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Investigating the Mechanisms Underlying Neuronal Death in Ischemia Using In Vitro Oxygen-Glucose Deprivation: Potential Involvement of Protein SUMOylation

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Abstract

It is well established that brain ischemia can cause neuronal death via different signaling cascades. The relative importance and interrelationships between these pathways, however, remain poorly understood. Here is presented an overview of studies using oxygen-glucose deprivation of organotypic hippocampal slice cultures to investigate the molecular mechanisms involved in ischemia. The culturing techniques, setup of the oxygen-glucose deprivation model, and analytical tools are reviewed. The authors focus on SUMOylation, a posttranslational protein modification that has recently been implicated in ischemia from whole animal studies as an example of how these powerful tools can be applied and could be of interest to investigate the molecular pathways underlying ischemic cell death.

Keywords

Ischemia; Organotypic cultures; Oxygen-glucose deprivation (OGD); Propidium iodide (PI); SUMOylation; SUMO; Posttranslational protein modification

Cerebral ischemia (stroke) is an extreme form of metabolic stress resulting from oxygen and glucose deprivation that most commonly occurs when the blood supply to a part of the brain is suddenly interrupted by occlusion of a vessel. Stroke is the third leading cause of death after coronary heart disease and cancer in the industrialized part of the world, and is also the single most common cause of severe disability (Dirnagl and others 1999; Lipton 1999). Within the European Union there are an estimated 1.1 million new stroke victims each year with an estimated total cost of €21.9 billion (Truelsen 2005). Furthermore, every 45 seconds someone in the United States has a stroke with an estimated direct and indirect cost of $62.7 billion per year (Rosamond and others 2007). No effective treatment to promote recovery from stroke exists; therefore, the understanding of the molecular mechanisms of cellular damage involved is a critically important area of research to find ways of tackling this highly debilitating condition. Moreover, because most people affected are older than 65, better understanding of ischemia-induced cell damage is an important area of research for the aging population.

Ischemia invokes a range of pathogenic mechanisms that are elicited by cellular energy depletion and involve increased extracellular and excitotoxic glutamate, calcium overload, mitochondrial dysfunction, and oxidative stress. In addition to their vital roles in normal

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cellular function, ischemia activates posttranslational protein modifications such as phosphorylation and ubiquitination. These ischemia-evoked changes in posttranslational modification are believed to play a major role in the pathological process proposed for acute and delayed ischemic neuronal cell death (Althausen and others 2001; DeGracia and Montie 2004; Paschen and others 2007). Recently another posttranslational protein modification, termed small ubiquitin-like modifier (SUMO) conjugation, has been shown to be involved in normal neuronal function and in ischemia. Like ubiquitin, SUMO is conjugated to the lysine residue of target proteins in a complex process (Fig. 1). Whereas ubiquitination usually targets proteins for degradation at the proteasome, SUMO conjugation modifies the interaction of target proteins with protein partners and thereby alters their subcellular localization, activity, and stability (Pichler and Melchior 2002; Gill 2003; Seeler and Dejean 2003; Johnson 2004; Hay 2005, 2007; Martin, Wilkinson, and others 2007; Mukhopadhyay and Dasso 2007; Zhao 2007). Importantly, SUMOylation is readily reversible by the SUMO-specific family of SENP proteases, which cleave SUMO proteins from their substrates, allowing cells to respond rapidly to varying cellular demands.

A broad range of animal models have been developed to study the mechanisms underlying ischemic damage in an attempt to identify targets and possible therapeutic strategies for stroke. Such models include in vivo models of focal and global ischemia, in vitro models of glutamate receptor-mediated excitotoxicity, “chemical ischemia,” and more “ischemic-like” oxygen-glucose deprivation (OGD)-induced damage in primary cell cultures as well as slice cultures or acute slices (Goldberg and Choi 1993; Dawson and others 1996; Mei and others 1996; Kalda and Zharkovsky 1999; Hossmann 2008). Here we review the currently available models for studies of mechanisms involved in stroke, focusing on the use of organotypic hippocampal slice cultures exposed to OGD. To illustrate how this in vitro model can be an invaluable tool to further understanding the molecular basis of ischemia, we use the example of very recent work investigating roles of protein SUMOylation. The effects of cerebral ischemia on SUMOylation are summarized and data from slice cultures are compared with experimental in vivo models, particularly in respect to the possible consequences for the affected cells.

In Vivo Models of Ischemia

To simulate the different clinical forms of brain ischemia, an array of experimental models have been established using different animals including rats, mice, gerbils, and pigs, which are now believed to be an excellent model for ischemic studies because the structure of the cortex closely resembles that of humans (Traystman 2003). These models allow the possibility of interrupting blood flow to the brain in several ways: acutely or slowly, completely or incompletely, focally or globally, permanently or transiently (Hossmann 2008). Global brain ischemia can be achieved by cardiac arrest or selective arrest of cerebral circulation. In the rat, the most widely established method of complete or near complete global brain ischemia is the coagulation of both vertebral arteries, followed one day later by the transient occlusion of both common carotid arteries (four-vessel occlusion; Pulsinelli and Brierley 1979). To study the effects of perinatal hypoxia-ischemia, unilateral common carotid artery ligation followed by exposure to systemic hypoxia (8% oxygen) in the postnatal day 7 rat has been proved an extremely useful model of the pathophysiology of pediatric stroke (Vannucci and Vannucci 2005). Another important model is the infusion of endothelin-1 near the middle cerebral artery. This procedure is undertaken in the conscious animal (Sharkey and others 1993), allowing acute and longterm behavioral studies (Callaway and others 1999). In addition to different surgical and experimental techniques, multiple animal models have also been used to investigate global interruption of cerebral blood flow (Levine and Payan 1966; Hossmann 2008).

Experimental focal brain ischemia is the most powerful model of ischemic stroke. According to epidemiological studies (Bogousslavsky and others 1988; Wolf and others 1992), focal ischemia in the territory of the middle cerebral artery is the most common cause of clinical stroke. In experimental stroke research, this situation is reflected by the preferential use of middle cerebral artery occlusion models in the rat or mouse. Furthermore, this model is advantageous compared with its permanent counterparts because it allows for reperfusion of the tissue after various periods of time after occlusion (usually between 30 and 120 minutes), thereby causing different severities of stroke.

The efficacy of any stroke model can be evaluated by estimating the tissue loss and functional deficits it causes. The extent of tissue damage is most commonly estimated by staining fresh brain tissue with tripenthyltetrazolium chloride (TTC) (Bederson 1986), which labels only viable cells. This gives a very quick, albeit rough, estimate of the size of the lesion. Another more sensitive method of lesion-size estimation is to count the number of surviving neurons using stereological sampling methods (Gundersen and others 1988). In this method, thin brain sections are immunohistochemically stained and the neurons counted or the remaining striatal area measured. Alternatively, one can use a ballistic approach that utilizes basic principles of light propagation combined with computerized image analysis in unstained slide-mounted sections that has been reported to be superior to more conventional staining approaches (Callaway and others 2000). When evaluating the efficacy of any stroke treatment in patients, the long-term functional outcome is of utmost importance. Therefore, estimating the functional deficits of the rats or mice following stroke is just as important as any measurement of tissue loss. Sensory, motor, and cognitive deficits after experimental stroke can be estimated using various behavioral tests (Schallert and others 2002).

In Vitro Models of Ischemia

To better understand the molecular injury pathways of brain ischemia, in vitro models have been used increasingly in recent years. In these models, primary neuronal cultures (Goldberg and Choi 1993), organotypic cultures (Vornov and others 1994; Cimarosti and others 2001; Cimarosti, Zamin, and others 2005; Cimarosti and others 2006), or acute brain tissue slices (Whittingham and others 1984; Cimarosti, Siqueira, and others 2005) are usually incubated in deoxygenated, glucose-free medium (OGD) to mimic the interruption of the supply of oxygen and nutrients to the brain parenchyma. Because in vivo cerebral ischemia often consists of both reversible ischemia and blood flow reperfusion, the cultures or slices are often incubated in normal medium in an oxygen atmosphere environment following the induction of in vitro ischemia to simulate the in vivo blood flow reperfusion period. Other ways to imitate in vivo ischemic conditions have used glutamate receptor mediated excitotoxicity (Choi 1987; Frandsen and others 1989) and “chemical ischemia” (Kume and others 2002).

Organotypic Slice Cultures

Slices of developing brain tissue can be grown for several weeks as organotypic slice cultures. This in vitro culture of postnatal brain provides a system in which the cytoarchitecture and connectivity of different anatomical regions, as well as the functional relationships and interactions with neighboring cell types (i.e., neurons and astrocytes), are relatively well preserved (Gähwiler and others 1997). Organotypic cultures are an invaluable intermediate between simpler cell lines and in vivo models. Moreover, judicious experimental design can result in slice culture studies requiring far fewer animals to be killed to gain sufficient reliable data than in vivo experiments.

