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GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE, APOPTOSIS, AND NEURODEGENERATIVE DISEASES*

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■ **Abstract** Increasing evidence supports the notion that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a protein with multiple functions, including its surprising role in apoptosis. GAPDH is overexpressed and accumulates in the nucleus during apoptosis induced by a variety of insults in diverse cell types. Knockdown of GAPDH using an antisense strategy demonstrates its involvement in the apoptotic cascade in which GAPDH nuclear translocation appears essential. Knowledge concerning the mechanisms underlying GAPDH nuclear translocation and subsequent cell death is growing. Additional evidence suggests that GAPDH may be an intracellular sensor of oxidative stress during early apoptosis. Abnormal expression, nuclear accumulation, changes in physical properties, and loss of glycolytic activity of GAPDH have been found in cellular and transgenic models as well as postmortem tissues of several neurodegenerative diseases. The interaction of GAPDH with disease-related proteins as well as drugs used to treat these diseases suggests that it is a potential molecular target for drug development.

INTRODUCTION

Recent research has revealed a small class of proteins whose members are endowed with multiple functions (for review, see 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a typical example. Historically, GAPDH has been

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considered a glycolytic enzyme with a key role in energy production. It has also been regarded as a product of a housekeeping gene whose transcript level remains constant under most experimental conditions, and it has been frequently used as an internal control in studying the regulation of gene expression. Mounting evidence, however, supports the view that the expression of GAPDH is regulated and that GAPDH is a protein with multiple intracellular localizations and diverse activities independent of its traditional role in glycolysis (for review, see 2, 3). These new activities include regulation of the cytoskeleton (4, 5), membrane fusion and transport (6–8), glutamate accumulation into presynaptic vesicles (9), and binding to low-molecular-weight G proteins (10). A role of GAPDH in nuclear function is also suggested by its ability to activate transcription in neurons (11), to export nuclear RNA (12), and to effect DNA repair (13). Particularly intriguing are the increasing reports that GAPDH is an integral part of various forms of apoptosis and may participate in neuronal death in some neurodegenerative diseases. The aim of this review is to provide evidence for the involvement of GAPDH in the apoptotic cascade, to discuss potential underlying mechanisms, and to evaluate the roles of GAPDH in preclinical models of neurodegeneration and human disease and in the pharmacological treatments of neurodegenerative diseases. Several reviews in these areas have appeared (2, 14–20).

EVIDENCE FOR A ROLE OF GAPDH IN APOPTOSIS

GAPDH Overexpression

Apoptosis, or programmed cell death, results from the actions of a genetically encoded suicide program that normally occurs in response to physiological or relatively mild stimuli (for review, see 21–23). Apoptotic cells display chromatin condensation, internucleosomal DNA cleavage, cytoplasmic shrinking, and plasma membrane blebbing, and they are phagocytized by microglia. This contrasts with necrosis, where cells swell and rupture, eliciting an inflammatory response. Mitochondria play a pivotal role in the genesis and propagation of apoptosis via events such as mitochondrial calcium accumulation, generation of free radicals, and, perhaps, activation of the permeability transition pore (for review, see 24). To date, four mitochondrial molecules mediating downstream cell-death pathways have been identified: cytochrome c, Smac/Diablo, apoptosis-inducing factor, and endonuclease G. Cytochrome c binds to Apaf-1, which, together with procaspase-9, forms apoptosomes that, in turn, cause activation of caspase-9, caspase-3, and others. Smac/Diablo binds to inhibitors of activated caspases, resulting in further caspase activation. Apoptosis-inducing factor and endonuclease G act via caspase-independent pathways to trigger cell death (for review, see 22, 25–27).

The involvement of GAPDH in apoptosis was first demonstrated in cultured cerebellar granule cells and cortical neurons undergoing spontaneous apoptosis (28, 29). A 38-kDa protein was found to be overexpressed prior to apoptosis and was soon identified as GAPDH by N-terminal sequencing. Direct evidence for its

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role in cell death came from studies using an antisense oligonucleotide knockdown strategy. Antisense oligonucleotides directed against either the translation initiation site or a segment of the coding region of GAPDH mRNA delay cell death; in contrast, the corresponding sense and scrambled oligonucleotides are ineffective (28, 29). Additionally, antisense, but not sense, oligonucleotides block the increase in GAPDH mRNA and protein preceding the apoptotic death with little or no effect on basal levels. Using similar approaches, GAPDH was found to be associated with apoptosis, but not necrosis, of cerebellar granule cells grown in culture medium with reduced concentrations of KCl (30).

The paradigm of cytosine arabinoside (AraC)-induced cell death has been used to further investigate the generality of the role of GAPDH in neuronal apoptosis and the molecular events associated with this process. AraC is a pyrimidine antimetabolite that has been used clinically for the treatment of acute leukemia. Freshly plated cerebellar granule cells exposed to AraC undergo rapid apopototic neuronal death that is preceded by an upregulation of the tumor suppressor protein p53 followed by an increase in levels of GAPDH and Bax, another proapoptotic protein (31). Again, the role of GAPDH in AraC-induced apoptosis was confirmed by antisense experiments (31, 32). Interestingly, a p53 antisense oligonucleotide not only suppresses apoptosis and decreases p53 and Bax mRNA induced by AraC, but also reduces the levels of upregulated GAPDH mRNA and protein. In the same study, it was shown that neurons prepared from p53-deficient mice are resistant to AraC neurotoxicity, and that p53 gene knockout also suppresses AraC-induced GAPDH expression. Moreover, infection of PC12 pheochromocytoma cells with an adenoviral vector containing the wild-type p53 gene dramatically increases GAPDH expression and triggers cell death (31). Taken together, these results lend support for the view that GAPDH is a novel target of p53, directly or indirectly regulated by this proapoptotic transcription factor, and could be one of the downstream apoptotic mediators. A scan of the rat GAPDH promoter reveals three potential p53 consensus binding sequences at positions –2008 to –1989, −1184 to –1165, and –1087 to –1068 from the ATG sequence and start site of translation, and suggests that p53 induces GAPDH expression directly.

