and this is believed to induce mechanical stress in the ER.

Hence, when an additional ER stress is superimposed, can a certain cascade, which results in cell death, be initiated? The cell death seen in cells that lack ORP150 requires *de novo* protein synthesis. This result indicates that cell death can be escaped by suppressing *de novo* protein synthesis and even lead us to hypothesize that a certain active cell death

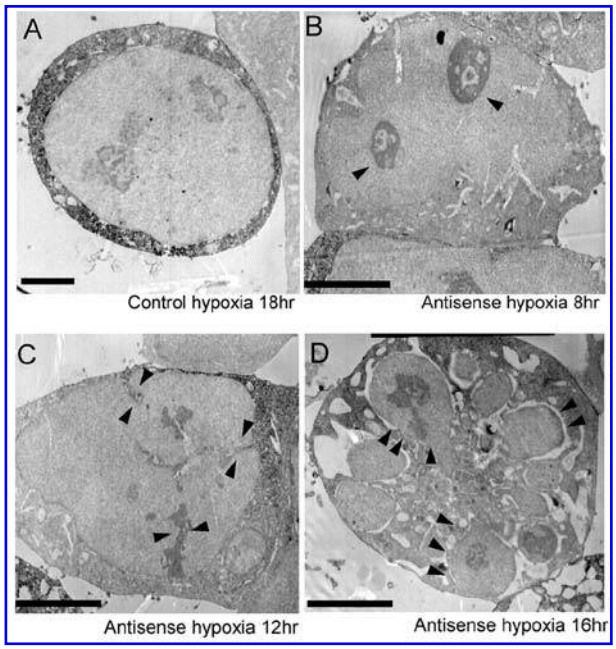

FIG. 9. Hypoxia-mediated cell death in ORP150-deficient cells. Hypoxia-mediated cell death is accelerated in mammalian cells deficient in ORP150. In permanently transfected A239 cells (derived from human embryonic kidney cells), which overexpress an antisense ORP150 sequence, induction of ORP150 is suppressed. Either wild-type (**A**) or ORP150-antisense transformant HEK cells (**B–D**) were exposed to hypoxia for ≤20 h, fixed, and studied with transmission electron microscopy. Micrographs were obtained from cells exposed to hypoxia for 20 h (**A**; wild type), or 16 h (**B**), 18 h (**C**) and 20 h (**D**) for ORP150-deficient HEK cells (bars, 4 μm). Electron microscopy of the ORP150-deficient cells subjected to hypoxia for 14–18 h showed fragmented nuclei with condensed chromatin (**C**), and formation of apoptotic bodies (**D**). Wild-type control cells harvested as long as 20 h after onset of hypoxia displayed normal nuclear morphology (**A**), suggesting a mechanism of cell death that is accelerated in ORP150-deficient cells.

pathway can be ignited by ER stress. The concept of cell death by so-called "apoptosis" has been established as a cell-death cascade accompanied by *de novo* protein synthesis. This is commonly observed in many cell types, and various factors that contribute to such cell death have been identified, with mitochondria as the starting point of the cell-death cascade (40). In this context, the concept of cell death initiated from the ER has been proposed, and accumulating experimental data support the existence of such a pathway (41), which is discussed later. As a first report, we note that in dengue fever, which is a viral disease that invades neurons, misfolded viral proteins accumulate in the ER, and cell death occurs in cultured neurons infected with the dengue virus (15).

ER stress, a novel target for understanding neuronal cell death in brain ischemia

The possible involvement of ER dysfunction in cell death, and especially that seen in neurons, has been largely neglected until recently, despite the central role of the ER to maintain cell functions. As well as being involved in the control of cellular calcium homeostasis, the ER is also the subcellular compartment in which the folding and processing of membrane and secretory proteins takes place. Because blocking of these processes is sufficient to cause cell damage, this indicates that they are crucial for normal cell functioning. The complex processes that take place in this subcellular compartment are, however, affected in different ways in various disorders, whereas it is ER calcium homeostasis that is disturbed in ischemia (80).

The ER plays an important role in the degradation of phagocytosed proteins and lipids, and in macrophages nuclear factor (NF)-κB is activated by oxygen radicals generated inside the ER. This observation suggests a possible role for the ER in cell death (76), and recently several pieces of evidence have accumulated that suggest a mechanism of ER-derived neuronal cell death (Fig. 10). A mutation in presenilin-1 (PS-1), which is frequently seen in Alzheimer