The basic requirements are simple: a stable substratum, culture medium, sufficient oxygenation, and incubation at a constant temperature of 36 °C (Fig. 2). Under the correct

conditions, neurons continue to differentiate and develop a tissue organization that closely resembles that observed in situ. Most often brain tissue from rats and mice, including transgenic mice (Teter and others 1999; Olsson and others 2004), has been used for organotypic slice cultures, but other species including rabbits (Savas and others 2001), pigs (Meyer and others 2000), and human fetal brain tissue (Bauer and others 2001; Walsh and others 2005) have also been used successfully. The brain area most commonly grown in slice culture is the hippocampus (Kristensen and others 2003; Noraberg 2004) because of its very well-characterized anatomy and because it is one of the brain regions most susceptible to stroke-induced damage.

The two main culturing methods for slices of brain tissue are the roller drum technique introduced by Gähwiler (Gähwiler 1981) and the interface cultures developed by Stoppini (Stoppini and others 1991). Slices maintained in stationary culture with the interface method are ideally suited to address questions requiring a three-dimensional structure, whereas slices cultured in roller tubes often remain the method of choice for imaging experiments.

Roller Drum Method

In roller tube cultures, the tissue is embedded in either a plasma clot or in a collagen matrix on glass coverslips and then undergoes continuous slow rotation (Gähwiler 1981). Oxygenation of these slices is assured because the slow rotation results in a continuous changing of the liquid-gas interface. The roller drum technique of slice cultures yields thin cultures that present certain advantages over other in vitro systems. Slice cultures of hippocampus retain important anatomical features and much of the synaptic organization of the intact tissue (Gähwiler 1981). Individual cells in roller drum cultures can be viewed with phase-contrast microscopes; the cultures are easily manipulated and diffusion barriers for exogenously applied substances are limited compared with resting cultures of the Stoppini type (Stoppini and others 1991). Also, long-term culturing permits recovery from dissection trauma and allows adaptation to the in vitro environment. Due to the flattening of the cultures to a quasi-monolayer, and their growth on glass coverslips, roller drum cultures are ideally suited for experiments using conventional light microscopical analysis. The disadvantages of this culture system, compared with interface cultures, include a lower degree of cellular organization and a partial loss of a distinct brain anatomy.

Interface Method

Most hippocampal slice cultures are now prepared using the interface method (Stoppini and others 1991) in which slices are placed at the air-medium interface on semiporous membranes and kept stationary during the entire culturing process. They obtain oxygen from above and nutrients from the medium below. Cultures are usually prepared from six- to eight-day-old (P6–8) rats, although attempts to culture adolescent or adult rat brain tissue have been made (Xiang and others 2000; Leutgeb and others 2003; Hassen and others 2004). Slices can be cultured from a few days up to 2 months (Gatherer and Sundström 1998; Routbort and others 1999; Bausch and McNamara 2000; Holopainen and others 2001; Xiang and others 2004). However, the most common length of time in culture is approximately two weeks. The laminated structure of the hippocampus and the characteristic morphological organization is well preserved and the maturation of different cell types, synaptic contacts, and receptor expression resembles that seen in vivo (Dailey and others 1994; Frotscher and others 1995; Gähwiler and others 1997; Holopainen and Lauren 2003).

Methods of Inducing Ischemic-like Conditions in Slices

Oxygen-Glucose Deprivation (OGD)—The most widely used method to induce stroke-like energy failure conditions in hippocampal slice cultures is to combine deprivation of oxygen and glucose. This insult can be accomplished in several ways, including the use of

anoxic chambers (Cimarosti and others 2001; Valentim 2003; Cimarosti, Jones, and others 2005; Cimarosti and others 2006) or submersion in glucose-free medium bubbled with nitrogen (Frantseva and others 1999). Importantly, the results obtained using OGD have proved consistent and reproducible between different labs suggesting that this approach can simulate the in vivo situation better than, for example, direct neuronal exposure to glutamate agonists to elicit excitotoxicity.

In organotypic hippocampal slices subjected to OGD, CA1 pyramidal cells are the most susceptible to cell death, whereas dentate gyrus (DG) granule cells are the most resistant (Fig. 3). The high susceptibility of CA1 combined with the high resistance of DG to OGD faithfully reproduces the selective vulnerability to ischemia in vivo (Newell and others 1990). In rodent models of transient ischemia, a brief insult induces selective neuronal death in the CA1 region, which develops over the following two to four days after ischemia, a phenomenon known as delayed neuronal cell death (Chen and others 1998). In organotypic hippocampal slice cultures, OGD induces neuronal death within 24 hours in the CA1 region, and the damage extends to the CA3 region during the following 72 hours (Cho and others 2004).

"Chemical Ischemia"—An alternative in vitro model that simulates certain aspects of ischemic brain injury is "chemical ischemia." In this model, sodium azide or sodium cyanide, inhibitors of oxidative metabolism, often together with 2-deoxyglucose, an inhibitor of glycolysis, are used to induce hypoxia and hypoglycemia in cultures (Bernaudin and others 1998; Imura and others 1999; Kume and others 2002). Sodium azide, either alone or combined with 2-deoxyglucose, has been used to induce chemical ischemia in cell cultures (Varming and others 1996; Grammatopoulos and others 2004), brain slices (Cavallini and others 2005), and in in vivo experiments (Bennett and others 1996; Vecsei and others 2001), but its precise mechanism of action remains unclear. The effects are usually attributed to cytochrome c oxidase—respiratory chain complex IV—inhibition (Duranteau and others 1998), although several other mechanisms (i.e., cell depolarization, binding to other metalloenzymes, and oxidation to nitric oxide by catalase) have not been ruled out (Bennett and others 1996; Lardinois and Rouxhet 1996; Varming and others 1996). Sodium cyanide provides a rapid and controllable loss of ATP (Vornov and others 1994; Myers and others 1995) and preserves the accessibility of cultures for subsequent manipulation. Several studies have shown that there is an activation of NMDA receptors and production of nitric oxide and other reactive oxygen species during cyanide toxicity (Patel and others 1991, 1993; Akira and others 1994; Vornov 1995; Gunasekar and others 1996), molecular mechanisms likely involved in neuronal delayed death after ischemia.

Glutamate and Excitotoxins—The pathophysiological role of glutamate in cerebral ischemia is based on the buildup of excess glutamate in the extracellular space (Benveniste and others 1984), which leads to activation of both ionotropic and metabotropic glutamate receptors (Choi 1992). Studies using glutamate-induced excitotoxicity mimic only one consequence of the energy failure associated with cerebral ischemia. However, this approach does enable investigation of the specific roles of individual glutamate receptors via the use of subtype-specific agonists or antagonists and receptor-modulating agents. Studies in hippocampal slice cultures exposed to *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainic acid (KA) are consistent with observations in vivo in terms of the susceptibilities of different populations of neurons (Nadler and others 1980; Moncada and others 1992; Bruce and others 1995; Kristensen and others 2001).

Quantification of Neuronal Cell Death

In most of slice culture studies investigating neurodegeneration, and in particular studies using OGD, the cellular uptake of the fluorescent dye propidium iodide (PI), which only enters and stains cell nuclei after loss of cell membrane integrity, has been used as a marker for dead or dying cells (Fig. 3; Macklis and Madison 1990; Noraberg and others 1999; Cimarosti and others 2001; Valentim and others 2003; Cimarosti, Zamin, and others 2005; Cimarosti and others 2006). Alternative markers of neurodegeneration include Fluoro-Jade (FJ), a fluorescent stain for degenerating neurons, lactate dehydrogenase (LDH) release to the culture medium, cell loss in Nissl cell staining, loss of immunohistochemical staining for microtubule-associated protein 2 (MAP2), and shrinkage of neuronal nuclei stained by Hoechst 33342 (bisbenzimide; Haug 1973; Vassault 1983; Schmued and others 1997; Noraberg and others 1999). Also, changes in the Timm sulfide silver staining, normally visualizing zinc-containing terminals that are abundant in the hippocampus, are commonly used to monitor the terminal fields of hippocampal pathways in the normal adult rat during development and after postlesional reorganization (Haug 1973; Zimmer and Gahwiler 1987).

Compared with LDH efflux, PI uptake has the advantage of directly depicting the susceptible hippocampal subfields and main cell types affected. LDH efflux, however, can be useful for measuring extensive cell death, because it does not reach its maximum level at a stage where PI uptake is already saturated. Consistent with the general concept that PI uptake requires cell membrane damage, whereas LDH release requires cell lysis, PI uptake appears to occur earlier than LDH efflux in the course of cell damage (Koh and Choi 1987; Macklis and Madison 1990; Noraberg and others 1999). Overall, PI uptake represents an earlier and more sensitive marker than LDH efflux and an invaluable marker for quantification of neuronal cell death in organotypic slice cultures. Staining with FJ (Schmued and others 1997) has confirmed that PI stains degenerating neurons. Immunocytochemical staining for MAP2, a marker of neuronal structural integrity, is also a very sensitive marker to neurotoxic effects in organotypic slice cultures (Noraberg and others 1999), but because it involves the use of antibody labelling is more complicated than PI staining.

Advantages and Disadvantages of In Vitro Models of Ischemia

In vitro models of ischemia have been widely used (Tanabe and others 1998; Cimarosti and others 2001; Carpenedo and others 2002; Cimarosti, Zamin, and others 2005; Noraberg and others 2005; Cimarosti and others 2006) and organotypic slice cultures offer many advantages over in vivo techniques to study the molecular mechanisms of brain ischemia. These include:

- immediate and direct access to the extracellular compartment due to the lack of a blood-brain barrier;
- elimination of any contribution from blood components; direct control of the environment, e.g., ion and nutrient availability, tissue temperature;
- tissue morphology relatively unchanged from the intact animal because intercellular connections are preserved; and
- excellent preparation for quantitative pharmacology, electrophysiology, and imaging studies.