GAPDH Nuclear Accumulation

Because upregulated GAPDH protein is present in the particulate (200×10^3 g pellet) fraction (28, 29, 32), subcellular fractionation studies using cultured neurons treated with AraC were performed to identify the sites of GAPDH accumulation (33). Immunoreactive GAPDH protein levels are markedly increased in the crude nuclear fraction and to a lesser extent in the crude mitochondrial fraction, but are unchanged in the crude fraction containing the endoplasmic reticulum. A similar conclusion concerning GAPDH nuclear translocation was reached in an independent study using confocal immunocytochemistry and subcellular fractionation of nonneuronal and neuronal cells subjected to various stresses, including dexamethasone treatment, nerve growth factor withdrawal, and aging of the cultures

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(34). Treatment with GAPDH antisense oligonucleotides abolished insult-induced GAPDH nuclear accumulation. Subsequent electron microscopic immunocytochemistry confirmed the accumulation of GAPDH in neurons undergoing apoptosis (35). Experiments using a GAPDH-green fluorescent protein (GFP) construct also showed movement of the GAPDH-GFP fusion protein from the cytosol to the nucleus shortly after exposure of cells to apoptosis-stimulating agents (36). A further study using highly purified nuclei from cerebellar granule cells treated with AraC showed that nuclear GAPDH accumulates over time with a concomitant degradation of lamin B1, a nuclear membrane protein, and caspase substrate (37). Nuclear accumulation is associated with a progressive decrease in the activity of uracil-DNA glycosylase, one of the nuclear functions of GAPDH (13). The nuclear glycolytic dehydrogenase activity is initially increased after AraC treatment and then decreases parallel to the DNA glycosylase activity. The same study shows that six isoforms of GAPDH with a similar molecular weight are present in the purified nuclei of cerebellar granule cells, and that all these nuclear isoforms are increased after AraC treatment, but only the more acidic isoforms are rapidly translocated. These GAPDH isoforms could be the results of posttranslational modifications, variants of alternative splicing, or products of distinct genes. Future studies using selective gene silencing, knockdown, or knockout may shed light on the differential roles of GAPDH isoforms in apoptosis.

There is a growing list of studies showing the involvement of GAPDH in cell death in various paradigms. These include androgen depletion-induced apoptosis of prostate epithelial cells (38); 1-methyl-4-phenylpyridinium (MPP^+)-induced death of mesencephalic dopaminergic neurons (39) and human neuroblastoma SK-N-SH cells (40); apoptosis induced by an endogenous dopaminergic neurotoxin, N-methyl-(R)-salsolinol, in human dopaminergic SH-SY5Y cells (41); induction of apoptosis (by staurosporine or MG 132) and oxidative stress (by H_2O_2 or FeCN, which is presumed to be ferricyanide) in neuroblastoma NB41A3 and nonneuronal cells (R6 fibroblasts) (42); and etoposide-induced apoptosis in cerebellar granule cells (43). Transfection of GAPDH into cells has been shown to induce cell death (44, 45) or facilitate cell death induced by apoptotic insults or oxidative stress (42). The latter study shows that nuclear localization of GAPDH is insufficient to induce apoptosis in NB41A3 cells. This can be explained by the fact that GAPDH also performs functions in the nucleus that are nonapoptotic (see below).

PERSPECTIVES CONCERNING GAPDH NUCLEAR TRANSLOCATION AND SUBSEQUENT APOPTOSIS

The mechanisms underlying the transport of GAPDH from the cytosol to the nucleus resulting in apoptotic death are poorly understood. In GT1-7 hypothalmic neurosecretory cells, treatment with thapsigargin, a selective inhibitor of calcium-ATPases of the endoplasmic reticulum, or buthionine sulfoximine, a specific γ-glutamyl-cysteine synthetase inhibitor, triggers GAPDH overexpression, nuclear

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translocation, and subsequent apoptosis; all three events are blocked by Bcl-2 overexpression (46). In human neuroblastoma cells, transfection-mediated Bcl-2 overexpression completely suppresses nuclear accumulation of GAPDH induced by N-methyl-(R)-salsolinol (41). In R6 cells overexpressing Bcl-2, the nuclear levels of endogenous GAPDH are markedly reduced compared with the wild-type control (42). These observations suggest that Bcl-2 may participate in the regulation of GAPDH nuclear translocation and this effect may be part of the protective mechanisms of Bcl-2 against apoptosis. However, Bcl-2 has no effect on transfection-induced nuclear accumulation of GAPDH-GFP fusion protein in R6 cells, indicating the complexity of the translocation process. The observation that GAPDH first localizes to the Golgi before proceeding to the nucleus following 6-hydroxydopamine treatment in neuroblastoma cells may be an indication of how GAPDH is transported to the nucleus (47).