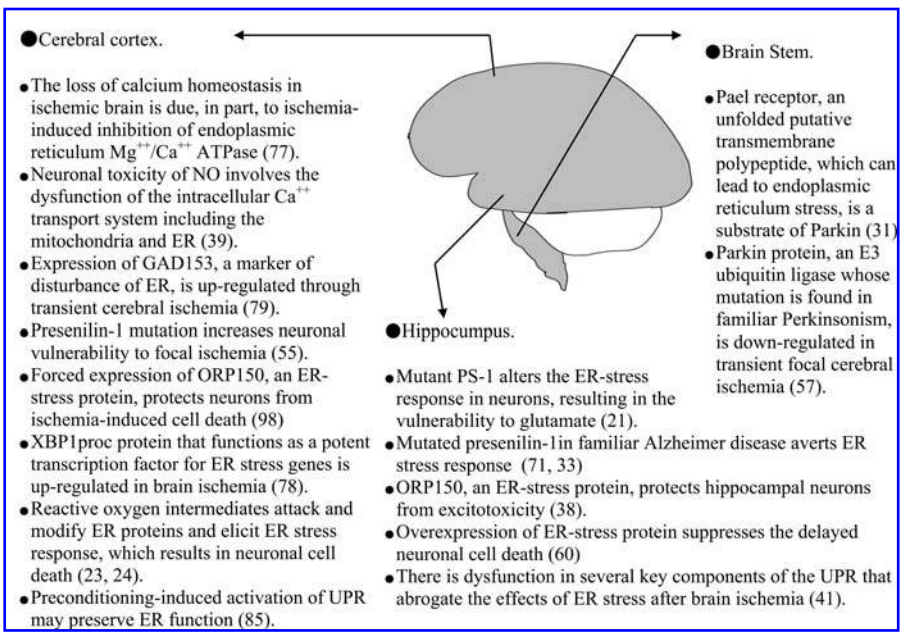

FIG. 10. Endoplasmic reticulum (ER) dysfunction and the pathogenesis of ischemic brain damage. Recent studies of dysfunction of the ER in neurodegenerative diseases and the possible contribution of such malfunctions to the pathogenesis of brain ischemic damage are summarized for various region of the brain.