As in in vivo models (Johansen and others 1986; Diemer and others 1992; Davoli and others 2002), CA1 pyramidal neurons are the most vulnerable cells in hippocampal slice cultures exposed to OGD (Newell and others 1990; Laake and others 1999). For many years, selective neuronal vulnerability has been presumed to reflect the inherent vulnerability and

connectivity of the neuronal population. However, despite progress in delineating these mechanisms, the long recognized selectivity of ischemic neuronal loss is still not fully understood, but it has been proposed that differences in astrocyte vulnerability or sensitivity to functional change are central to differential regional neuronal loss (Ouyang and others 2007). The typical time course of neuronal cell death following global ischemia in vivo, which involves delayed inflammatory and apoptotic components (Abe and others 1995; Kirino 2000), is not easy to replicate in slice cultures although this might be achievable by culturing of hippocampal slices from adolescent or adult rats or mice (Xiang and others 2000; Hassen and others 2004). Furthermore, the integrated mechanisms of ischemic brain damage and the effect of drug interventions are readily studied in rodent in vivo models, which for these purposes are more suitable than in vitro models (Ginsberg and Busto 1989). The intact brain preserves the blood-brain barrier, the complex neural networks, and interactions among neurons and nonneuronal cells; however, this complexity does not permit detailed studies of particular molecular mechanisms and isolated cellular events. For in vitro screening of drugs, dispersed cell culture systems are widely used; however, the loss of tissue-specific architecture, mechanical and biochemical cues, and cell-cell communication is not representative of the cellular environment found in organisms. Organotypic slice cultures with preservation of the basal cellular and connective organization and several fundamental in vivo-like characteristics are an attractive additional tool. They have intermediate levels of complexity between primary cell cultures and intact animals. In addition, many synaptic components are expressed at steady levels in long-term hippocampal slice cultures, allowing for detailed investigations of synaptic function (Bahr and others 1995).

Organotypic cultures are also well suited for both confocal imaging and viral transgenesis (Fig. 4). Although they are not much more transparent than brain tissue of a young animal, they are much thinner than any conventional acutely cut slice as they flatten with time in culture. The thickness of a slice in culture depends on the thickness of the original slice and the time in vitro. Usually at the time of the experiment only a few cell layers remain. Compared with acute slices, slice cultures are more mechanically stable, and they have flat, relatively clean surfaces that reduce light scattering, giving a better clarity of image.

Slice cultures are relatively amenable to expression of exogenous proteins via various methods of DNA delivery. They can be transduced with viral vectors, and it is also possible to combine the virally mediated gene expression with application of dyes to reveal details of intercellular connectivity (Kasparov and others 2002). The ability to genetically manipulate the organotypic cultures also makes them an attractive model for studying the cellular and molecular mechanisms of synaptic plasticity. Virus-mediated gene transfer of β-galactosidase or green fluorescent protein (GFP) has been efficiently used in slice cultures, and the infected slices showed no cytotoxic effects (Miyaguchi and others 2000). Alternatively, individual neurons in brain slice cultures can be transfected using micropipettes loaded with plasmid DNA expression constructs (Neumann and others 1999). Electroporation-mediated transfection has been used to visualize GFP-tagged proteins to analyze subcellular trafficking and targeting in single living neurons in cultured slices (Rathenberg and others 2003). Moreover, transgenic mice, expressing fluorescent proteins in neurons and glia, provide new opportunities for real-time microscopic monitoring of degenerative and regenerative structural changes. Organotypic cultures from these transgenic mice, combined with detailed visualization by time-lapse fluorescence microscopy, have great potential for investigating both major irreversible and minor reversible structural changes in neurons and glia induced by neurotoxins and other neurodegenerative compounds and conditions (Haber and others 2006; Noraberg and others 2007).

Study of the Molecular Mechanisms Involved in Brain Ischemia

Preconditioning, Postconditioning, and Hypothermia

Several studies have shown that exposure of animals to a mild sublethal ischemic insult, known as preconditioning, induces resistance to a subsequent otherwise lethal insult, a phenomenon known as tolerance (Tasaki and others 1997). In neonatal (P6–8) hippocampal slice cultures and in cultures from juvenile (P20–30) rats, preconditioning by exposure to 15 and 5 minutes of OGD, respectively, induced tolerance to a subsequent prolonged OGD period (Xu and others 2002; Hassen and others 2004). The molecular mechanisms leading to this increased neuronal resistance to ischemic injury are still not entirely clear, but a number of possible induction pathways, including activation of glutamate receptors and changes in the concentration of intracellular free calcium, have been proposed (Dirnagl and others 2003; Bickler and Fahlman 2004; Gidday 2006).

Recently, it has been hypothesized that modified reperfusion following a prolonged ischemic episode may also confer ischemic neuroprotection, a phenomenon termed postconditioning (Pignataro and others 2008). Postconditioning has been shown to reduce ischemic volume both in vivo and in vitro, with levels of neuroprotection equivalent to that observed in models of ischemic preconditioning. The postconditioning stimulus appears to result in the prolonged activation of Akt, a prosurvival protein kinase that has been associated with mediating ischemic protection in the heart and in the brain (Yano and others 2001), and has also been shown to mediate intrinsic protection to ischemia in models of ischemic preconditioning. Postconditioning may represent a novel endogenous neuroprotective approach that, due to its postevent time window of effectiveness, has translational relevance to reperfusion and thrombolitic treatments in acute brain ischemia.

Reducing the temperature of the brain is one of the few ways to clinically reduce ischemic damage in patients (Gagliardi 2000). Protection by low temperature has been demonstrated experimentally in vivo (Johansen and others 1993; Olsson and others 2003), and hypothermia has also been shown to reduce OGD-induced neuronal cell death in hippocampal slice cultures (Frantseva and others 1999; Feiner and others 2005). Modest decreases in brain temperature confer robust protection, both in vivo and in vitro (Ginsberg and others 1992; Bruno and others 1994; Barone and others 1997; Maier and others 2001). Although the neuroprotective effects of hypothermia have been attributed to reductions in the metabolic rate (Chopp and others 1989), glutamate accumulation (Globus and others 1988; Busto and others 1989; Lo and Steinberg 1992), and reductions in excitotoxicity (Bruno and others 1994), the detailed mechanisms remain unknown.

SUMOylation

An intriguing recent observation is that massive increases in protein SUMOylation occur in hibernating animals, suggesting that increase in SUMO conjugation could be a protective response, shielding neurons from damage caused by low blood flow during hibernation torpor (Lee and others 2007). This hypothesis has been supported by the observation that both transient and permanent global or focal cerebral ischemia induce a rapid, dramatic, and long-lasting increase in SUMO conjugation (Cimarosti and others 2008; Yang and others 2008a, 2008b). After transient focal cerebral ischemia, increased SUMO conjugation was particularly prominent in neurons located at the border of the ischemic territory where SUMO-conjugated proteins translocated to the nucleus (Yang and others 2008b). We also have data showing that protein SUMOylation increases in organotypic slice cultures following OGD with a biphasic time course and particularly for higher molecular weight proteins (Fig. 5). Many SUMO conjugation target proteins are transcription factors (Seeler and Dejean 2003; Heun 2007). Further, multiple, as yet unknown, synaptic proteins are

conjugated by SUMO (Martin, Nishimune, and others 2007). For the majority of substrates, the functional consequences of SUMO attachment are not well understood. Nonetheless, it is becoming increasingly apparent that protein SUMOylation is involved in diverse cellular processes beyond its roles in the nucleus including cell signaling, plasma membrane depolarization, and signal transduction (Johnson 2004; Wilson and Rosas-Acosta 2005; Kerscher and others 2006; Martin, Wilkinson, and others 2007; Scheschonka and others 2007). Thus, the increased SUMO conjugation induced by cerebral ischemia is likely to have a major effect on the fate of cells exposed to a transient reduction or interruption of blood supply, and the SUMOylation process could provide an exciting new target for therapeutic intervention.

SUMO transgenic animals are not available yet with which to investigate the role of SUMO conjugation in the pathological process triggered by cerebral ischemia and culminating in neuronal cell death, to establish whether it is a protective or toxic stress response, or simply an epiphenomenon not directly related to the pathological process. Because intact SUMOylation pathways are an absolute requirement for viability in eukaryotic cells (Hayashi and others 2002; Nacerddine and others 2005), the production of simple knockout animals is unlikely to be a viable option, although conditional knockouts may prove useful. Therefore, in vitro models such as organotypic cultures exposed to OGD will be a valuable method for the investigation of the potential neuroprotective effects of SUMOylation. Genetically manipulating the levels of SUMO, by overexpressing recombinant SUMO or enzymes involved in the SUMO conjugation pathway, such as SENP (deconjugating enzyme) or Ubc9 (conjugating enzyme), in the slice cultures exposed to OGD would allow evaluation of the effects of SUMOylation on neuronal function and survival. It has recently been shown that overexpressing SUMO increased the tolerance of neuroblastoma SHSY5Y cells to transient OGD, whereas blocking SUMO conjugation, by expressing a dominant negative form of the SUMO-conjugating enzyme Ubc9, increased the extent of cell death (Lee and others 2007). Conversely knocking down SUMO, SENP, and/or Ubc9 by RNAi approaches will also provide valuable information.

