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Excitotoxic and post-ischemic neurodegeneration: Involvement of transglutaminases

Review Article

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Summary. Neurodegeneration induced by excitotoxicity is a common feature in various neurological disorders. This pathological condition is caused by prolonged stimulation of glutamate receptor subtypes, followed by both intracellular Ca^{2+} overload and activation of specific genes, resulting in synthesis of enzymes involved in cell stress response.

Using experimental *in vitro* models of excitotoxicity, we demonstrated that glutamate exposure up-regulated tissue transglutaminase in primary cultures of both cerebellar granule cells and astrocytes. These changes were consequent to receptor-mediated Ca^{2+} influx, as demonstrated by the inhibition with selective antagonists, MK-801 and GYKI 52466. Early increases in different transglutaminase isoforms were also observed in global cerebral ischemia, which closely resembles neuronal damage caused by NMDA receptor activation.

These findings agree with a postulated role for transglutaminases in molecular mechanisms of several neurodegenerative diseases. Indeed, increased cross-linking reactions could be of pathologic relevance, as part of biochemical changes observed in neurological disorders.

Keywords: Transglutaminases – Excitotoxicity – Neurodegenerative diseases – Ischemia – Cerebellar granule neurons – Astroglial cells

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; GYKI 52466, 1-(4′-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzo-diazepine; KA, kainate; MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo [*a, d*]-cyclohepten-5,10-imine hydrogen maleate; NMDA, N-methyl-D-aspartic acid; ROS, reactive oxygen species; TGase (s), transglutaminase (s)

Introduction

Excitotoxicity was originally reported to term the ability of glutamate and structurally related excitatory amino acids to destroy neurons (Olney, 1986). Many studies have suggested an involvement of excitotoxicity in the pathogenesis of human neurodegenerative diseases induced by acute insults, such as hypoxia, hypoglycemia, sustained epilepsy, and trauma (Doble, 1999; Meldrum, 2000). In addition, it is currently accepted that massive glutamate release, with consequent prolonged activation of ionotropic and metabotropic glutamate receptors, contributes remarkably to neuronal death (Doble, 1999). Neurodegeneration mainly results from the activation of free radicals generating enzymes, production of reactive oxygen species (ROS), and increased extracellular Ca^{2+} influx. This latter event, in turn, leads to over-activation of numerous enzymes, such as protein kinases, phospholipases, proteases and endonucleases (Lewen et al., 2000; Lok and Martin, 2002). In recent years, the study of excitotoxicity has been carried out in a variety of *in vitro* models, including cell and tissue cultures, retina and brain slices. The evidence obtained in these simplified systems has generally been consistent with earlier *in vivo* observations, contributing to define underlying mechanism of excitotoxic cell death. Cell damage produced by brief, intense exposure to glutamate can be associated with distinct events: (i) immediate neuronal cell swelling, dependent on the alteration of Na^+/Cl^- influx; (ii) delayed cell degeneration dependent on excessive Ca^{2+} overload, triggered by glutamate receptor agonists (Choi, 1992). Under certain conditions, cell swelling observed *in vitro* can be characterized as a temporary event, as cell volume homeostasis can be recovered and neurons survive. On the contrary, Ca^{2+}-dependent component of neurotoxicity produces

irreversible neuronal injury, and can be induced by glutamate or related agonists in different *in vitro* and *in vivo* models, leading to neuronal death in vulnerable brain regions. Different susceptibility to excessive glutamate release may depend on the type of glutamate receptors expressed in neurons (Doble, 1999).

High glutamate concentration in inter-synaptic spaces is a feature common to various neuropathological conditions and neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease, and amiotrophic lateral sclerosis. Numerous studies show evidence for the accumulation of insoluble oligomers and polymers of disease specific proteins that initiate or contribute to neuronal dysfunction (Selkoe, 2002; Lai et al., 2004). In this context, many findings showing increases in Ca^{2+}-dependent transglutaminase (TGase) reactions could be of pathogenetic relevance. Thus, TGases may contribute to formation of protein aggregates and to biochemical changes occurring in cell damage and neuronal loss.