It has been reported that GAPDH binds to a nuclear localization signal (NLS)-containing protein, Siah, to initiate the GAPDH translocation to the nucleus (48). GAPDH appears to stabilize Siah following their association, and thereby enhances Siah-mediated proteolytic cleavage of its nuclear substrates, such as N-CoR, to trigger apoptosis. Siah is also a target of p53 induction (49, 50) and can trigger apoptosis either alone (49) or in conjunction with Pw1/Peg3 (51). In addition, Siah acts as an E3 ubiquitin-ligase in the ubiquitination/proteasome degradation pathway for Numb and DCC (52). Although GAPDH is known to interact with microtubules and microfilaments (53) and binds to a variety of proteins linked to neurodegenerative diseases (see below), there is no evidence yet supporting the notion that these proteins mediate the transport of GAPDH to the nucleus. In NIH 3T3 fibroblast cells, serum depletion-induced GAPDH import to the nucleus is a reversible process; readdition of serum or stimulation with growth factors causes GAPDH export from the nucleus (54). The GAPDH nuclear export requires the activity of phosphatidylinositol 3-kinase but is not mediated by exportin 1 (54, 55). However, a novel exportin1-dependent nuclear export signal (NES) comprising 13 amino acids of the C-terminal domain of GAPDH has been identified (56). Truncation or mutation of this NES abrogates exportin1 binding and causes nuclear accumulation of GAPDH-GFP fusion protein expressed in colorectal adenocarcinoma cells. This suggests that GAPDH would be a nuclear protein were it not for its NES, and that the cytoplasmic localization of GAPDH is an active rather than a passive process. Reversible GAPDH nuclear translocation following its overexpression has also been found in human monocytes infected with vaccinia virus (57).

It appears that there is a change in the structure of the GAPDH protein following its nuclear translocation. For example, sodium nitropruside (an NO donor) -induced NAD labeling of nuclear GAPDH is decreased by 60% in cerebellar granule cells after AraC treatment (37), suggesting that the active site of GAPDH may be covalently modified, denatured, or improperly folded. GAPDH is present as a tetramer in the cytoplasm where the enzyme catalyzes its glycolytic activity. However, the uracil glycosylase activity of GAPDH is associated with the

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monomer form of GAPDH in the nucleus (58). It is still unresolved as to whether a change in the oligomeric state is required for GAPDH nuclear translocation. In cerebellar granule cell cultures treated with SYM-2081, a purported glutamate uptake inhibitor and receptor agonist, GAPDH is rapidly accumulated in the nucleus, and chromatin immunoprecipitation reveals that GAPDH complexes with acetylated histone H3, including Lys9 acetylated histone (59). Pretreatment with valproate causes a reduction in levels of nuclear GAPDH with a concomitant increase in acetylated histone in the immuno-complex and neuroprotection against SYM-2081-induced excitotoxicity. These results suggest that valproate suppresses excitotoxicity-induced GAPDH nuclear accumulation by weakening the interactions between GAPDH and histone through its hyperacetylation. A recent study shows that GAPDH is a key component of the coactivator complex OCA-S that is essential for S phase–dependent histone H2B transcription (60). GAPDH binds directly to Oct-1, exhibits potent transactivation potential, and is essential for S phase–specific H2B transcription in vivo and in vitro. The binding of GAPDH to Oct-1 is stimulated by NAD^+ but inhibited by NADH, raising the possibility that redox regulation could be important for the nuclear function of GAPDH (see below). Taken together, it is conceivable that the structural and functional changes of GAPDH following nuclear translocation result in alteration in nuclear function in a gain-of-toxicity manner, thereby contributing to its proapoptotic activities. GAPDH is also found in a nuclear protein complex involved in DNA repair of mismatched base pairs, where GAPDH, through its ability to bind DNA, acts as a sensor of altered base pairing in mismatched base pairs (61).

IS GAPDH AN INTRACELLULAR SENSOR OF OXIDATIVE STRESS?

It has been hypothesized that GAPDH serves as an intracellular sensor of oxidative stress and may play an early and pivotal role in the cascade leading to cell death (43). As described in the preceding section, the binding of Siah to GAPDH stabilizes Siah, and this event appears to be crucial for GAPDH nuclear translocation. The group reported that the GAPDH-Siah interaction is influenced by the oxidative modification on GAPDH at Cys149. The addition of antioxidants to the culture media inhibits GAPDH-Siah binding and GAPDH-dependent apoptotic death of neurons (43). Transfection with wild-type GAPDH increases Siah levels, whereas the GAPDH C149S mutant does not. This is an attractive hypothesis for several reasons. Active site Cys149 in GAPDH is made more reactive by the removal of its sulfhydryl proton to form a highly reactive thiolate group (cys-S^-) that is essential for its enzymatic activity and its subsceptibility to modification by electrophiles (oxidants). These include oxidation by either reduced glutathione or S-nitrosylated glutathione to form mixed disulfides such as enzyme-S-S-glutathione (62), S-nitrosylation to form enzyme-S-NO (62), transition metal bonding to form enzyme-S-Metal (63), attack of bound NADH by nitrosonium ion (NO^+) to form an

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enzyme-NADH adduct (64), and mono-ADP-ribosylation by an alternate route of the latter mechanism (64, 65). This mono-ADP-ribosylation is not to be confused with the enzymatic ADP-ribosylation of an arginine of GAPDH in the presence of ARF-1 catalyzed by Brefeldin A or stimulated by ER stress (66). Additionally, direct and indirect reactions of reactive oxygen species to form carbonyls are also possible (67).