Elucidating the mechanisms underlying ischemia-induced SUMO conjugation and clarifying its suggested neuroprotective role could help to design strategies to block or activate this reaction and thus to intervene with the fate of postischemic neurons. One approach to dissect out such a specific molecular mechanism is to explore in vitro models and then test clearer hypotheses on in vivo models, which are more likely to subsequently translate into neuroprotective therapies of the future.

Concluding Remarks

Recent studies in animals found that one particular cellular process, called SUMOylation and which involves the conjugation of SUMO proteins to other proteins, is significantly activated following stroke. Future progress, however, is likely to require reliable in vitro models of ischemia that enable researchers to manipulate the SUMOylation pathway and investigate the effects of such manipulations on cell survival. Organotypic hippocampal slice cultures exposed to OGD have been widely used as an in vitro stroke model, showing similarities with the in vivo models of ischemia. This in vitro model of ischemic lesion is likely to prove an invaluable tool to study the molecular mechanisms involved in brain ischemia, and especially to further understanding the role of ischemic-induced SUMOylation.

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References

Abe K, Aoki M, Kawagoe J, Yoshida T, Hattori A, Kogure K, et al. Ischemic delayed neuronal death. A mitochondrial hypothesis. Stroke. 1995; 26:1478–89. [PubMed: 7631357]

Akira T, Henry D, Baldwin RA, Wasterlain CG. Nitric oxide participates in excitotoxic mechanisms induced by chemical hypoxia. Brain Res. 1994; 645:285–90. [PubMed: 7520342]

Althausen S, Mengesdorf T, Mies G, Olah L, Nairn AC, Proud CG, et al. Changes in the phosphorylation of initiation factor eIF-2alpha, elongation factor eEF-2 and p70 S6 kinase after transient focal cerebral ischaemia in mice. J Neurochem. 2001; 78:779–87. [PubMed: 11520898]

Bahr BA, Kessler M, Rivera S, Vanderklish PW, Hall RA, Mutneja MS, et al. Stable maintenance of glutamate receptors and other synaptic components in long-term hippocampal slices. Hippocampus. 1995; 5:425–39. [PubMed: 8773255]

Barone FC, Feuerstein GZ, White RF. Brain cooling during transient focal ischemia provides complete neuroprotection. Neurosci Biobehav Rev. 1997; 21:31–44. [PubMed: 8994207]

Bauer M, Meyer M, Sautter J, Gasser T, Ueffing M, Widmer HR. Liposome-mediated gene transfer to fetal human ventral mesencephalic explant cultures. Neurosci Lett. 2001; 308:169–72. [PubMed: 11479015]

Bausch SB, McNamara JO. Synaptic connections from multiple subfields contribute to granule cell hyperexcitability in hippocampal slice cultures. J Neurophysiol. 2000; 84:2918–32. [PubMed: 11110821]

Bederson J, Pitts L, Germano S, Nishimura M, Davis R, Bartkowski H. Evaluation of 2, 3, 5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke. 1986; 17:1304–08. [PubMed: 2433817]

Bennett MV, Pellegrini-Giampietro DE, Gorter JA, Aronica E, Connor JA, Zukin RS. The GluR2 hypothesis: Ca(++)-permeable AMPA receptors in delayed neurodegeneration. Cold Spring Harb Symp Quant Biol. 1996; 61:373–84. [PubMed: 9246466]

Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem. 1984; 43:1369–74. [PubMed: 6149259]

Bernaudin M, Nouvelot A, MacKenzie ET, Petit E. Selective neuronal vulnerability and specific glial reactions in hippocampal and neocortical organotypic cultures submitted to ischemia. Exp Neurol. 1998; 150:30–39. [PubMed: 9514820]

Bickler PE, Fahlman CS. Moderate increases in intracellular calcium activate neuroprotective signals in hippocampal neurons. Neuroscience. 2004; 127:673–83. [PubMed: 15283966]

Bogousslavsky J, Van Melle G, Regli F. The Lausanne Stroke Registry: analysis of 1,000 consecutive patients with first stroke. Stroke. 1988; 19:1083–92. [PubMed: 3413804]

Bruce AJ, Sakhi S, Schreiber SS, Baudry M. Development of kainic acid and N-methyl-D-aspartic acid toxicity in organotypic hippocampal cultures. Exp Neurol. 1995; 132:209–19. [PubMed: 7540554]

Bruno VM, Goldberg MP, Dugan LL, Giffard RG, Choi DW. Neuroprotective effect of hypothermia in cortical cultures exposed to oxygen-glucose deprivation or excitatory amino acids. J Neurochem. 1994; 63:1398–1406. [PubMed: 7523591]

Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. Stroke. 1989; 20:904–10. [PubMed: 2568705]

Callaway JK, Knight MJ, Watkins DJ, Beart PM, Jarrot B. Delayed treatment with AM-36, a novel neuroprotective agent, reduces delayed neuronal damage after endothelin-1 induced middle cerebral artery occlusion in conscious rats. Stroke. 1999; 30:2704–12. 2000. [PubMed: 10583001]

Callaway JK, Knight MJ, Watkins DJ, Beart PM, Jarrot B, Delaney PM. A novel, rapid, computerised method for quantitation of neuronal damage in a rat model of stroke. J Neurosci Methods. 2000; 102:53–60. [PubMed: 11000411]

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Carpenedo R, Meli E, Peruginelli F, Pellegrini-Giampietro DE, Moroni F. Kynurenine 3-mono-oxygenase inhibitors attenuate post-ischemic neuronal death in organotypic hippocampal slice cultures. J Neurochem. 2002; 82:1465–71. [PubMed: 12354294]

Cavallini S, Marti M, Marino S, Selvatici R, Beani L, Bianchi C, et al. Effects of chemical ischemia in cerebral cortex slices. Focus on nitric oxide. Neurochem Int. 2005; 47:482–90.

Chen J, Nagayama T, Jin K, Stetler RA, Zhu RL, Graham SH, et al. Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. J Neurosci. 1998; 18:4914–28. [PubMed: 9634557]

Cho S, Liu D, Fairman D, Li P, Jenkins L, McGonigle P, et al. Spatiotemporal evidence of apoptosis-mediated ischemic injury in organotypic hippocampal slice cultures. Neurochem Int. 2004; 45:117–27. [PubMed: 15082229]

Choi DW. Ionic dependence of glutamate neurotoxicity. J Neurosci. 1987; 7:369–79. [PubMed: 2880938]

Choi DW. Excitotoxic cell death. J Neurobiol. 1992; 23:1261–76. [PubMed: 1361523]

Chopp M, Knight R, Tidwell CD, Helpern JA, Brown E, Welch KM. The metabolic effects of mild hypothermia on global cerebral ischemia and recirculation in the cat: comparison to normothermia and hyperthermia. J Cereb Blood Flow Metab. 1989; 9:141–48. [PubMed: 2921288]

Cimarosti H, Lindberg C, Bomholt SF, Ronn LC, Henley JM. Increased protein SUMOylation following focal cerebral ischemia. Neuropharmacology. 2008; 54:280–89. [PubMed: 17991493]

Cimarosti H, Jones NM, O'Shea RD, Pow DV, Salbego C, Beart PM. Hypoxic preconditioning in neonatal rat brain involves regulation of excitatory amino acid transporter 2 and estrogen receptor alpha. Neurosci Lett. 2005; 385:52–7. [PubMed: 15927375]

Cimarosti H, Rodnight R, Tavares A, Paiva R, Valentim L, Rocha E, et al. An investigation of the neuroprotective effect of lithium in organotypic slice cultures of rat hippocampus exposed to oxygen and glucose deprivation. Neurosci Lett. 2001; 315:33–6. [PubMed: 11711208]

Cimarosti H, Zamin LL, Frozza R, Nassif M, Horn AP, Tavares A, et al. Estradiol protects against oxygen and glucose deprivation in rat hippocampal organotypic cultures and activates Akt and inactivates GSK-3beta. Neurochem Res. 2005; 30:191–99. [PubMed: 15895822]

Cimarosti H, Siqueira IR, Zamin LL, Nassif M, Balk R, Frozza R, et al. Neuroprotection and protein damage prevention by estradiol replacement in rat hippocampal slices exposed to oxygenglucose deprivation. Neurochem Res. 2005; 30:583–89. [PubMed: 16076028]

Cimarosti H, O'Shea RD, Jones NM, Horn AP, Simao F, Zamin LL, et al. The effects of estradiol on estrogen receptor and glutamate transporter expression in organotypic hippocampal cultures exposed to oxygen-glucose deprivation. Neurochem Res. 2006; 31:483–90. [PubMed: 16758356]

Dailey ME, Buchanan J, Bergles DE, Smith SJ. Mossy fiber growth and synaptogenesis in rat hippocampal slices in vitro. J Neurosci. 1994; 14:1060–78. [PubMed: 8120613]