TGases

TGases catalyze a Ca^{2+}-dependent acyl-transfer reaction between the γ-carboxamide group of peptide-bound glutamine residues and various primary amines, most commonly the ε-amino group of lysine residues, or amino groups of polyamines. The reaction results in cross-linked proteins or incorporated polyamines into proteins, respectively (Griffin et al., 2002), leading to formation of insoluble polymers resistant to breakage and proteolysis. In mammals, nine different TGase genes have been identified so far, encoding biochemically and immunologically distinct TGases (Grènard et al., 2001): TGase 1 (membrane, particulate), TGase 2 (tissue TGase) (ubiquitous, soluble), TGase 3 (epithelia, soluble), TGase 4 (prostate, soluble), TGase 5 (epithelia, soluble), TGase 6, TGase 7, factor XIII-a (plasma) and band 4.2 protein, the latter lacking enzymatic activity. Different TGases are involved in a wide range of biological events, including blood coagulation, wound healing and cell differentiation. However, despite a close relationship between expression and distribution of enzyme isoforms in different cell types, recent findings have highlighted the overlap of TGase genes expression patterns, which leads to proteins with functional redundancy.

Under physiological conditions, TGase-mediated reactions are associated with long-term potentiation, synapse formation, and tissue differentiation. However, interest in TGases is further stimulated by the evidence of their increased activity in a number of neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease and different forms of amiotrophic lateral sclerosis (Citron et al., 2002). Several efforts have been made to characterize the role of TGase substrates involved in the pathogenesis of diseases. Further, the presence of ε (γ-glutamyl)lysine isodipeptides in body fluids, following cell damage and breakdown, has been considered a useful marker of tissue damage under pathological conditions (Jeitner et al., 2001; Bonelli et al., 2002).

Although TGase 2 appears the most inducible form among TGases in the brain areas, where it is expressed in neurons as well as in glial cells, the mechanisms leading to its up-regulation are still unclear. Retinoids are considered the most potent inducers of TGase 2, in cerebellar granule cells. Here, we consider recent results, suggesting that exposure of neuronal cells to high concentrations of excitatory amino acids promotes Ca^{2+} accumulation, leading, in turn, to enzyme activation and enhanced TGase 2 expression, responsible for increased cross-linking of proteins associated to cell damage and tissue degradation.

Tissue TGase and cell damage

In recent years, growing evidence has shown that TGase 2 (tissue TGase) may be involved in apoptosis occurring in different cell types. In particular, increases in TGase 2 by different inducers (i.e. retinoids, dexamethasone, p53) have been implicated in programmed cell death. In these cases, TGase 2 was up-regulated in dying cells, leading to production of protein polymers/envelopes associated to death pathways (Piacentini et al., 1994; Melino and Piacentini, 1998). It has also been suggested that TGase 2 is important to prevent loss of intracellular compounds by protein cross-linking inside apoptotic cells, before cell destruction by phagocytosis (Fesus, 1998). In Swiss 3T3 fibroblasts, evidence was given for increases in TGase 2, causing changes in the mitochondrial membrane polarization, and acting as a sensitizer of death stimuli (Piacentini et al., 2002). Nevertheless, some findings suggested that TGase 2 is not a crucial component of the main pathway of the apoptotic program. Indeed, it has been reported that the TGase 2 gene disruption did not produce developmental anomalies in mice, which were viable and phenotypically normal (De Laurenzi and Melino, 2001; Nanda et al., 2001). Interestingly, it is possible to hypothesize that the redundancy of other TGase isoforms can compensate for the lack of TGase 2 protein. However, the expression of TGase 2 in cells, in which calcium homeostasis was rapidly modified, caused massive accumulation of cross-linking products resulting in cell death.