In vivo, active site modification of GAPDH is most likely to arise from nitric oxide–derived products produced in response to oxidative stress (68). The formation of mixed disulfides, S-nitrosylation, and transition metal bonding are reversible and protective. The remaining modifications are irreversible. In either case, the enzyme activity is inhibited (69). Glucose metabolism continues, however, through the pentose phosphate shunt, which produces the NADPH used by glutathione reductase to recycle oxidized glutathione to its reducing form. It also uncouples glucose metabolism from the production of ATP and oxidative intermediates (70, 71). Thus, under oxidative conditions, GAPDH can act as a switch to redirect glucose metabolism to a more appropriate pathway. Under mild conditions, GAPDH activity can be recovered with the return of a reducing redox potential aided by the presence of reduced glutathione. Irreversibly modified GAPDH is ubiquitinated and degraded by proteasomes (72, 73). GAPDH, like histone H2A, actin, α-crystallin, and α-lactalbumin, requires a non-N-end rule E2 and HSC 70 for this process (74, 75). Oxidized active site cysteine also alters the affinity of GAPDH for its cofactors (76). The normal high affinity of GAPDH for NAD^+ is dramatically decreased, whereas the affinity for NADH is increased. The active site lies at the end of a twofold axis fold in the tertiary structure of GAPDH that accommodates the binding of the cofactor NAD^+. This is known as the Rossmann fold and is shared by a number of NAD^+-linked dehydrogenases (77). The negatively charged thiolate ion forms an ionic bond with the positively charged NAD^+ and stabilizes the GAPDH structure. This fold also accommodates the binding of nucleic acids, as bound NAD^+ or NADH blocks the binding of nucleic acids (78). Because the binding of NAD^+ stabilizes GAPDH tertiary structure, oxidation of the active site cysteine destabilizes the normal tetrameric form of the enzyme, and dimers, monomers, and denatured protein can be detected in the nucleus, as was shown in HeLa cells (76).

In addition, GAPDH has been found to associate with Nm23-H1, a nucleoside diphosphate kinase, to form an enzyme exhibiting dehydrogenase, nucleoside diphosphate kinase, and phosphotransferase activities (79). The complex is a tetramer composed of dimeric GAPDH and dimeric Nm23-H1. The separate enzymatic activities of the two proteins are unaffected by the association, as is the binding of NADH to GAPDH. Both Nm23-H1 and GAPDH are also components of the OCA-S complex involved in activation of histone H2B transcription mentioned above (60). In this complex, NAD^+ enhances association of the complex to the H2B promoter, whereas NADH is inhibitory. Thus, normal enzymatic activity, cofactor binding, and tetrameric quaternary structure do not exclude GAPDH from the nucleus, nor does the enhanced binding of nucleic acids resulting from

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active site oxidation appear to be required for at least three nuclear functions of GAPDH. This does not, however, exclude all mechanisms of nuclear translocation involving active site oxidation of GAPDH, and an explanation and function for GAPDH translocation to the nucleus during oxidative stress remains to be found.

The function of a sensor is to detect and signal changes in intracellular conditions. In addition to nuclear translocation, increased GAPDH gene expression with oxidation of the GAPDH active site cysteine has been observed (80). Could the oxidation of GAPDH Cys149 signal transcriptional activation of its own gene? Increased NADPH levels resulting from inhibition of GAPDH enable thioredoxin to become reduced, which in turn, through Ref-1, reduces a putative Cys275-Cys135 bridge within p53, enhances DNA binding, and activates the transcription of several proteins, including, presumably, GAPDH (31, 81). Reduced thioredoxin, through Ref-1, also enhances the function of NF-κB, AP-1, and HIF-1. It has long been thought that p53 mediates oxidative stress-induced apoptosis (82). Recently, however, it has become apparent that p53 acts through another protein, p66Shc, to mediate apoptosis (83). Overexpression of p66Shc potentiates overexpressed p53-mediated apoptosis in DLD-1 colorectal cancer cells, whereas overexpressed p53 induces no apoptosis in p66Shc$-/-$ mouse embryonic fibroblast cells. Overexpressed p66Shc alone has little effect and hence acts downstream of p53. Under high oxidative stress, p66Shc plays a permissive role in cytochrome c release and collapse of the mitochondrial membrane potential (84). Exactly how p53 and/or GAPDH interact with p66Shc is still unclear.

The one property of GAPDH that ties the multiple functions, locations, and ligands of this protein with its putative role as a sensor of oxidative stress is its capacity to bind NAD^+. Direct induction of GAPDH expression by p53 suggests that GAPDH plays a role in the function of p53, but the details of this role are unknown. Perhaps GAPDH provides NAD^+ to the NAD^+-dependent protein deacetylase, Sirt1. The human homologue of yeast Sir2a, Sirt1, inhibits p53 by removing the acetyl group from Lys382 in the C-terminal tail of p53 (85). This action results in resistance to stress and enhanced survival (86). Because irreversible oxidation of GAPDH Cys149 dramatically reduces its affinity for NAD^+, it could prevent delivery of the cofactor to Sirt1 and result in unchecked p53 activity toward apoptosis. This function could explain why GAPDH is translocated to the nucleus under conditions of cellular stress.

POTENTIAL ROLES OF GAPDH IN THE PATHOPHYSIOLOGY OF NEURODEGENERATIVE DISEASES

There is a general consensus that the pathophysiology of a variety of neurodegenerative diseases involves excessive apoptosis in distinct brain areas. For example, cytochrome c release, caspase activation, and apoptosis in the striatum have been documented in Huntington's disease (HD), an autosomal dominant neurodegenerative disease caused by an expansion of a sequence of repeated CAG triplets

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(coding for glutamine) in the gene of huntingtin, (reviewed in 87). The hallmarks of apoptosis, such as DNA fragmentation, caspase activation, and induction of apoptosis-related genes, have been found in brain neurons associated with the deposits of β-amyloid peptide in Alzheimer's disease (AD) (for review, see 27). Growing evidence also links Parkinson's disease (PD) with apoptotic death of dopaminergic neurons in the substantia nigra (reviewed in 20, 27, 87). In brain ischemia, the activation of caspases 1, 3, 8, 9, and 11 and apoptosis have been reported in the ischemic penumbra, where hypoxia and energy depletion are not as severe (reviewed in 87–89). The participation of GAPDH in apoptosis in vitro suggests significant roles in human pathophysiology. This section reviews literature reporting abnormal expression and intracellular localization of GAPDH and implicating the involvement of GAPDH in the apoptosis and neurodegeneration observed in HD, AD, PD, and ischemia.