Davoli MA, Ren Y, Zhu Y, Fourtounis J, Jones C, Robertson GS, et al. Automated analysis of global ischemia-induced CA1 neuronal death using terminal UTP nick end labeling (TUNEL). J Neurosci Methods. 2002; 115:55–61. [PubMed: 11897363]

Dawson VL, Kizushi VM, Huang PL, Snyder SH, Dawson TM. Resistance to neurotoxicity in cortical cultures from neuronal nitric oxide synthase-deficient mice. J Neurosci. 1996; 16:2479–87. [PubMed: 8786424]

DeGracia DJ, Montie HL. Cerebral ischemia and the unfolded protein response. J Neurochem. 2004; 91:1–8. [PubMed: 15379881]

Diemer NH, Jørgensen MB, Johansen FF, Sheardown M, Honoré T. Protection against ischemic hippocampal CA1 damage in the rat with a new non-NMDA antagonist, NBQX. Acta Neurol Scand. 1992; 86:45–9. [PubMed: 1325729]

Dirnagl U, Simon RP, Hallenbeck JM. Ischemic tolerance and endogenous neuroprotection. Trends Neurosci. 2003; 26:248–54. [PubMed: 12744841]

Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 1999; 22:391–7. [PubMed: 10441299]

Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. J Biol Chem. 1998; 273:11619–24. [PubMed: 9565580]

Europe PMC Funders Author Manuscripts

Feiner JR, Bickler PE, Estrada S, Donohoe PH, Fahlman CS, Schuyler JA. Mild hypothermia, but not propofol, is neuroprotective in organotypic hippocampal cultures. Anesth Analg. 2005; 100:215–25. [PubMed: 15616081]

Frandsen A, Drejer J, Schousboe A. Direct evidence that excitotoxicity in cultured neurons is mediated via N-methyl-D-aspartate (NMDA) as well as non-NMDA receptors. J Neurochem. 1989; 53:297–9. [PubMed: 2566655]

Frantseva MV, Carlen PL, El-Beheiry H. A submersion method to induce hypoxic damage in organotypic hippocampal cultures. J Neurosci Methods. 1999; 89:25–31. [PubMed: 10476680]

Frotscher M, Zafirov S, Heimrich B. Development of identified neuronal types and of specific synaptic connections in slice cultures of rat hippocampus. Prog Neurobiol. 1995; 45:143–64. [PubMed: 7598766]

Gagliardi RJ. Neuroprotection, excitotoxicity and NMDA antagonists. Arq Neuropsiquiatr. 2000; 58:583–8. [PubMed: 10920427]

Gähwiler BH. Organotypic monolayer cultures of nervous tissue. J Neurosci Methods. 1981; 4:329–42. [PubMed: 7033675]

Gähwiler BH, Capogna M, Debanne D, McKinney RA, Thompson SM. Organotypic slice cultures: a technique has come of age. Trends Neurosci. 1997; 20:471–7. [PubMed: 9347615]

Gatherer M, Sundström LE. Mossy fibre innervation is not required for the development of kainic acid toxicity in organotypic hippocampal slice cultures. Neurosci Lett. 1998; 253:119–22. [PubMed: 9774164]

Gidday JM. Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci. 2006; 7:437–48. [PubMed: 16715053]

Gill G. Post-translational modifications by the small ubiquitin-related modifier SUMO has big effects on transcription factor activity. Curr Opin Genet Dev. 2003; 13:108–13. [PubMed: 12672486]

Ginsberg MD, Busto R. Rodent models of cerebral ischemia. Stroke. 1989; 20:1627–42. [PubMed: 2688195]

Ginsberg MD, Sternau LL, Globus MY, Dietrich WD, Busto R. Therapeutic modulation of brain temperature: relevance to ischemic brain injury. Cerebrovasc Brain Metab Rev. 1992; 4:189–225. [PubMed: 1389956]

Globus MY, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. Neurosci Lett. 1988; 91:36–40. [PubMed: 2902538]

Goldberg MP, Choi DW. Combined oxygen and glucose deprivation in cortical cell culture: calcium-dependent and calcium-independent mechanisms of neuronal injury. J Neurosci. 1993; 13:3510–24. [PubMed: 8101871]

Grammatopoulos TN, Morris K, Bachar C, Moore S, Andres R, Weyhenmeyer JA. Angiotensin II attenuates chemical hypoxia-induced caspase-3 activation in primary cortical neuronal cultures. Brain Res Bull. 2004; 62:297–303. [PubMed: 14709344]

Gunasekar PG, Sun PW, Kanthasamy AG, Borowitz JL, Isom GE. Cyanide-induced neurotoxicity involves nitric oxide and reactive oxygen species generation after N-methyl-D-aspartate receptor activation. J Pharmacol Exp Ther. 1996; 277:150–5. [PubMed: 8613912]

Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. Apmis. 1988; 96:379–94. [PubMed: 3288247]

Haber M, Zhou L, Murai KK. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. J Neurosci. 2006; 26:8881–91. [PubMed: 16943543]

Hassen GW, Tian D, Ding D, Bergold PJ. A new model of ischemic preconditioning using young adult hippocampal slice cultures. Brain Res Brain Res Protoc. 2004; 13:135–43. [PubMed: 15296850]

Haug FM. Heavy metals in the brain. Adv Anat Embryol Cell Biol. 1973; 47:1–70. [PubMed: 4139881]

Hay RT. SUMO: a history of modification. Mol Cell. 2005; 18:1–12. [PubMed: 15808504]

Hay RT. SUMO-specific proteases: a twist in the tail. Trends Cell Biol. 2007; 17:370–76. [PubMed: 17768054]

Europe PMC Funders Author Manuscripts

Hayashi T, Seki M, Maeda D, Wang W, Kawabe Y, Seki T, et al. Ubc9 is essential for viability of higher eukaryotic cells. Exp Cell Res. 2002; 280:212–21. [PubMed: 12413887]

Heun P. SUMOrganization of the nucleus. Curr Opin Cell Biol. 2007; 19:350–5. [PubMed: 17467254]

Holopainen IE, Lauren HB, Romppanen A, Lopez-Picon FR. Changes in neurofilament protein-immunoreactivity after kainic acid treatment of organotypic hippocampal slice cultures. J Neurosci Res. 2001; 66:620–629. [PubMed: 11746382]

Holopainen IE, Lauren HB. Neuronal activity regulates GABAA receptor subunit expression in organotypic hippocampal slice cultures. Neuroscience. 2003; 118:967–74. [PubMed: 12732242]

Hossmann K-A. Cerebral ischemia: models, methods and outcomes. Neuropharmacology. 2008 in press.

Imura T, Shimohama S, Sato M, Nishikawa H, Madono K, Akaike A, et al. Differential expression of small heat shock proteins in reactive astrocytes after focal ischemia: possible role of beta-adrenergic receptor. J Neurosci. 1999; 19:9768–79. [PubMed: 10559386]

Johansen FF, Jørgensen MB, Diemer NH. Ischemic CA-1 pyramidal cell loss is prevented by preischemic colchicines destruction of dentate gyrus granule cells. Brain Res. 1986; 377:344–7. [PubMed: 2873869]

Johansen FF, Tønder N, Berg M, Zimmer J, Diemer NH. Hypothermia protects somatostatinergic neurons in rat dentate hilus from zinc accumulation and cell death after cerebral ischemia. Mol Chem Neuropathol. 1993; 18:161–72. [PubMed: 7682076]

Johnson ES. Protein modification by SUMO. Annu Rev Biochem. 2004; 73:355–82. [PubMed: 15189146]

Kalda A, Zharkovsky A. Metabotropic glutamate receptor agonists protect from oxygen-glucose deprivation-and colchicine-induced apoptosis in primary cultures of cerebellar granule cells. Neuroscience. 1999; 92:7–14. [PubMed: 10392826]

Kasparov S, Teschemacher AG, Paton JF. Dynamic confocal imaging in acute brain slices and organotypic slice cultures using a spectral confocal microscope with single photon excitation. Exp Physiol. 2002; 87:715–24. [PubMed: 12447451]

Kerscher O, Felberbaum R, Hochstrasser M. Modification of proteins by ubiquitin and ubiquitin-like proteins. Annu Rev Cell Dev Biol. 2006; 22:159–80. [PubMed: 16753028]

Kirino T. Delayed neuronal death. Neuropathology. 2000; 20(Suppl):S95–7. [PubMed: 11037198]

Koh JY, Choi DW. Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay. J Neurosci Methods. 1987; 20:83–90. [PubMed: 2884353]

Kristensen BW, Noraberg J, Zimmer J. Comparison of excitotoxic profiles of ATPA, AMPA, KA and NMDA in organotypic hippocampal slice cultures. Brain Res. 2001; 917:21–44. [PubMed: 11602227]

Kristensen BW, Noer H, Gramsbergen JB, Zimmer J, Noraberg J. Colchicine induces apoptosis in organotypic hippocampal slice cultures. Brain Res. 2003; 964:264–78. [PubMed: 12576187]