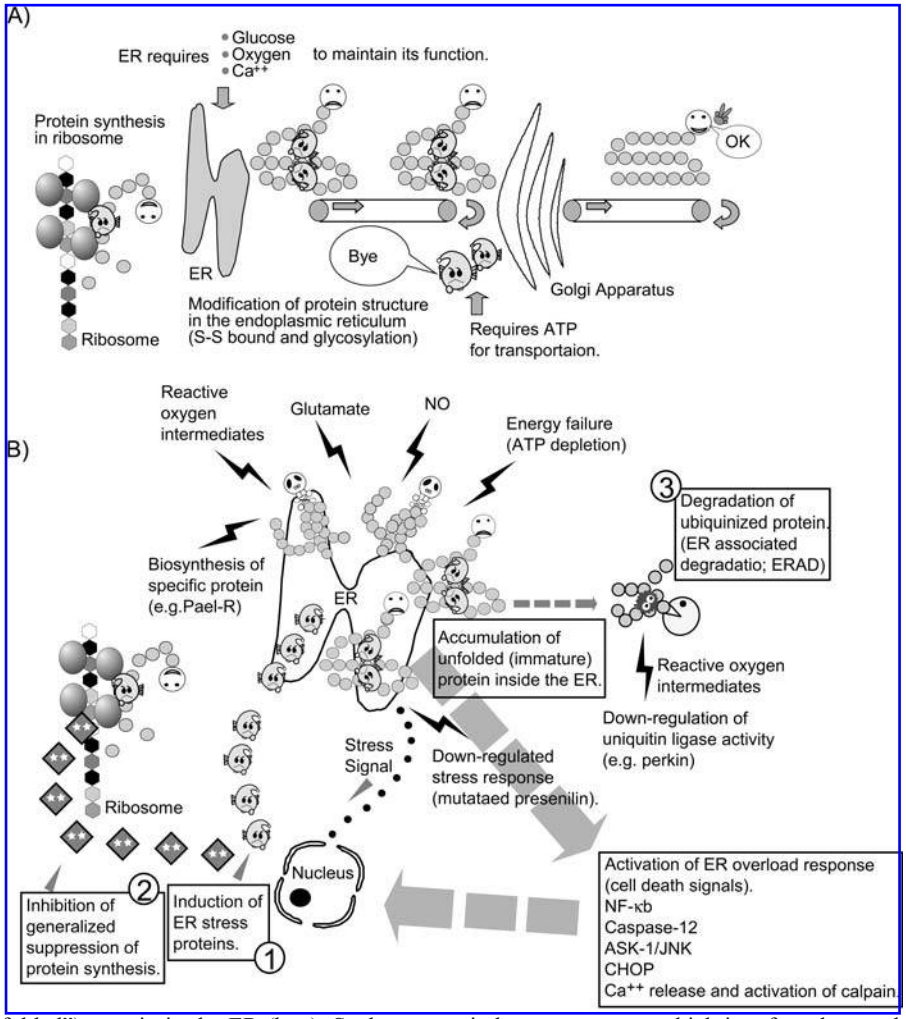

FIG. 11. Role of molecular chaperones in the endoplasmic reticulum (ER) and the unfolded protein response (UPR). In the ER, molecular chaperones play a pivotal role in the maintenance of ER function. The molecular chaperons in the ER share common motifs with HSPs and are referred to as glucose-regulated proteins (GRPs) or oxygen-regulated proteins (ORPs), because their expression is markedly enhanced by deprivation of environmental glucose or oxygen. Whereas HSPs mediate the quality control of proteins in the cytosol, GRPs/ORPs participate in the maturation of proteins that pass through the ER–Golgi system. These proteins include secretory proteins and cell-surface receptors, which play a crucial role in intercellular signal transmission. Protein modification and maturation performed in the ER requires glucose, oxygen, and Ca^{2+}, whereas protein transport in the Golgi system and thereafter is mediated by ATP (**A**). Although deprivation of glucose and oxygen decelerates this pathway, various other factors, which are specific to neurons, also cause serious dysfunction of the ER, resulting in the accumulation of immature (or "unfolded") protein in the ER (box). Such stresses induce a response, which is referred to as the "unfolded protein response," that directly increases the expression of molecular chaperones in the ER. The term "unfolded protein response" is used for GRPs/ORPs in analogy to the "heat-shock response" for HSPs. The ER triggers this response, which consists of three pathways (box): (1) Induction of stress proteins in the ER, (2) generalized suppression of protein synthesis, and (3) activation of a protein degradation pathway (ERAD), to maximize the folding capacity of the ER. The failure of this response ultimately results in activation of the cell-death pathway (box).

disease, reduces the ER stress response (33, 71). In knockin mice carrying a mutant PS-1, the ER stress response is especially suppressed in neurons, which results in neuronal vulnerability to stresses such as exposure to glutamate (21) and ischemic stress (56). These observations suggest that the ER may play a central role in stress-induced cell death in neurons, both under ischemic conditions and in neurodegenerative diseases. Consistent with this, transgenic mice that overexpress ORP150 are resistant to ischemic stress (98), as well as to neuronal cell death caused by excitatory amino acids (38). Proteins involved in cell death have also been cloned, such as CHOP (12, 103), Perk (33), and caspase-12 (66).

Moreover, recent studies that have been performed mainly in Paschen's group suggest that ischemic neuronal cell damage is worsened by a "neuron-specific" stress response in the ER (see Fig. 10). For instance, it has been reported that loss of calcium homeostasis in the ischemic brain is due, in part, to ischemia-induced inhibition of ER Mg^{2+}/Ca^{2+} ATPase (77). Expression of ER-stress markers, such as GAD153 (79) and XBP1 protein (78), has also been observed. It has been reported that the neuronal toxicity of nitric oxide (NO) involves dysfunction of the intracellular Ca^{2+} transport system in the mitochondria and ER (39), suggesting that hypoxia and glutamate, the two major factors that induce ischemic brain damage *in vivo*, target the same cellular organelle, the ER. Since the discovery of the parkin gene (35), which is responsible for the pathogenesis of autosomal recessive juvenile parkinsonism, the ER-stress response has been considered to be involved in parkinsonism (31). Because parkin participates in ER-associated protein degradation as a ubiquitin ligase, the downregulation of this gene in transient focal ischemia also suggests that a neuronal ER response in the

brainstem may be involved in the pathogenesis of parkinsonism (57). This evidence demonstrates that ER function is compromised in acute and chronic diseases of the brain and further indicates that severe disturbances to ER function are sufficient to induce cell injury.

Intervention of neuronal cell death in ischemia

The ER plays a major role in the maintenance of cellular function in eukaryotes by providing an environment for complex processes such as calcium storage and calcium signaling and for processing and folding of newly synthesized membrane and secretory proteins. To maintain its function, the ER can sense an alteration in both the intracellular and extracellular environment and initiate a cellular response to severe forms of stress (Fig. 11). Cell injury may develop under conditions in which ER calcium homeostasis and/or folding or processing of proteins is disturbed, leading to the accumulation of unfolded or immature proteins inside the ER. This conformational stress in the ER is relieved by induction of ER-stress proteins (the unfolded protein response) accompanied by generalized protein synthesis and the activation of a proteolysis pathway; ER-associated degradation (ERAD). In neurons, various factors may induce ER dysfunction, including biosynthesis of specific proteins such as Pael receptors (31), energy failure that interferes with ATP-dependent protein transport, nitric oxide (NO) and reactive oxygen intermediates, and genetic defects that suppress the ER-stress response. Furthermore, downregulation of ERAD-associated enzymes may accelerate ER dysfunction. In case of a failure to maximize the ER folding capacity, the ER overload response is activated. This includes expression of NF-κB (99), caspase-12 (90), ASK-1 (69), C/EBP homologous protein (CHOP) (75), and Ca^{2+} release followed by activation of the calpain pathway (38). Knowledge of the exact mechanisms underlying ER dysfunction in different disorders of the brain will improve our chances of finding specific therapeutic strategies to protect neurons from ischemia-induced cell death.