Huntington's Disease and Other Polyglutamine-Connected Brain Disorders

Several pioneering studies have shown that GAPDH binds to proteins containing polyglutamine tracts associated with several neurodegenerative diseases. These include huntingtin for HD (90), atrophin for dentatorubral-pallidoluysian atrophy (DRPLA) (90), ataxin for spinocerebellar ataxia type-1 (SCA-1) (91), and androgen receptor for spinobulbar muscular atrophy (91). Specific binding of GAPDH to the polyglutamine stretch of huntingtin and atrophin depends on the number of glutamines in their polyglutamine tracts (90). GAPDH binds in vitro to both normal and mutant huntingtin with a preference for cleaved fragments of the protein. GAPDH binding sites for both ataxin-1 and androgen receptors are located in the N-terminal polyglutamine-containing domain but do not depend on the length of the polyglutamine tract (91). Although of potentially great importance, the functional significances of these interactions with GAPDH remain unelucidated. A recent postmortem study shows that the extent of cerebral white matter damage in DRPLA correlates with GAPDH immunoreactivity (92). An increase in GAPDH immunostaining of endothelial cells, astrocytes, and oligodendrocytes has been observed in this disease, and the abnormal staining appears to depend on the severity of cerebral white matter damage. It has also been reported that profuse GAPDH granular deposits were found in neuronal nuclei in the pontine region of the postmortem brain of patients with spinocerebellar ataxia type-3 (Machado-Joseph disease), but only weak and diffuse GAPDH staining was detected in the cytoplasm of neurons in control brain (19). Although not described in that report, similar granule nuclear GAPDH deposits were also found in the DRPLA brain.

The interaction of huntingtin with GAPDH, resulting in either loss of normal GAPDH function or gain of toxic function, is one possible cause of the pathology of HD, which is characterized by hyperkinetic involuntary movement, cognitive impairment, and depression. Several attempts have been made to investigate

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whether there is a change in the glycolytic activity of GAPDH in the HD state. In the initial study, GAPDH was not significantly altered in homogenates of frontal and parietal cortex, caudate putamen, or cerebellum of human postmortem HD brain samples (93). A subsequent study showed, however, a small but significant decrease (−12%) in GAPDH glycolytic activity in homogenates of the caudate nucleus of HD patients (94). As discussed in a previous section, nuclear translocation of GAPDH in vitro is associated with decreased glycolytic activity of nuclear fractions (37). Thus, in future studies, the measurements of GAPDH activity in nuclear preparations rather than whole homogenates from postmortem HD brains are warranted. HD fibroblasts subjected to metabolic stress by withholding fresh medium, on the other hand, increased their GAPDH glycolytic activity by only threefold, compared to an eightfold increase in control fibroblasts (95). In another study, GAPDH activity of HD fibroblasts decreased by 33% in the nuclear fraction but not in the postnuclear fraction, when compared with age-matched controls (96). This decrease in nuclear glycolytic activity of HD fibroblasts is associated with the nuclear appearance of a high-molecular-weight GAPDH-immunopositive species (97). This high-molecular-weight GAPDH-immunopositive species was not found in whole-cell sonicates of HD fibroblasts, which have normal glycolytic activity. It is unknown whether these changes are related to the transglutaminase-catalyzed, polyglutamine domain-dependent inactivation of GAPDH reported in a cell-free study (98). Striatal neurodegeneration in HD patients is accompanied by the appearance of nuclear inclusions of mutant huntingtin (for review, see 23, 87). It has been proposed that, through binding to the polyglutamine stretch of this disease-causing protein, GAPDH functions as a molecular chaperon in the nuclear translocation of huntingtin (34, 35). However, there are not, as yet, sufficient experimental data to substantiate this interesting hypothesis.

An increasing number of reports demonstrate that the expression in mice of mutant huntingtin with expanded polyglutamine tracts leads to neuronal loss and shows phenotypes of neurological disorders similar to those found in HD (99–104). Studies of abnormal GAPDH expression and localization have been conducted utilizing the brains of transgenic mice that express full-length huntingtin cDNA with 89 CAG repeats and display neurodegeneration in brain areas including the striatum and cerebral cortex (100). The majority of the transgenic mice show a strong increase in GAPDH immunofluorescence that increases with age, compared with wild-type mice. The wild-type mice show an even and predominantly cytoplasmic distribution of GAPDH (105). Increased GAPDH immunostaining in transgenic mice occurs in cells of specific brain regions such as the caudate putamen, globus pallidus, neocortex, and hippocampus. Double-staining experiments revealed that GAPDH overexpression occurs in neurons but not glial cells. Subcellular fluorescence microscopy demonstrated that GAPDH accumulates in the nuclei of neurons in these brain regions (Figure 1, see color insert). Nuclear accumulation is associated with the loss of medium-sized and small neurons in the caudate putamen and neurons in layers V and VI of the neocortex (105). The marked increase of GAPDH in the cytoplasm and nuclei of neurons suggests that GAPDH is involved in the apoptotic cascade in the transgenic mouse model of HD.