The mechanism by which excitotoxic injury produces cell death is still not fully understood. In ischemia models, for example, excitotoxic neuronal damage has been shown to be either sensitive or insensitive to protein synthesis inhibition (Wiessner et al., 2000; Snider et al., 2001). Therefore, a role for *de novo* protein synthesis in the expression of apoptotic cascade in excitotoxicity is uncertain. In particular, there is much evidence to show that apoptotic cell death should be associated with early ischemic events. Indeed, numerous findings suggest that some proteins could be translated post-ischemia. Cytoskeletal damage also occurs in early post-ischemia, before the degeneration of neuronal cell bodies. This early modification, following ischemia and excitotoxicity, contrasts with the appearance of an organized cytoskeleton in neuronal apoptosis. It is noteworthy that TGase 2 appears to be induced not only by apoptotic but also ''necrobiotic'' cells (Fesus, 1998; Nicholas et al., 2003). This is consistent with the principle that insults, causing necrosis by direct cell destruction, can induce apoptosis if the cell initially survives (Carson and Ribeiro, 1993). In particular, increases in protein cross-linking may be directly associated with mechanisms leading to extensive cell death in some brain regions.

In all useful models of global cerebral ischemia, neurodegeneration in selectively vulnerable regions is morphologically indistinguishable from neuronal death caused by excitotoxicity, and, specifically, closely resembles the neuronal death evoked by N-methyl-D-aspartic acid (NMDA) receptor activation in the adult brain (Martin et al., 1998).

TGase and excitotoxic damage in cell cultures

Experiments, carried out with different agonists of glutamate receptor subtypes, have shown that stimulation of NMDA receptors can increase the activity of numerous enzymes, triggering protein–protein interactions deleterious for cell homeostasis, leading to excitotoxic neuronal death (Doble, 1999). Indeed, brief exposures of cerebellar granule cells to NMDA give onset to different calcium-dependent processes, mainly evoked by NMDA receptor activation (Griffiths et al., 1998; Tenneti and Lipton, 2000). We recently demonstrated, in primary cultures of cerebellar granule cells, an early increase in TGase activity and TGase 2 expression, after excitotoxic injury produced by a 30 min treatment with NMDA (100 μM). Further, these increases were strongly reduced by 5-methyl-10,11-dihydro-5*H*-dibenzo [*a, d*]-cyclohepten-5,10-imine hydrogen maleate (MK-801), a non-competitive blocker of Ca^{2+} influx through receptor gated ion channel. In contrast, 1-(4′-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzo-diazepine (GYKI 52466), a selective inhibitor of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)/kainate (KA) receptors, was not able to abolish the observed effects on TGase (Fig. 1). This suggests that changes, induced by NMDA exposure, were specifically dependent on NMDA receptor activation (Ientile