CONCLUSION

Conventionally, it has been considered that neuronal cell death in the ischemic brain is an irreversible phenomenon that is due to the collapse of energy metabolism in the cell. However, the expression of stress proteins such as HSPs in ischemic foci shows that the defense mechanism is organized as a dynamic stress response, accompanied by protein neogenesis. Furthermore, as discussed in the latter part of this review, studies of the consequences of deterioration in ER function and identification of mechanisms causing ER dysfunction under pathologic brain conditions will be of significance. Such studies will help to determine whether ischemia-induced neuronal cell death is indeed caused by ER dysfunction and will facilitate the search for drugs capable of directly blocking pathologic processes at an early stage. It will be of importance to modulate the response to achieve optimal brain conditions for rescue of neurons from impairment during the kind of cellular metabolic crisis that is seen in cerebrovascular disease.

ABBREVIATIONS

ER, endoplasmic reticulum; GRP, glucose-regulated protein; HSP, heat-shock protein; ORP, oxygen-regulated protein.

REFERENCES

1. Akins PT and Atkinson RP. Glutamate AMPA receptor antagonist treatment for ischaemic stroke. *Curr Med Res Opin* 18(suppl 2): s9–s13, 2002.
2. Anegawa NJ, Lynch DR, Verdoorn TA, and Pritchett DB. Transfection of N-methyl-D-aspartate receptors in a nonneuronal cell line leads to cell death. *J Neurochem* 64: 2004–2012, 1995.
3. Beere HM and Green DR. Stress management: heat shock protein-70 and the regulation of apoptosis. *Trends Cell Biol* 11: 6–10, 2001.
4. Beere HM. Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J Clin Invest* 115: 2633–2639, 2005.
5. Benveniste H. The excitotoxin hypothesis in relation to cerebral ischemia. *Cerebrovasc Brain Metab Rev* 3: 213–245, 1991.
6. Busto R, Dietrich WD, Globus MY, and Ginsberg MD. The importance of brain temperature in cerebral ischemic injury. *Stroke* 20: 1113–1114, 1989.
7. Busto R, Dietrich WD, Globus MY, Valdes I, Scheinberg P, and Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J Cereb Blood Flow Metab* 7: 729–738, 1987.
8. Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, and Ginsberg MD. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 20: 904–910, 1989.
9. Chittajallu R, Braithwaite SP, Clarke VR, and Henley JM. Kainate receptors: subunits, synaptic localization and function. *Trends Pharmacol Sci* 20: 26–35, 1999.
10. Corbett EF, Michalak M. Calcium, a signaling molecule in the endoplasmic reticulum? *Trends Biochem Sci* 25: 307–311, 2000.
11. Coyle JT and Puttfarchen P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262: 689–695, 1993.
12. Crozat A, Aman P, Mahl N, and Ron D. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature* 363: 640–644, 1993.
13. Culmsee C, Junker V, Kremers W, Thal S, Plesnila N, and Krieglstein J. Combination therapy in ischemic stroke: synergistic neuroprotective effects of memantine and clenbuterol. *Stroke* 35: 1197–1202, 2004.
14. David JC, Lry J, and Grongnet JF. Perinatal expression of heat-shock protein 27 in brain regions and nonneural tissues of the piglet. *J Mol Neurosci* 15: 109–120, 2000.
15. Despres P, Flam M, Ceccaldi PE, and Deubel V. Human isolates of dengue type 1 virus induce apoptosis in mouse neuroblastoma cells. *J Virol* 70: 4090–4096, 1996.
16. Dienel GA, Kiessling M, Jacewicz M, and Pulsinelli WA. Synthesis of heat shock proteins in rat brain cortex after transient ischemia. *J Cereb Blood Flow Metab* 6: 505–510, 1986.
17. Duchen MR. Mitochondria and calcium: from cell signalling to cell death. *J Physiol* 529: 57–68, 2000.
18. Easton DP, Kaneko Y, and Subjeck JR. The hsp110 and Grp1 70 stress proteins: newly recognized relatives of the Hsp70s. *Cell Stress Chaperones* 5: 276–290, 2000.
19. Felipo V, Hermenegildo C, Montoliu C, Llansola M, and Minana MD. Neurotoxicity of ammonia and glutamate: molecular mechanisms and prevention. *Neurotoxicology* 19: 675–681, 1998.
20. Gill R, Foster AC, and Woodruff GN. Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. *J Neurosci* 7: 3343–3349, 1987.
21. Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, and Mattson MP. Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. *Nat Med* 5: 101–106, 1999.