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Alzheimer's Disease

AD is the most common cause of progressive neurodegeneration and is characterized by dementia and memory loss. The deposition of β-amyloid protein in the cerebral cortex and hippocampus is one of the most distinct morphological features in AD (reviewed in 106). β-Amyloid peptide derives from irregular proteolytic processing of β-amyloid precursor protein (β-APP). The deposition of β-amyloid peptide is associated with neuronal loss in these brain regions and is, at least in part, due to apoptosis. Initial in vitro studies found GAPDH binding to the cytoplasmic carboxyl terminal of β-amyloid precursor protein (β-APP) (107). Such binding could alter the normal processing of β-APP to produce β-amyloid protein. A significant nuclear role for the C terminus of β-APP has also been suggested (108, 109). The recognition of GAPDH by a monoclonal antibody raised against amyloid plaques from the brains of patients with AD indicates the presence of GAPDH in these plaques and suggests an interaction between GAPDH and β-APP (110, 111). A more recent preliminary report suggests that cotransfection of COS-7 cells with GAPDH and wild-type β-APP cDNAs induces synergistic cytotoxicity (112).

The initial postmortem study showed a significant (19%) reduction in GAPDH glycolytic activity in the homogenates of the temporal cortex of AD patients (94). However, a subsequent study failed to detect a change in GAPDH activity in homogenates of the frontal, temporal, parietal, and occipital lobes of AD brains, although there was a significant increase in activity in the same brain regions in Down's syndrome patients (113). Such a discrepancy could be related to the fact that whole-cell homogenates, rather than subcellular fractions were used in the activity measurements. The presence of multiple cell types in a given brain region might have also masked potential changes in a specific cell population. Using fibroblasts from AD patients, it has been reported that GAPDH glycolytic activity is decreased by approximately 27% in both postnuclear and nuclear fractions compared with age-matched controls (58, 96). A high-molecular-weight species of GAPDH-immunoreactivity was detected exclusively in the postnuclear fraction of AD fibroblasts (58). The latter displayed reduced GAPDH activity and was not present in postnuclear fractions from control subjects. Whether the shift in molecular weight reflects GAPDH binding to β-APP is unknown. Interestingly, the association of GAPDH with a high-molecular-weight species was not detected in sonicated AD whole-cell extracts, which exhibited normal levels of GAPDH activity. This suggests that GAPDH is weakly bound to the high-molecular-weight protein complex and that dissociation of GAPDH from the high-molecular-weight complex restores its glycolytic activity. In the postmortem AD brain, nuclear aggregated GAPDH in neurons of affected areas has been found (114). It is also noteworthy that potential drugs for treating AD, such as tetrahydroaminoacridine (THA) and ONO-1603, effectively suppress GAPDH overexpression and nuclear translocation in rat brain neurons undergoing apoptosis in cultures (115, 116). Moreover, THA and another antidementia drug, donepezil, inhibit AraC-induced increase in GAPDH promoter activity (114).

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Parkinson's Disease

PD is characterized by the loss of catecholaminergic neurons, including those in the substantia nigra compacta and noradrenergic neurons in the locus coeruleus. The first implication that GAPDH is involved in the pathogenesis of PD is from the observation that CGP 3466 (also known as TCH 346), a structurally related analog of R-(-)-deprenyl, protects human neuroblastoma PAJU cells from apoptosis induced by rotenone, a toxin producing PD-like neuropathology (44). The MAO-B inhibitor, (-)-deprenyl, slows neurodegeneration and reduces the clinical deficits of PD, HD and AD. Unlike (-)-deprenyl, CGP 3466 does not inhibit MAO-B activity, but like (-)-deprenyl, binds to GAPDH. These results are compatible with the view that GAPDH, rather than MAO-B, is the target of deprenyl-like compounds effective against PD neuropathology. An independent study suggested that deprenyl-like compounds inhibit apoptosis by inducing GAPDH to dissociate from its usual tetrameric form to a dimer, and thereby interfere with GAPDH nuclear translocation (117). Nuclear localization of GAPDH monomers and dimmers were readily detected in HeLa cells, however, following GAPDH active site oxidation (76). R-2HMP (R-2-heptylmethyl-pargylamine) and other aliphatic pargylamines bind GAPDH, prevent GAPDH overexpression, and block p53-dependent apoptosis (16, 17, 118). Other studies show that apoptosis of neurons or related cell-lines induced by dopaminergic toxins such as MPP^+, 6-hydroxydopamine, or N-methyl-(R)-salsolinol involves GAPDH overexpression and nuclear translocation, and these effects are prevented by GAPDH antisense oligonucleotides and anti-PD drugs (39–41, 47). In total, these observations suggest that GAPDH is a potential molecular target of drugs used to treat PD and other neurodegenerative diseases.

Despite the number of cell culture studies of the role of GAPDH in apoptosis, knowledge concerning GAPDH changes in the PD brain is limited. This may be because there is still no clear consensus that apoptosis contributes to the loss of dopaminergic neurons in PD. Interestingly, an accumulation of GAPDH was found in the nuclei of melanized neurons of the nigra in postmortem brain sections from PD patients, whereas GAPDH was found only in the cytoplasm of melanized cells of age-matched control sections (119). Nuclear inclusion bodies, known as Marinesco's bodies, are immunoreactive for GAPDH in numerous nigral neurons from PD, but not control brains. Many cytoplasmic inclusion bodies, known as Lewy bodies, in the melanized neurons of PD brain are also immunopositive for GAPDH, although it is unknown whether all Lewy bodies are immunopositive. Moreover, GAPDH appears to be colocalized with Bax and caspase3 in melanized neurons of the PD nigra. Although a direct link between PD and GAPDH-mediated apoptosis is still undetermined, these results suggest a potential role of GAPDH nuclear accumulation in dopaminergic cell death in the PD brain.