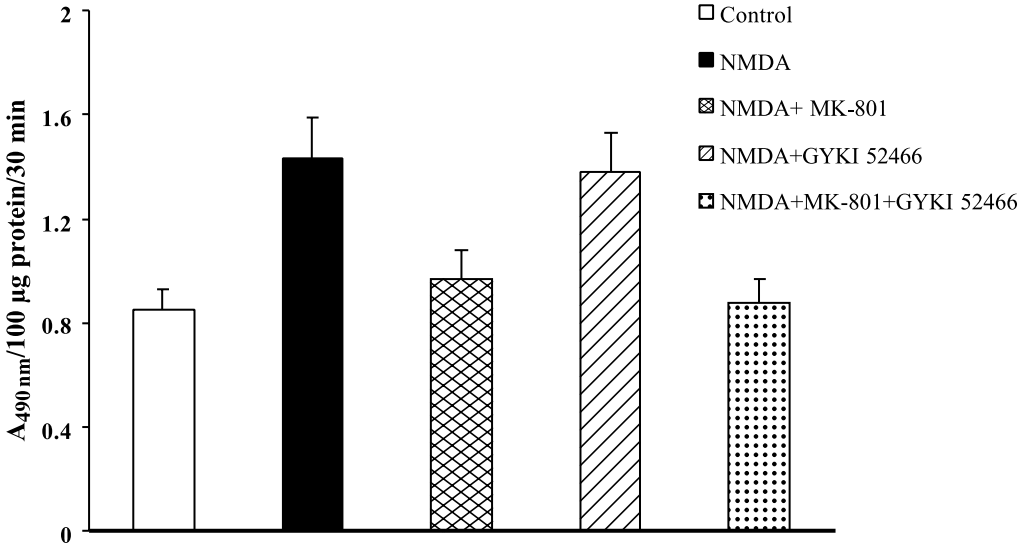

Fig. 1. Effect of NMDA and glutamate receptor subtype inhibitors on TGase activity in primary cultures of neurons. Cerebellar granule cells were treated for 30 min with NMDA (100 μM), in the presence or absence of inhibitors, MK-801 (10 μM), a non-competitive blocker of Ca^{2+} influx through ion channel associated with NMDA receptor, and GYKI 52466 (100 μM), a selective antagonist of AMPA/KA receptor. Enzyme activity was measured by ELISA with FITC antibody, evaluating TGase-mediated incorporation of fluorescein cadaverine into cell proteins

Table 1. Effect of extracellular Ca^{2+} removal on TGase activity in cultured neurons exposed to NMDA

	TGase activity ($A_{490\,nm}/100\,\mu g$ protein/30 min)
NMDA (100 μM) + Ca^{2+} (2.3 mM)	1.8 ± 0.2
NMDA + EGTA (2 mM) − Ca^{2+}	0.7 ± 0.06

Cerebellar granule cells were treated for 30 min with NMDA in Mg^{2+}-free Locke's solution, in the presence or absence of ethylene glycol tetra-acetic acid (EGTA). Choline chloride replaced sodium chloride in the medium, in order to prevent cell swelling caused by simultaneous removal of Ca^{2+} and Mg^{2+}. After cell lysis, TGase activity was measured by ELISA, using fluorescein cadaverine as substrate and fluorescein isothiocyanate (FITC) antibody. Values are means ± SEM of triplicate measurements for three different experiments

et al., 2002). Our findings may be relevant considering that, in different cell systems, the up-regulation of TGase 2 was considered a differential modulator of apoptosis in a stimuli-dependent manner (Tucholsky and Johnson, 2002).

It has also been reported that cerebellar granule cells, maintained under depolarizing conditions (KCl 25 mM), were susceptible to NMDA-induced neurodegeneration (Cox et al., 1990; Xia et al., 1995). The observation that increased levels of TGase activity are dependent on NMDA-induced Ca^{2+} influx demonstrated the role of NMDA receptor in these effects, as confirmed by the inhibition with MK-801 (Table 1).

In recent years, several findings have demonstrated that excitotoxic cell damage is involved in astrocyte activation *in vivo* and *in vitro* (Condorelli et al., 1999; Ahlemeyer et al., 2002). Indeed, primary cultures of astrocytes respond to excitotoxic stimulus by different transduction mechanisms (Matute et al., 2002), including influx of Ca^{2+} (Charles et al., 1991) as well as increased expression of immediate early genes (McNaughton and Hunt, 1992; Condorelli et al., 1993). In this context, our results demonstrated that, in cultured astrocytes, TGase was increased by 100 μM glutamate exposure (24 h). The highest levels of enzyme activity were reached in late differentiated cells (14 DIV). The different effects of glutamate treatment on TGase activation, during cell differentiation, can be explained considering that the enzyme is involved in the formation of focal adhesion and cytoskeleton stabilisation (Perry et al., 1995; Chowdhury et al., 1997), critical processes during nervous cell development. Indeed, confocal laser scanning microscopy analysis showed that TGase substrates were localised in both cytosolic and membrane regions. Further, the influence of physiological potentiation of activated glutamate receptors, in relation to cell differentiation stage, should also be considered. Glutamate-increased TGase activity was in part reduced by GYKI 52466, the inhibitor of AMPA/KA receptors, demonstrating a receptor-mediated effect. This is consistent with the presence of ligand-gated channel family of glutamate receptors in primary cultures of astrocytes (Condorelli et al., 1999). It is well known that also in astroglial cells the prolonged activation of glutamate receptors is associated with increases in intracellular calcium levels (Charles et al., 1991). Under our experimental conditions, excitotoxic stimulus in cultured astrocytes (14–21 DIV) produced an increase in intracellular calcium levels, and an active nuclear translocation of TGase 2 (Campisi et al., 2003). This is relevant, considering a postulated role for TGase 2 in neurodegenerative diseases. In particular, nuclear inclusions in brains of subjects affected by Huntington's disease were associated with high levels of increased TGase activity in different cell compartments. Indeed, cytosolic and intranuclear inclusions, which occur in neurodegeneration, are likely to play a role in the progression of disease. Therefore, TGase 2 may contribute to the formation, growth and/or stabilization of protein aggregates (Gentile et al., 1998; Cooper et al., 1999, 2002), which, in turn, may lead to impairment of energy metabolism, resulting in excitotoxic cell death (Cassarino and Bennett, 1999; Butterfield and Kanski, 2001).