22. Harding HP, Zhang Y, Bertolotti A, Zeng H, and Ron D. Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell* 5: 897–904, 2000.
23. Hayashi T, Saito A, Okuno S, Ferrand-Drake M, Dodd RL, and Chan PH. Damage to the endoplasmic reticulum and activation of apoptotic machinery by oxidative stress in ischemic neurons. *J Cereb Blood Flow Metab* 25: 41–53, 2005.
24. Hayashi T, Saito A, Okuno S, Ferr-Drake M, Dodd RL, Nishi T, Maier CM, Kinouchi H, and Chan PH. Oxidative damage to the endoplasmic reticulum is implicated in ischemic neuronal cell death. *J Cereb Blood Flow Metab* 23: 1117–1128, 2003.
25. Heacock CS and Sutherland RM. Induction characteristics of oxygen regulated protein. *Int J Radiat Oncol Biol Phys* 12: 1287–1290, 1986.
26. Hertz L. Astrocytes. In: *Handbook of Neurochemistry,* Vol 1, 2nd ed. Edited by Lajtha A. 1981, pp. 603–661.
27. Higashi T, Takechi H, Uemura Y, Kikuchi H, and Nagata K. Differential induction of mRNA species encoding several classes of stress proteins following focal cerebral ischemia in rats. *Brain Res* 650: 239–248, 1994.
28. Hori O, Matsumoto M, Maeda M, Kuwabara K, Ueda H, Ohtsuki T, Kinishita T, Ogawa S, Kamada T, and Stern D. Exposure of astrocytes to hypoxia/reoxygenation results in intracellular glucose depletion and enhanced expression of glucose-regulated proteins (GRPs). *J Neurochem* 66: 973–979, 1996.
29. Ikeda J, Kaneda S, Kuwabara K, Ogawa S, Kobayashi T, Matsumoto M, Yura T, and Yanagi H. Cloning and expression of cDNA encoding the human 150 kDa oxygen regulated protein, ORP150. *Biochem Biophys Res Commun* 230: 94–99, 1997.
30. Ikonomidou C and Turski L. Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurol* 6: 383–386, 2002.
31. Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, and Takahashi R. An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. *Cell* 105: 891–902, 2001.
32. Imuta N, Ogawa S, Maeda Y, Kuwabra K, Hori O, Ueda H, Yanagihara Y, and Tohyama T. Induction of 72 kDa inducible heat shock protein (HSP72) in cultured rat astrocytes after energy depletion. *J Neurochem* 70: 550–555, 1998.
33. Katayama T, Imaizumi K, Sato N, Miyoshi K, Kudo T, Hitomi J, Morihara T, Yoneda T, Gomi F, Mori Y, Nakano Y, Takeda J, Tsuda T, Itoyama Y, Murayama O, Takashima A, St George-Hyslop P, Takeda M, and Tohyama M. Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. *Nat Cell Biol* 1: 479–485, 1999.
34. Kinouchi H, Sharp FR, Hill MP, Koistinaho J, Sagar SM, and Chan PH. Induction of 70-kDa heat shock protein and hsp70 mRNA following transient focal cerebral ischemia in the rat. *J Cereb Blood Flow Metab* 13: 105–115, 1993.
35. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, and Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392: 605–608, 1998.
36. Kitagawa K, Matsumoto M, Tagaya M, Hata R, Ueda H, Niinobe M, Handa N, Fukunaga R, Kimura K, Mikoshiba K, and Kamada T. Ischemic tolerance phenomenon found in the brain. *Brain Res* 528: 21–24, 1990.
37. Kitagawa K, Matsumoto M, Tagaya M, Kuwabara K, Hata R, Handa N, Fukunaga R, Kimura K, and Kamada T. Hyperthermia-induced neuronal protection against ischemic injury in gerbils. *J Cereb Blood Flow Metab* 11: 449–452, 1991.
38. Kitao Y, Ozawa K, Miyazaki M, Tamatani M, Kobayashi T, Yanagi H, Okabe M, Ikawa M, Yamashima T, Tohyama M, Stern D, Hori O, and Ogawa S. Expression of 150 kDa oxygen regulated protein (ORP150), a molecular chaperone in the endoplasmic reticulum, rescues hippocampal neurons from glutamate toxicity. *J Clin Invest* 108: 1439–1450, 2001.
39. Kohno K, Higuchi T, Ohta S, Kohno K, Kumon Y, and Sakaki S. Neuroprotective nitric oxide synthase inhibitor reduces intracellular calcium accumulation following transient global ischemia in the gerbil. *Neurosci Lett* 224: 17–20, 1997.