Stroke and Hypoxia

Stroke is a major cause of mortality and morbidity worldwide, and is one of the neurodegenerative diseases linked to glutamate excitotoxicity. The significant

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increase in extracellular glutamate in the brain following ischemia and the brain damage that occurs as a result are well recognized (for review, see 87, 89, 120). Few studies address the involvement of GAPDH in the ischemic brain. In a rat focal ischemia stroke model, nuclear accumulation of GAPDH has been found in the ischemic core of the parietal cortex of rats subjected to 2 h of middle cerebral artery occlusion without reperfusion (121). During subsequent reperfusion, GAPDH immunostaining in the ischemic core decreases but cytoplasmic and nuclear staining in the penumbra becomes detectable. The increase in nuclear GAPDH immunoreactivity persists up to 48 h with a concomitant decrease in cytoplasmic reactivity. Double labeling of GAPDH-positive cells with TUNEL suggests an association of GAPDH overexpression/nuclear accumulation with excitotoxicity-induced apoptotic death. Cell culture studies have provided insights into mechanisms by which GAPDH is induced by hypoxia. A pioneering study has shown that hypoxia stimulates GAPDH overexpression in the cytoplasm and nucleus of endothelial cells at both transcriptional and posttranscriptional levels (122). Subsequent studies identified at least two hypoxia-responsive elements in the GAPDH gene promoter (123, 124). Hypoxia induces an elevation of GAPDH protein in the cytosolic, nuclear, and particulate fractions by 4.0-, 2.3-, and 4.2-fold, respectively, in a mouse brain capillary endothelial cell line (125). Little or no increase in GAPDH glycolytic activity was found in these fractions, however, suggesting a dynamic steady state or an inactivation of newly induced GAPDH. The same study also shows that GAPDH expression is suppressed by inhibiting the activation of nonselective Ca^{2+} channels, Na^{+}/Ca^{2+} exchanger, Ca^{2+}/calmodulin-dependent kinase, and c-Jun-dependent AP-1 binding. GAPDH has been shown to interact directly with heat shock proteins (HSP), and in particular, with HSP70 (61, 126). HSP70 has a major role in protection against ischemia-induced brain damage (88, 127, 128). Therefore, if GAPDH is involved in the apoptotic death induced by ischemia/hypoxia, then GAPDH binding-induced inactivation of cytoprotective HSP70 and nuclear translocation may be involved as well.

CONCLUSIONS AND FUTURE DIRECTIONS

The involvement of GAPDH in apoptosis was first demonstrated in primary cultures of brain neurons, and this finding was soon expanded to numerous apoptotic paradigms in diverse cell types, including neurons and nonneuronal cells. Several lines of evidence also suggest that GAPDH may be an intracellular sensor of oxidative stress during the early phase of the apoptotic cascade. Irreversible oxidation of the active site cysteine of GAPDH triggers major changes in cellular homeostasis. Enhanced expression, nuclear accumulation, changes in the apparent molecular size, and a decrease in the glycolytic activity of GAPDH have also been observed in some cellular, rodent, and transgenic mouse models as well as postmortem brain tissues of several neurodegenerative diseases, including HD, AD, PD, and stroke/hypoxia. Table 1 lists cellular and rodent models as well as neurodegenerative diseases that have been linked to GAPDH abnormalities. Interactions

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TABLE 1 Changes in expression, intracellular localization, and glycolytic activity of GAPDH are associated with diverse apoptotic paradigms in multiple cell types, as well as rodent models and postmortem brain tissues of neurodegenerative diseases*

Apoptotic stimuli or disease	Cell types or tissues
Spontaneous cell death, AraC toxicity, K^+ depletion, excitotoxicity, etoposide	Cerebellar granule cells, cerebral cortical neurons
Nerve growth factor withdrawal, serum deprivation, dexamethasone stimulation	PC 12 cells, HEK 293 cells, S49 lymphoma cells
Androgen deprivation	Prostate epithelial cells
Thapsigargin, buthionine sulfoximine	GT1-7 hypothalmic neurosecretory cells
Dopaminergic toxins (MPP^+, rotenone, 6-hydroxydopamine, N-methyl-(R)-salsolinol)	Mesencephalic dopaminergic neurons; human neuroblastoma PAJU, SK-N-SH, and SH-SY5Y cells
Staurosporine, oxidative stress	Neuroblastoma NB41A3, R6 fibroblasts
Hypoxia, middle cerebral artery occlusion (a rat stroke model)	Bovine and rodent endothelial cells, rat brain penumbra
HD, DRPLA, Machado-Joseph disease	Postmortem brains, fibroblasts, transgenic mouse brain of HD
AD	Postmortem brains, fibroblasts
PD	Postmortem brains, cellular models

*See text for references.

of GAPDH with some disease-related proteins such as polyglutamine-containing mutant disease proteins and β-APP have been reported. Whether such interactions occur in the affected brain areas during pathological states and play a role in nuclear transport of the GAPDH complex need to be rigorously demonstrated. Drugs used to treat PD, HD, and AD can bind GAPDH and/or suppress its overexpression, and these actions correlate with their neuroprotective effects, suggesting that GAPDH may be a therapeutic target for disease interventions and future drug design and development.