Although further experiments on glutamate-evoked effects in glial cells should be carried out, there is increasing evidence for cell damage in brain regions, executed by intracellular caspases (Tenneti and Lipton, 2000). In particular, astrocyte cell death, occurring during *in vitro* ischemia and AMPA receptor-mediated toxicity, as well as during induced oxidative stress, has been associated with proteolytic activation of caspase-3 (Chen et al., 2001; Yu et al., 2001; Liu et al., 2002). We have recently demonstrated that glutamate-evoked increases in TGase activity and TGase 2 expression paralleled the activation of caspase pathway (Ientile et al., 2003). Notably, increases in caspase-3, following excitotoxic insult, were prevented by AMPA/KA receptor blockage with specific inhibitors. This suggests that caspase-3 activation may also be due to intracellular Ca^{2+} overload, triggered by glutamate receptor stimulation. Furthermore, it has been hypothesized that TGase inhibitors are able to reduce cell damage associated with increases in aggregates formation. In particular, cell death was partially suppressed by cystamine and monodansylcadaverine (Igarashi et al., 1998). Indeed, under our experimental conditions, increases in both TGase and caspase were reduced after

incubation with cystamine (1 mM) prior to glutamate treatment, suggesting that changes in these enzymatic activities may be concomitant events subsequent to excitotoxic injury.

These results demonstrated the direct effects of TGase inhibitors on the activation of pro-apoptotic caspase-3 caused by excitotoxic injury. Therefore, it can be suggested that excessive glutamate leads to astrocyte cell death, an event which, if occurring *in vivo*, could impair brain functions, due to neuronal toxicity resulting from loss of buffering capacity by astrocytes. This is noteworthy, since increases in extracellular glutamate have been clinically related to different neurodegenerative diseases, in which alterations of astroglial cells may contribute to cell damage.

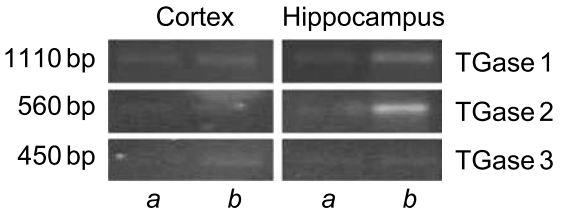

Fig. 3. Differential expression of multiple TGase isoforms in a gerbil model of global cerebral ischemia. RT-PCR analysis of TGase expression was performed at the time of peaking TGase activity, both in sham operated (a) and ischemic gerbil brains (b), with specific primers for TGase 1, TGase 2, TGase 3 and TGase 5. Different mRNA amounts were found for all TGase isoforms, except for TGase 5. A strong up-regulation of TGase 2, the most abundantly expressed, and TGase 1, was observed in ischemic hippocampus compared to cerebral cortex. In contrast, low expression levels were found for TGase 3, and no changes were observed, both in hippocampus and in cerebral cortex, after ischemic insult

TGases in cerebral ischemia

Different experimental models have been used to study biochemical features of brain cell damage following ischemic insults. Numerous events, such as release of excitatory amino acids, ROS production, and intracellular Ca^{2+} overload, are involved in the progression of ischemic neuronal loss, an active process caused by transient or permanent reduction of the cerebral blood flow. In addition, activation of several enzymes may be part of biochemical processes leading to neurodegeneration (Martin et al., 1998; Lipton, 1999).