Kume T, Nishikawa H, Taguchi R, Hashino A, Katsuki H, Kaneko S, et al. Antagonism of NMDA receptors by sigma receptor ligands attenuates chemical ischemia-induced neuronal death in vitro. Eur J Pharmacol. 2002; 455:91–100. [PubMed: 12445574]

Laake JH, Haug FM, Wieloch T, Ottersen OP. A simple in vitro model of ischemia based on hippocampal slice cultures and propidium iodide fluorescence. Brain Res Brain Res Protoc. 1999; 4:173–84. [PubMed: 10446412]

Lardinois OM, Rouxhet PG. Peroxidatic degradation of azide by catalase and irreversible enzyme inactivation. Biochim Biophys Acta. 1996; 1298:180–90. [PubMed: 8980644]

Lee YJ, Miyake S, Wakita H, McMullen DC, Azuma Y, Auh S, et al. Protein SUMOylation is massively increased in hibernation torpor and is critical for the cytoprotection provided by ischemic preconditioning and hypothermia in SHSY5Y cells. J Cereb Blood Flow Metab. 2007; 27:950–62. [PubMed: 16955077]

Leutgeb JK, Frey JU, Behnisch T. LTP in cultured hippocampal-entorhinal cortex slices from young adult (P25-30) rats. J Neurosci Methods. 2003; 130:19–32. [PubMed: 14583401]

Levine S, Payan H. Effects of ischemia and other procedures on the brain and retina of the gerbil (Meriones unguiculatus). Exp Neurol. 1966; 16:255–62. [PubMed: 5928981]

Lipton P. Ischemic cell death in brain neurons. Physiol Rev. 1999; 79:1431–568. [PubMed: 10508238]

Lo EH, Steinberg GK. Effects of hypothermia on evoked potentials, magnetic resonance imaging, and blood flow in focal ischemia in rabbits. Stroke. 1992; 23:889–93. [PubMed: 1595110]

Macklis JD, Madison RD. Progressive incorporation of propidium iodide in cultured mouse neurons correlates with declining electrophysiological status: a fluorescence scale of membrane integrity. J Neurosci Methods. 1990; 31:43–6. [PubMed: 2308380]

Maier CM, Sun GH, Kunis D, Yenari MA, Steinberg GK. Delayed induction and long-term effects of mild hypothermia in a focal model of transient cerebral ischemia: neurological outcome and infarct size. J Neurosurg. 2001; 94:90–6. [PubMed: 11147904]

Martin S, Wilkinson KA, Nishimune A, Henley JM. Emerging extranuclear roles of protein SUMOylation in neuronal function and dysfunction. Nat Rev Neurosci. 2007; 8:948–59. [PubMed: 17987030]

Martin S, Nishimune A, Mellor JR, Henley JM. SUMOylation regulates kainate-receptor-mediated synaptic transmission. Nature. 2007; 447:321–5. [PubMed: 17486098]

Mei JM, Chi WM, Trump BF, Eccles CU. Involvement of nitric oxide in the deregulation of cytosolic calcium in cerebellar neurons during combined glucose-oxygen deprivation. Mol Chem Neuropathol. 1996; 27:155–66. [PubMed: 8962600]

Meyer M, Johansen J, Gramsbergen JB, Johansen TE, Zimmer J. Improved survival of embryonic porcine dopaminergic neurons in coculture with a conditionally immortalized GDNF-producing hippocampal cell line. Exp Neurol. 2000; 164:82–93. [PubMed: 10877918]

Miyaguchi K, Maeda Y, Collin C, Sihag RK. Gene transfer into hippocampal slice cultures with an adenovirus vector driven by cytomegalovirus promoter: stable co-expression of green fluorescent protein and lacZ genes. Brain Res Bull. 2000; 51:195–202. [PubMed: 10718511]

Moncada C, Lekieffre D, Arvin B, Meldrum B. Effect of NO synthase inhibition on NMDA- and ischaemia-induced hippocampal lesions. Neuroreport. 1992; 3:530–2. [PubMed: 1382661]

Mukhopadhyay D, Dasso M. Modification in reverse: the SUMO proteases. Trends Biochem Sci. 2007; 32:286–95. [PubMed: 17499995]

Myers KM, Fiskum G, Liu Y, Simmens SJ, Bredesen DE, Murphy AN. Bcl-2 protects neural cells from cyanide/aglycemia-induced lipid oxidation, mitochondrial injury, and loss of viability. J Neurochem. 1995; 65:2432–40. [PubMed: 7595537]

Nacerddine K, Lehembre F, Bhaumik M, Artus J, Cohen-Tannoudji M, Babinet C, et al. The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev Cell. 2005; 9:769–79. [PubMed: 16326389]

Nadler JV, Perry BW, Gentry C, Cotman CW. Degeneration of hippocampal CA3 pyramidal cells induced by intraventricular kainic acid. J Comp Neurol. 1980; 192:333–59. [PubMed: 7400401]

Neumann E, Kakorin S, Toensing K. Fundamentals of electroporative delivery of drugs and genes. Bioelectrochem Bioenerg. 1999; 48:3–16. [PubMed: 10228565]

Newell DW, Malouf AT, Franck JE. Glutamate-mediated selective vulnerability to ischemia is present in organotypic cultures of hippocampus. Neurosci Lett. 1990; 116:325–30. [PubMed: 1978744]

Noraberg J. Organotypic brain slice cultures: an efficient and reliable method for neurotoxicological screening and mechanistic studies. Altern Lab Anim. 2004; 32:329–37. [PubMed: 15651916]

Noraberg J, Jensen CV, Bonde C, Montero M, Nielsen JV, Jensen NA, et al. The developmental expression of fluorescent proteins in organotypic hippocampal slice cultures from transgenic mice and its use in the determination of excitotoxic neurodegeneration. Altern Lab Anim. 2007; 35:61–70. [PubMed: 17411353]

Noraberg J, Poulsen FR, Blaabjerg M, Kristensen BW, Bonde C, Montero M, et al. Organotypic hippocampal slice cultures for studies of brain damage, neuroprotection and neurorepair. Curr Drug Targets CNS Neurol Disord. 2005; 4:435–52. [PubMed: 16101559]

Noraberg J, Kristensen BW, Zimmer J. Markers for neuronal degeneration in organotypic slice cultures. Brain Res Brain Res Protoc. 1999; 3:278–90. [PubMed: 9974143]

Olsson T, Cronberg T, Rytter A, Asztély F, Fredholm BB, Smith ML, et al. Deletion of the adenosine A1 receptor gene does not alter neuronal damage following ischaemia in vivo or in vitro. Eur J Neurosci. 2004; 20:1197–1204. [PubMed: 15341591]

Olsson T, Wieloch T, Smith ML. Brain damage in a mouse model of global cerebral ischemia. Effect of NMDA receptor blockade. Brain Res. 2003; 982:260–9.

Ouyang YB, Voloboueva LA, Xu LJ, Giffard RG. Selective dysfunction of hippocampal CA1 astrocytes contributes to delayed neuronal damage after transient forebrain ischemia. J Neurosci. 2007; 27:4253–60. [PubMed: 17442809]

Paschen W, Proud CG, Mies G. Shut-down of translation, a global neuronal stress response: mechanisms and pathological relevance. Curr Pharm Des. 2007; 13:1887–1902. [PubMed: 17584115]

Patel MN, Yim GK, Isom GE. N-Methyl-D-aspartate receptors mediate cyanide-induced cytotoxicity in hippocampal cultures. Neurotoxicology. 1993; 14:35–40. [PubMed: 8103209]

Patel MN, Ardelt BK, Yim GK, Isom GE. Cyanide induces Ca(2+)-dependent and -independent release of glutamate from mouse brain slices. Neurosci Lett. 1991; 131:42–4. [PubMed: 1686477]

Pichler A, Melchior F. Ubiquitin-related moifier SUMO1 and nucleocytoplasmic transport. Traffic. 2002; 3:381–7. [PubMed: 12010456]

Pignataro G, Meller R, Inoue K, Ordonez AN, Ashley MD, Xiong Z, et al. In vivo and in vitro characterization of a novel neuroprotective strategy for stroke: ischemic postconditioning. J Cereb Blood Flow Metab. 2008; 28:232–41. [PubMed: 17882162]

Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke. 1979; 10:267–72. [PubMed: 37614]

Rathenberg J, Nevian T, Witzemann V. High-efficiency transfection of individual neurons using modified electrophysiology techniques. J Neurosci Methods. 2003; 126:91–8. [PubMed: 12788505]

Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, American Heart Association Statistics Committee and Stroke Statistics Subcommittee. et al. Heart disease and stroke statistics— 2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2007; 115:e69–171. [PubMed: 17194875]

Routbort MJ, Bausch SB, McNamara JO. Seizures, cell death, and mossy fiber sprouting in kainic acid-treated organotypic hippocampal cultures. Neuroscience. 1999; 94:755–65. [PubMed: 10579566]

Savas A, Warnke PC, Ginap T, Feuerstein TJ, Ostertag CB. The effects of continuous and single-dose radiation on choline uptake in organotypic tissue slice cultures of rabbit hippocampus. Neurol Res. 2001; 23:669–75. [PubMed: 11547941]

Schallert, T.; Woodlee, M.; Fleming, SM. Disentangling multiple types of recovery from brain injury. In: Krieglstein, J.; Klumpp, S., editors. Pharmacology of cerebral ischemia. Medpharm Scientific Publishers; Stuttgart: 2002. p. 201-16.