40. Korsmeyer J. Regulators of cell death. *Trends Genet* 11: 101–105, 1995.
41. Kumar R, Krause GS, Yoshida H, Mori K, and DeGracia DJ. Dysfunction of the unfolded protein response during global brain ischemia and reperfusion. *J Cereb Blood Flow Metab* 23: 462–471, 2003.
42. Kwak S and Weiss JH. Calcium-permeable AMPA channels in neurodegenerative disease and ischemia. *Curr Opin Neurobiol* 16: 281–287, 2006.
43. Lee AS. Mammalian stress response: induction of the glucose regulated protein family. *Curr Opin Cell Biol* 4: 267–273, 1992.
44. Lee MS, Kwon YT, Li M, Peng J, Friedler RM, and Tsai LH. Neurotoxicity induces cleavage of p35 to p25 by calpain. *Nature* 405: 360–364, 2000.
45. Lin HY, Masso-Welch P, Di YP, Cai JW, Shen JW, and Subjeck JR. The 170-kDa glucose-regulated stress protein is an endoplasmic reticulum protein that binds immunoglobulin. *Mol Biol Cell* 4: 109–119, 1993.
46. Ma E and Haddad GG. Anoxia regulates gene expression in the central nervous system of *Drosophila melanogaster*. *Brain Res Mol Brain Res* 46: 325–328, 1997.
47. Macario AJ, Conway de Macario E. Stress and molecular chaperones in disease. *Int J Clin Lab Res* 30: 49–66, 2000.
48. MacManus JP and Linnik MD. Gene expression induced by cerebral ischemia: an apoptotic perspective. *J Cereb Blood Flow Metab* 17: 815–832, 1997.
49. Maeda Y, Matsumoto M, Ohtsuki T, Kuwabara K, Ogawa S, Hori O, Shui DY, Konoshita T, Kamada T, and Stern D. Hypoxia-reoxygenation mediated induction of interleukin-6 in cultured rat astrocytes and expression in ischemic gerbil brain: a paracrine mechanism enhancing neuron-survival. *J Exp Med* 180: 2297–2308, 1994.
50. Matsumoto K, Yamada K, Kohmura E, Kinoshita A, and Hayakawa T. Role of pyruvate in ischaemia-like conditions on cultured neurons. *Neurol Res* 16: 460–464, 1994.
51. Matsumoto M, Hatakeyama T, Yamamoto K, and Yanagihara T. A combined method for measurement of cerebral blood flow and immunohistochemistry for investigation of cerebral ischemia. *Brain Res* 424: 231–238, 1987.
52. Matsuo N, Ogawa S, Imai Y, Takagi T, Tohyama M, Stern D, and Wanaka A. Cloning of a novel RNA binding polypeptide (RA301) induced by hypoxia/reoxygenation. *J Biol Chem* 270: 28216–28222, 1995.
53. Matsuo N, Ogawa S, Takagi T, Wanaka A, Mori T, Matsuyama T, Pinsky D, Stern D, and Tohyama T. Cloning of a putative vesicle transport-related protein, RA410, from cultured rat astrocytes and its expression in ischemic rat brain. *J Biol Chem* 272: 16438–16444, 1997.
54. Matsushita K, Matsuyama T, Nishimura H, Takaoka T, Kuwabara K, Tsukamoto Y, Sugita M, and Ogawa S. Marked, sustained expression of a novel 150-kDa oxygen regulated stress protein, in severely ischemic mouse neurons. *Mol Brain Res* 60: 98–106, 1998.
55. Mattson MP, Zhu H, Yu J, and Kindy MS. Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo and to hypoxia and glucose deprivation in cell culture: involvement of perturbed calcium homeostasis. *J Neurosci* 20: 1358–1364, 2000.
56. Mattson MP. Apoptosis in neurodegenerative disorders. *Nat Rev Mol Cell Biol* 1: 120–129, 2000.
57. Mengesdorf T, Jensen PH, Mies G, Aufenberg C, and Paschen W. Down-regulation of parkin protein in transient focal cerebral ischemia: a link between stroke and degenerative disease? *Proc Natl Acad Sci U S A* 99: 15042–15047, 2002.
58. Mitani A, Imon H, Iga K, Kubo H, and Kataoka K. Gerbil hippocampal extracellular glutamate and neuronal activity after transient ischemia. *Brain Res Bull* 25: 319–324, 1990.
59. Mitani A and Kataoka K. Critical levels of extracellular glutamate mediating gerbil hippocampal delayed neuronal death during hypothermia: brain microdialysis study. *Neuroscience* 42: 661–670, 1991.
60. Miyazaki M, Ozawa K, Hori O, Kitao Y, Matsushita K, Ogawa S, and Matsuyama T. Expression of ORP150 (150 kDa oxygen