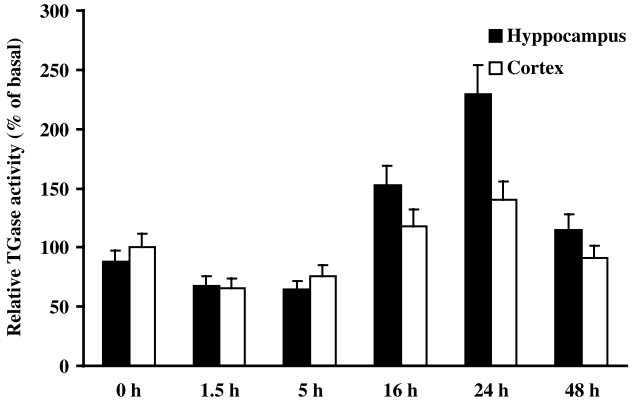

Fig. 2. Changes in TGase activity in a gerbil model of global cerebral ischemia. Following 3 min occlusion, TGase activity was measured in whole homogenates of ischemic gerbil brains by detecting the incorporation of [^{3}H]-putrescine into N, N'-dimethylcasein, throughout 48 h of reperfusion. Compared to sham operated brains (basal activity), an early, strong activation of TGase was observed in hippocampus, with the highest levels of enzyme activity at 24 h of reperfusion, while minor changes were found in cerebral cortex. Results are mean values ± SEM of triplicate measurements from three different experiments

Interestingly, TGase reaction was associated with stress response in brain areas susceptible to ischemic damage. In a model of global cerebral ischemia, we evaluated changes in TGase activity and expression, following a 3 min occlusion. The time course of enzyme activity, throughout 48 h of reperfusion, provided evidence for increased TGase reactions in the early stages of ischemic injury, at least in the hippocampus (Fig. 2). In fact, compared to sham operated brains, TGase resulted strongly activated in ischemic hippocampus homogenates at 24 h of reperfusion, while only minor modifications in enzyme activity were observed in cerebral cortex. Further, we firstly provided robust evidence for the presence of different TGase isoforms in ischemic brain regions (Fig. 3). Among several known TGases, TGase 2 was the most abundantly expressed, and was four-fold increased in ischemic hippocampus, at 24 h following reperfusion. In contrast, no significant variations were observed in the cortex, both in sham-operated and ischemic brains. Likewise, TGase 1 was two-fold increased in ischemic hippocampus, while no significant differences were found in ischemic cortex. Such differences in expression may depend on a more direct responsiveness of TGase 2 vs TGase 1 transcription regulatory elements to increasing Ca^{2+} concentrations (Polakowska et al., 1999). Indeed, the activation of Ca^{2+} channels is sharply increased in hippocampus during and after ischemic events (Lipton, 1999). Low mRNA levels were found for TGase 3, and particularly in hippocampus of both ischemic and sham-operated gerbil brains (Fig. 3). This was probably due to partially overlapping expression patterns of TGase 2 and TGase 3. Similarly, undetectable TGase 5 mRNA levels could be consequent to a total overlap, usually occurring in different cell types, of expression patterns of TGase 2 and TGase 5 (Grènard et al., 2001).

However, different enzyme activities are susceptible to changes in cell signalling, following the ischemia/reperfusion period. In particular, the increases in TGase activity, following ischemic injury, were due to up-regulation of TGase 2 and TGase 1, and, most likely, to a calpain-mediated processing of TGase 1 into the high specific activity form, significantly contributing to total enzyme activity. In fact, calpain, a calcium-dependent protease, plays a pivotal role in the hippocampal damage induced by excitatory amino acids (Paschen et al., 1990). The observed region-specific changes in TGases in infarction were probably dependent on different susceptibility to ischemia of examined brain areas. In fact, it is well known that hippocampus is more severely damaged by ischemic insult, while brain cortex is only moderately affected (Lipton, 1999). However, it has been reported that the expression of proteins, promoting cell survival or delayed cell death in models of cerebral ischemia, is often altered in cell type-specific patterns.