Scheschonka A, Tang Z, Betz H. Sumoylation in neurons: nuclear and synaptic roles? Trends Neurosci. 2007; 30:85–91. [PubMed: 17241677]

Schmued LC, Albertson C, Slikker W Jr. Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res. 1997; 751:37–46. [PubMed: 9098566]

Seeler JS, Dejean A. Nuclear and unclear functions of SUMO. Nat Rev Cell Biol. 2003; 4:690–9.

Sharkey J, Ritchie IM, Kelly PA. Perivascular microapplication of endothelin-1: a new model of focal cerebral ischaemia in the rat. J Cereb Blood Flow Metab. 1993; 13:865–71. [PubMed: 8360292]

Stoppini L, Buchs PA, Muller D. A simple method for organotypic cultures of nervous tissue. J Neurosci Methods. 1991; 37:173–82. [PubMed: 1715499]

Tanabe M, Gahwiler BH, Gerber U. Effects of transient oxygenglucose deprivation on G-proteins and G-protein-coupled receptors in rat CA3 pyramidal cells in vitro. Eur J Neurosci. 1998; 10:2037–45. [PubMed: 9753091]

Tasaki K, Ruetzler CA, Ohtsuki T, Martin D, Nawashiro H, Hallenbeck JM. Lipopolysaccharide pre-treatment induces resistance against subsequent focal cerebral ischemic damage in spontaneously hypertensive rats. Brain Res. 1997; 748:267–70. [PubMed: 9067475]

Europe PMC Funders Author Manuscripts

Teter B, Xu PT, Gilbert JR, Roses AD, Galasko D, Cole GM. Human apolipoprotein E isoform-specific differences in neuronal sprouting in organotypic hippocampal culture. J Neurochem. 1999; 73:2613–6. [PubMed: 10582625]

Traystman RJ. Animal models of focal and global cerebral ischemia. ILAR J. 2003; 44:85–95. [PubMed: 12652003]

Truelsen T, Ekman M, Boysen G. Cost of stroke in Europe. Eur J Neurol. 2005; 12(Suppl 1):78–84. [PubMed: 15877785]

Valentim LM, Rodnight R, Geyer AB, Horn AP, Tavares A, Cimarosti H, et al. Changes in heat shock protein 27 phosphorylation and immunocontent in response to preconditioning to oxygen and glucose deprivation in organotypic hippocampal cultures. Neuroscience. 2003; 118:379–86. [PubMed: 12699774]

Vannucci RC, Vannucci SJ. Perinatal hypoxic-ischemic brain damage: evolution of an animal model [review]. Dev Neurosci. 2005; 27:81–6. [PubMed: 16046840]

Varming T, Drejer J, Frandsen A, Schousboe A. Characterization of a chemical anoxia model in cerebellar granule neurons using sodium azide: protection by nifedipine and MK-801. J Neurosci Res. 1996; 44:40–6. [PubMed: 8926628]

Vassault, A. Lactate Dehydrogenase. UV-method with pyruvate and NADH. In: Bergmeyer, HU.; Bergmeyer, J.; Grossl, M., editors. Methods of enzymatic analysis. 3rd ed. John Wiley & Sons; New York: 1983. p. 118-26.

Vecsei L, Tajti J, Klivenyi P, Pinter S, Karg E. Sodium azide treatment decreases striatal and cortical concentrations of alphatocopherol in rats. J Neural Transm. 2001; 108:273–8. [PubMed: 11341478]

Vornov JJ. Toxic NMDA-receptor activation occurs during recovery in a tissue culture model of ischemia. J Neurochem. 1995; 65:1681–91. [PubMed: 7561865]

Vornov JJ, Tasker RC, Coyle JT. Delayed protection by MK-801 and tetrodotoxin in a rat organotypic hippocampal culture model of ischemia. Stroke. 1994; 25:457–64. discussion 464–5. [PubMed: 8303757]

Walsh K, Megyesi J, Hammond R. Human central nervous system tissue culture: a historical review and examination of recent advances. Neurobiol Dis. 2005; 18:2–18. [PubMed: 15649692]

Whittingham TS, Lust DW, Passonneau JV. An in vitro model of ischemia: metabolic and electrical alterations in the hippocampal slice. J Neurosci. 1984; 4:793–802. [PubMed: 6323646]

Wilson VG, Rosas-Acosta G. Wrestling with SUMO in a new arena. Sci STKE. 2005; 2005:pe32. [PubMed: 15985640]

Wolf P, D'Agostino R, O'Neal M, Sytkowski P, Kase C, Belanger A, et al. Secular trends in stroke incidence and mortality. The Framingham Study. Stroke. 1992; 23:1551–5.

Xiang Z, Hrabetova S, Moskowitz SI, Casaccia-Bonnefil P, Young SR, Nimmrich VC, et al. Long-term maintenance of mature hippocampal slices in vitro. J Neurosci Methods. 2000; 98:145–54. [PubMed: 10880828]

Xiang Z, Yuan M, Hassen GW, Gampel M, Bergold PJ. Lactate induced excitotoxicity in hippocampal slice cultures. Exp Neurol. 2004; 186:70–7. [PubMed: 14980811]

Xu GP, Dave KR, Vivero R, Schmidt-Kastner R, Sick TJ, Pérez-Pinzón MA. Improvement in neuronal survival after ischemic preconditioning in hippocampal slice cultures. Brain Res. 2002; 952:153–8. [PubMed: 12376175]

Yang W, Sheng H, Warner DS, Paschen W. Transient global cerebral ischemia induces a massive increase in protein sumoylation. J Cereb Blood Flow Metab. 2008a; 28:269–79. [PubMed: 17565359]

Yang W, Sheng H, Warner DS, Paschen W. Transient focal cerebral ischemia induces a dramatic activation of small ubiquitinlike modifier conjugation. J Cereb Blood Flow Metab. 2008b; 28:892–6. [PubMed: 18167501]

Yano S, Morioka M, Fukunaga K, Kawano T, Hara T, Kai Y, et al. Activation of Akt/protein kinase B contributes to induction of ischemic tolerance in the CA1 subfield of gerbil hippocampus. J Cereb Blood Flow Metab. 2001; 21:351–60. [PubMed: 11323521]

Zhao J. Sumoylation regulates diverse biological processes. Cell Mol Life Sci. 2007; 64:3017–33. [PubMed: 17763827]

Zimmer J, Gahwiler BH. Growth of hippocampal mossy fibers: a lesion and coculture study of organotypic slice cultures. J Comp Neurol. 1987; 264:1–13. [PubMed: 2445790]

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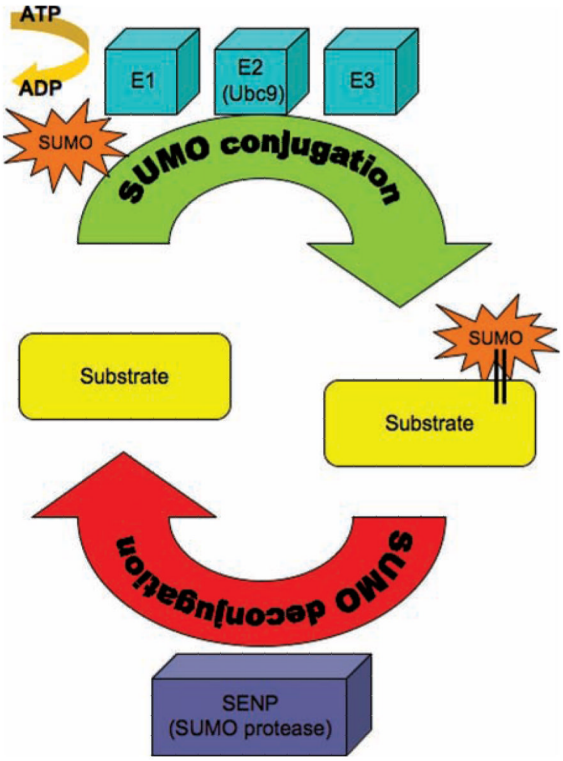

Fig. 1.

Schematic of the SUMOylation pathway. SUMOylation comprises three enzymatic steps that culminate in the formation of an isopeptide bond, between the carboxyl group of the C-terminal glycine of SUMO and the substrate ε-amino group of a specific lysine residue. The initial step in SUMOylation is ATP dependent and involves the activation of the C-terminus of the SUMO protein by the enzyme E1. Once activated, the SUMO protein is transferred to a SUMO-conjugating enzyme (E2) called Ubc9. Ubc9 binds substrate proteins directly and, in conjunction, with one of the several SUMO protein ligases (E3s), subsequently mediates the transfer of the SUMO protein to its target protein. However, it should be noted the involvement of E3 is not always required for efficient SUMOylation. Importantly, despite being a covalent modification substrate, SUMOylation is highly labile and readily reversible by the SUMO-specific family of SENP proteases. This highly dynamic system allows cells to respond rapidly to varying cellular demands. SUMO = small ubiquitin-like modifier.