Despite the advancement in knowledge concerning the proapoptotic effects of GAPDH and the crucial role of GAPDH nuclear translocation in the apoptotic process, their precise molecular mechanisms remain to be defined. The observations that GAPDH is positively regulated by p53 and is colocalized with Bax and caspases and that Bcl-2 overexpression blocks GAPDH nuclear translocation suggest that GAPDH may act by perturbing the p53-Bax-Bcl-2-caspase pathway. It appears that GAPDH nuclear translocation is integral to the apoptotic cascade and that protein-protein interactions may be crucial in mediating this nuclear transport. Although some GAPDH chaperone proteins have been proposed, future studies are necessary to substantiate their roles in nuclear translocation. GAPDH exists in multiple isoforms that are differentially translocated to the nucleus following

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apoptotic insults. Selective gene silencing and/or knockout/knockdown of these isoforms will shed light on their potentially distinct involvements in apoptosis. It seems possible that GAPDH translocation to the nucleus is not the sole mechanism whereby its proapoptotic effect is mediated. Translocation of GAPDH to other intracellular organelles such as the mitochondria warrants future investigation, particularly considering that this organelle plays a pivotal role in apoptosis and that GAPDH is also enriched in the crude mitochondrial fraction of neurons undergoing apoptosis. The apparent contradictions as to whether GAPDH glycolytic activity is decreased in the postmortem HD and AD brains could be related to the fact that whole-cell homogenates rather than subcellular fractions were used in the analysis. It would seem necessary to reexamine changes in glycolytic activity using various subcellular fractions derived from the postmortem brains of patients with these diseases. The success in demonstrating dramatic changes in GAPDH overexpression and nuclear accumulation in distinct brain neurons of HD transgenic mice raises the possibility that the transgenic disease model may be a useful tool to elucidate the role of GAPDH in mediating neurodegeneration and the pathophysiology of human diseases.

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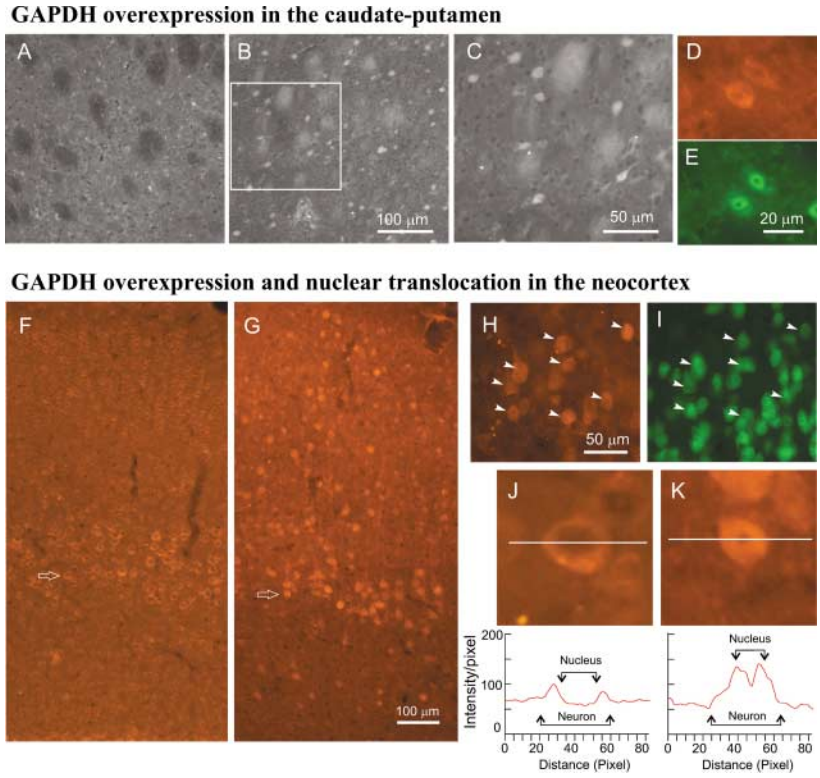

Figure 1 Overexpression and nuclear accumulation of GAPDH in the brain of transgenic (Tg) mice expressing huntingtin gene with 89 CAG repeats. (*A*) Relatively weak GAPDH immunostaining in the caudate putamen region of a wild-type (WT) mouse. (*B*, *C*) An increase in GAPDH immunofluorescent expression in selective populations in the caudate putamen of a Tg mouse, as compared with WT age-matched control in (*A*). The boxed area in (*B*) is expanded in (*C*). Note the nuclear accumulation of immunofluorescence in Tg mouse cells. (*D*, *E*) Double immunostaining of GAPDH-reactive cells in red (*D*) with the neuronal marker NeuN in green (*E*). (*F*, *G*) Immunofluorescent staining shows an increase in GAPDH expression in GAPDH immunoreactivity in specific cell populations in the neocortex of Tg mice (*G*), as compared with the control (*F*). Note the accumulation of immunofluorescence in the nuclei of Tg mouse cells. (*H*, *I*) Double immunostaining of GAPDH-reactive cells (*H*) and for the neuronal marker NeuN (*I*). Arrowheads indicate cells double-immunostained for GAPDH and NeuN. (*J*, *K*) Representative images of subcellular distributions of GAPDH immunoreactivity at the level of neocortical layer V in WT (*J*) and Tg (*K*) mice, respectively. Bottom panels show the fluorescent intensity profiles of GAPDH-expressing neurons in the top panels. Note that the highest fluorescent intensity occurs in the nuclear portion of the intensity profile as delineated by arrows. Results are modified from Senatorov et al. 2003 (105). Reprinted with permission of the publisher.