regulated protein) in the hippocampus suppresses delayed neuronal cell death. *J Cereb Blood Flow Metab* 22: 979–987, 2002.
61. Miyazawa T, Tamura A, Fukui S, and Hossmann KA. Effect of mild hypothermia on focal cerebral ischemia: review of experimental studies. *Neurol Res* 25: 457–464, 2003.
62. Mody I and MacDonald JF. NMDA receptor-dependent excitotoxicity: the role of intracellular Ca2+ release. *Trends Pharmacol Sci* 16: 356–359, 1995.
63. Monaghan DT, Holets VR, Toy DW, and Cotman CW. Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding sites. *Nature* 306: 176–179, 1983.
64. Mulle C, Sailer A, Perez-Otano I, Dickinson-Anson H, Castillo PE, Bureau I, Maron C, Gage FH, Mann JR, Bettler B, and Heinemann SF. Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. *Nature* 392: 601–605, 1998.
65. Munro S and Pelham HR. An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* 46: 291–300, 1986.
66. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, and Yuan J. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* 403: 98–103, 2000.
67. Neale JH, Bzdega T, and Wroblewska B. N-Acetylaspartylglutamate: the most abundant peptide neurotransmitter in the mammalian central nervous system. *J Neurochem* 75: 443–452, 2000.
68. Nikam SS and Kornberg BE. AMPA receptor antagonists. *Curr Med Chem* 8: 155–170, 2001.
69. Nishitoh H, Matsuzawa A, Tobiume K, Saegusa K, Takeda K, Inoue K, Hori S, Kakizuka A, and Ichijo H. ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev* 16: 1345–1355, 2002.
70. Nishizawa Y. Glutamate release and neuronal damage in ischemia. *Life Sci* 69: 369–381, 2001.
71. Niwa M, Sidrauski C, Kaufman RJ, and Walter P. A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response. *Cell* 99: 691–702, 1999.
72. Noh KM, Yokota H, Mashiko T, Castillo PE, Zukin RS, and Bennett MV. Blockade of calcium-permeable AMPA receptors protects hippocampal neurons against global ischemia-induced death. *Proc Natl Acad Sci U S A* 102: 12230–12235, 2005.
73. Nusser Z. AMPA and NMDA receptors: similarities and differences in their synaptic distribution. *Curr Opin Neurobiol* 10: 337–341, 2000.
74. Ooboshi H, Ibayashi S, Takano K, Sadoshima S, Kondo A, Uchimura H, and Fujishima M. Hypothermia inhibits ischemia-induced efflux of amino acids and neuronal damage in the hippocampus of aged rats. *Brain Res* 884: 23–30, 2000.
75. Oyadomari S, Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ* 11: 381–389, 2004.
76. Pahl HL and Baeuerle PA. Endoplasmic reticulum-induced signal transduction and gene expression. *Trends Cell Biol* 7: 50–55, 1997.
77. Parsons JT, Churn SB, and DeLorenzo RJ. Ischemia-induced inhibition of calcium uptake into rat brain microsomes mediated by Mg^{2+}/Ca^{2+} ATPase. *J Neurochem* 68: 1124–1134, 1997.
78. Paschen W, Aufenberg C, Hotop S, and Mengesdorf T. Transient cerebral ischemia activates processing of xbp1 messenger RNA indicative of endoplasmic reticulum stress. *J Cereb Blood Flow Metab* 23: 449–461, 2003.
79. Paschen W, Gissel C, Linden T, Althausen S, and Doutheil J. Activation of gadd153 expression through transient cerebral ischemia: evidence that ischemia causes endoplasmic reticulum dysfunction. *Brain Res Mol Brain Res* 60: 115–122, 1998.
80. Paschen W. Endoplasmic reticulum dysfunction in brain pathology: critical role of protein synthesis. *Curr Neurovasc Res* 1: 173–181, 2004.
81. Pelham RBH. Speculation on the functions on the major heat shock and glucose-regulated proteins. *Cell* 46: 959–961, 1986.
82. Pellegrini-Giampietro DE, Gorter JA, Bennett MV, and Zukin RS. The GluR2 (GluR-B) hypothesis: $Ca^{(2+)}$-permeable AMPA receptors in neurological disorders. *Trends Neurosci* 20: 464–470, 1997.
83. Pellegrini-Giampietro DE, Zukin RS, Bennett MV, Cho S, and Pulsinelli WA. Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats. *Proc Natl Acad Sci U S A* 89: 10499–10503, 1992.
84. Rossi DJ, Oshima T, and Attwell D. Glutamate release in severe brain ischemia is mainly by reversed uptake. *Nature* 403: 316–321, 2000.
85. Schneeloch E, Wenkel S, Mies G, and Paschen W. Spreading depression activates unfolded protein response. *Neurosci Lett* 368: 37–40, 2004.
86. Schousboe A. Role of astrocytes in the maintenance and modulation of glutamatergic and GABAergic neurotransmission. *Neurochem Res* 28: 347–352, 2003.
87. Sharp FR, Lu A, Tang Y, and Millhorn DE. Multiple molecular penumbras after focal cerebral ischemia. *J Cereb Blood Flow Metab* 20: 1011–1032, 2000.
88. Sharp FR, Massa SM, and Swanson RA. Heat-shock protein protection. *Trends Neurosci* 22: 97–99, 1999.
89. Sheardown MJ, Nielsen EO, Hansen AJ, Jacobsen P, and Honore T. 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: a neuroprotectant for cerebral ischemia. *Science* 247: 571–574, 1990.
90. Shibata M, Hattori H, Sasaki T, Gotoh J, Hamada J, and Fukuuchi Y. Activation of caspase-12 by endoplasmic reticulum stress induced by transient middle cerebral artery occlusion in mice. *Neuroscience* 118: 491–499, 2003.
91. Sidrauski C, Chapman R, and Walter P. The unfolded protein response: an intracellular signalling pathway with many surprising features. *Trends Cell Biol* 8: 245–249, 1998.
92. Siesjo BK, Agardh CD, and Bengtsson F. Free radicals and brain damage. *Cerebrovasc. Brain Metab Rev* 1: 165–211, 1989.
93. Siesjo BK and Bengtsson F. Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. *J. Cereb Blood Flow Metab* 9: 127–140, 1989.
94. Siesjoe BK. Mechanisms of ischemic brain damage. *Crit Care Med* 16: 954–963, 1988.
95. Simon RP, Swan JH, Griffiths T, and Meldrum BS. Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science* 226: 850–852, 1984.
96. Southard JH and Belzer FO. Organ preservation. *Annu Rev Med* 46: 235–247, 1995.
97. Takaba T and Inoue K. Past and present in myocardial protection. *Ann Thorac Cardiovasc Surg* 6: 3–8, 2000.
98. Tamatani M, Matsuyama T, Yamaguchi A, Mitsuda N, Tsukamoto Y, Taniguchi T, Che YH, Ozawa K, Hori O, Nishimura H, Yamashita A, Okabe M, Yanagi H, Stern DM, Ogawa S, and Tohyama M. ORP150 protects against hypoxia/ischemia-induced neuronal death. *Nat Med* 7: 317–323, 2001.
99. Tamatani M, Ogawa S, and Tohyama T. Roles of Bcl-2 and the ICE family proteases in hypoxia-induced neuronal cell death: a possible neuroprotective mechanism of peptide growth factors. *Mol Brain Res* 58: 27–39, 1998.
100. Ting J and Lee AS. Human gene encoding the 78,000-dalton glucose-regulated protein and its pseudogene: structure, conservation, and regulation. *DNA* 7: 275–286, 1988.
101. Tsukamoto Y, Kuwabara K, Hirota S, Ikeda J, Stern D, Yanagi H, Matsumoto M, Ogawa S, and Kitamura Y. 150 kDa oxygen regulated protein (ORP150) is expressed in human atherosclerotic plaques and allows mononuclear phagocytes to withstand cellular stress on exposure to hypoxia and modified LDL. *J Clin Invest* 98: 1930–1941, 1996.
102. Vannucci SJ, Mummery R, Hawkes RB, Rider CC, and Beesley PW. Hypoxia-ischemia induces a rapid elevation of ubiquitin conjugate levels and ubiquitin immunoreactivity in the immature rat brain. *J Cereb Blood Flow Metab* 18: 376–385, 1998.