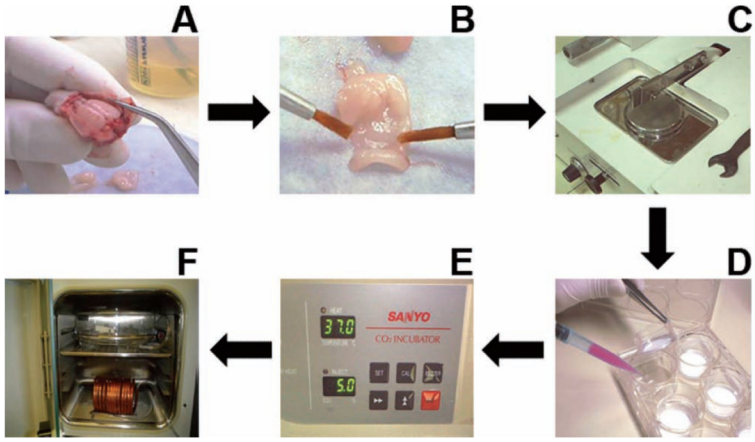

Fig. 2.

Slice preparation and culturing followed by oxygen-glucose deprivation (OGD). (*A*) Decapitate rats one by one with large scissors and remove the brain quickly. (*B*) Isolate the hippocampi. (*C*) Section transversely at 400 μm by McIlwain tissue chopper, and place the slices in chilled Hanks' balanced salt solution (HBSS). (*D*) Place six slices on each membrane insert in culture trays with six wells, each well containing 1 mL of culture medium (see composition below). (*E*) Place culture trays in incubator at 36 °C with 5% CO_2. Change the medium twice a week for two weeks. After 14 days in vitro, expose the cultures to OGD. Transfer the inserts to sterilized petri dishes and rinse twice with OGD medium (see composition below). Incubate the inserts in 1 mL of OGD medium for 10 minutes. Exchange for OGD medium previously bubbled with nitrogen for 15 minutes. (*F*) Transfer the petri dishes containing the slice cultures to an anaerobic chamber. Close the chamber and inject a mixture of N_2 with 5% CO_2 for 10 minutes at 8 L/min. Keep the chamber in the incubator for 45 minutes at 37 °C. Remove the dishes from the chamber and wash the slices twice with HBSS. Return to culture medium and incubate for 24 hours. Add 7.5 mM propidium iodide (PI) and incubate for 1 hour. Examine the cultures in an inverted fluorescent microscope fitted with a rhodamine filter. Photograph the slices and analyze using Scion Image software. Culture medium: minimum essential medium (50%), horse serum (25%), and HBSS (25%) supplemented with (mM, final): glucose 36, glutamine 2, HEPES 25, $NaHCO_3$ 4, and penicillin/streptomycin 1% (pH 7.3). OGD medium composed of (mM): $CaCl_2$ 1.26, KCl 5.36, NaCl 136.89, sucrose 36.08, KH_2PO_4 0.44, Na_2HPO_4 0.34, $MgCl_2$ 0.49, $MgSO_4$ 0.44, HEPES 25, and penicillin/streptomycin 1% (pH 7.2).

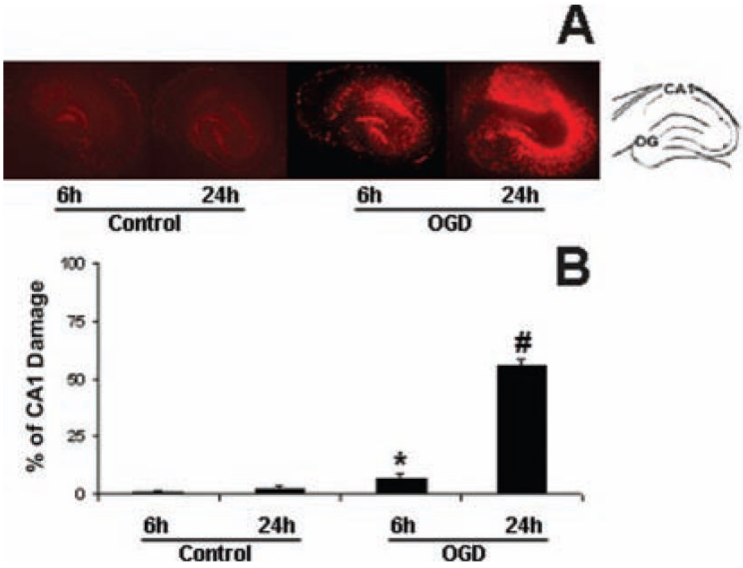

Fig. 3.

Propidium iodide (PI) staining indicating cell death following oxygen-glucose deprivation (OGD). After 45 minutes of OGD, cultures were returned to culture medium and incubated in the presence of O_2 to mimic the reperfusion that usually follows an ischemic episode in vivo. (*A*) Photographs of the slices showing PI staining in control and OGD slices at 6 and 24 hours' exposure to OGD. (*B*) Quantification of cellular damage in CA1 performed by using Scion Image software (www.scioncorp.com). The area where PI fluorescence was detectable above background levels was determined using the "density slice" option of the software and compared with total CA1 area to obtain the percentage of damage. Data represent mean ± SEM values, n = 15 slices per group. *indicates significant difference from control cultures; #indicates significant difference from all other groups (one-way ANOVA followed by Duncan's test, $P < .001$).

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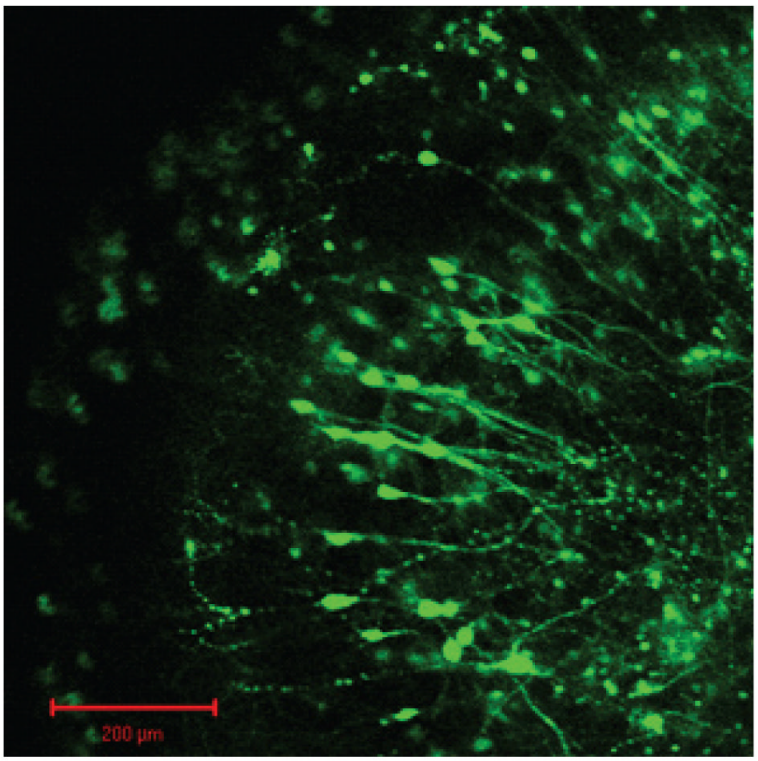

Fig. 4.
Expression of GFP Sindbis virus in pyramidal CA1 neurons of long-term organotypic hippocampal slice cultures. Fourteen-day in vitro P8 hippocampal slices were infected overnight by bath applying approximately 2 μL of virus directly onto the slice.

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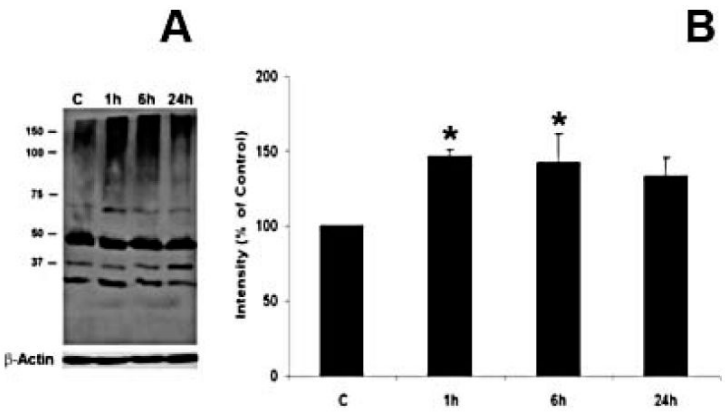

Fig. 5.

Changes in protein conjugation by SUMO-2/3 following oxygen-glucose deprivation (OGD). Organotypic hippocampal slice cultures were exposed to 45 minutes of OGD and then permitted 1, 6, or 24 hours of recovery. Protein SUMOylation was evaluated by Western blot analysis. Most strikingly, there is a marked increase in SUMOylation of higher molecular weight proteins that run as a smear on the gel at 1 hour and 6 hours that diminishes by 24 hours. (*A*) Representative pattern of SUMO-2/3 immunoreactivity detected using anti-SUMO-2/3 antibody from Zymed. (*B*) Cumulative SUMO-2/3 results showing quantified data from separate immunoblots using slices from four different cultures. The results are presented as percentages of control ± SEM. *indicates significant difference from control cultures (one-way ANOVA followed by Duncan's test, $P < .05$).

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