103. Wang XZ, Ron D. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP kinase. *Science* 272: 1347–1349, 1996.
104. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress protein, and implications for medicine and disease. *Physiol Rev* 72: 1063–1081, 1992.
105. Wieloch T. Neurochemical correlates to selective neuronal vulnerability. *Prog Brain Res* 63: 69–85, 1985.
106. Yagita Y, Kitagawa K, Taguchi A, Ohtsuki T, Kuwabara K, Mabuchi T, Matsumoto M, Yanagihara T, and Hori M. Molecular cloning of a novel member of the HSP110 family of genes, ischemia-responsive protein 94 kDa (irp94), expressed in rat brain after transient forebrain ischemia. *J Neurochem* 72: 1544–1551, 1999.
107. Yenari MA. Heat shock proteins and neuroprotection. *Adv Exp Med Biol* 513: 281–299, 2002.

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3. Tomomi Gotoh, Motoyoshi Endo, Yuichi Oike. 2011. Endoplasmic Reticulum Stress-Related Inflammation and Cardiovascular Diseases. *International Journal of Inflammation* **2011**, 1-8. [CrossRef]
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5. Eunkuk Park, Gi Ja Lee, Samjin Choi, Seok Keun Choi, Su Jin Chae, Sung Wook Kang, Hun-Kuk Park. 2010. Correlation between extracellular glutamate release and neuronal cell death in an eleven vessel occlusion model in rat. *Brain Research* **1342**, 160-166. [CrossRef]
6. Laura V. González Bosc, Thomas Resta, Benjimen Walker, Nancy L. Kanagy. 2010. Mechanisms of intermittent hypoxia induced hypertension. *Journal of Cellular and Molecular Medicine* **14**:1-2, 3-17. [CrossRef]
7. Bernard A. Tarr, Jeremy S. Rabinowitz, Mubdiul Ali Imtiaz, Timothy J. DeVoogd. 2009. Captivity reduces hippocampal volume but not survival of new cells in a food-storing bird. *Developmental Neurobiology* **69**:14, 972-981. [CrossRef]
8. Fang Cao, Ryuji Hata, Pengxiang Zhu, Michinobu Niinobe, Masahiro Sakanaka. 2009. Up-regulation of syntaxin1 in ischemic cortex after permanent focal ischemia in rats. *Brain Research* **1272**, 52-61. [CrossRef]
9. T.V. Arumugam, T.M. Woodruff, J.D. Lathia, P.K. Selvaraj, M.P. Mattson, S.M. Taylor. 2009. Neuroprotection in stroke by complement inhibition and immunoglobulin therapy. *Neuroscience* **158**:3, 1074-1089. [CrossRef]
10. Sofiyan Saleem, Abdullah Shafique Ahmad, Takayuki Maruyama, Shuh Narumiya, Sylvain Doré. 2009. PGF2# FP Receptor Contributes to Brain Damage Following Transient Focal Brain Ischemia. *Neurotoxicity Research* **15**:1, 62-70. [CrossRef]
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12. MoonHee Lee, Sung-Jin Lee, Hyo-Jung Choi, Yong-Wook Jung, Jørgen Frøkiær, Søren Nielsen, Tae-Hwan Kwon. 2008. Regulation of AQP4 protein expression in rat brain astrocytes: Role of P2X7 receptor activation. *Brain Research* **1195**, 1-11. [CrossRef]
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