Conclusions

Some evidences demonstrate increases in TGases in some neurodegenerative diseases. Although it has been emphasized that TGase 2 (tissue TGase) may be linked to molecular mechanisms of programmed cell death, in different models of cell damage, recent findings sustained the possibility that the enzyme is not operating during the apoptotic process associated with neuronal death. However, direct and indirect observations suggest that TGase-dependent protein cross-linking may be part of the molecular mechanisms involved in neurological diseases.

Increased activation of glutamate receptors, by high levels of glutamate in synaptic spaces, is a common event to several neuropathological conditions. Interestingly, we observed an early up-regulation of TGase 2 in our experimental *in vitro* models of glutamate-evoked excitotoxicity, as well as *in vivo* models of cerebral ischemia. In these cases, rises in TGase 2 expression after excitotoxic insult were dependent on direct responsiveness of TGase 2 transcription regulatory elements to increasing Ca^{2+} concentrations. These findings indicate that TGase 2 plays an important role in biochemical pathways associated with response to cell injury. However, TGase involvement in physiological cell death has led to the conclusion that, while in some cell types enzyme activation is lethal, in others its role is not directly related to the act of killing. In fact, in certain conditions, TGase induction is an important and beneficial part of the death process, and should be facilitated to avoid leakage of macromolecules and inflammation, even in those cases when the enzyme is originally not part of the killing pathway.

Indeed, further studies are needed to better understand the role of different TGase isoforms in the brain response to excitotoxicity, and to elucidate the possible involvement of early genes in the regulation of TGases expression. Moreover, the characterization of TGase substrates in neuronal cells, and other observations on TGase reaction, as a biochemical marker in pathological conditions, should be addressed towards this area of neurobiology.

References

Ahlemeyer B, Kölker S, Zhu Y, Hoffmann GF, Krieglstein J (2002) Increase in glutamate-induced neurotoxicity by activated astrocytes involves stimulation of protein kinase C. J Neurochem 82: 504–515

Bonelli RM, Aschoff A, Niederwieser G, Heuberger C, Jirikowski G (2002) Cerebrospinal fluid tissue transglutaminase as a biochemical marker for Alzheimer's disease. Neurobiol Dis 11: 106–110

Butterfield DA, Kanski J (2001) Brain protein oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins. Mech Aging Dev 122: 945–962

Campisi A, Caccamo D, Raciti G, Cannavò G, Macaione V, Currò M, Macaione S, Vanella A, Ientile R (2003) Glutamate-induced increases in transglutaminase activity in primary cultures of astroglial cells. Brain Res 978: 24–30

Carson DA, Ribeiro JM (1993) Apoptosis and disease. Lancet 341: 1251–1254

Cassarino DS, Bennett JP Jr (1999) An evaluation of the role of mitochondria in neurodegenerative diseases: mitochondrial mutations and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration. Brain Res Rev 29: 1–25

Charles AC, Merrill JE, Dirksen ER, Sanderson MJ (1991) Intercellular signalling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. J Neurosci 6: 983–992

Chen SH, Liu SH, Liang YC, Lin JK, Lin-Shiau SY (2001) Oxidative stress and c-Jun-amino-terminal kinase activation involved in apoptosis of primary astrocytes induced by disulfiram-Cu(2+) complex. Eur J Pharmacol 414: 177–188

Choi DW (1992) Excitotoxic cell death. J Neurobiol 23: 1261–1276

Chowdhury ZA, Barsigian C, Chalupowicz GD, Bach TL, Garcia-Manero G, Martinez J (1997) Colocalization of tissue transglutaminase and stress fibers in human vascular smooth muscle cells and human umbilical vein endothelial cells. Exp Cell Res 231: 38–49

Citron BA, Suo Z, SantaCruz K, Davies PJA, Qin F, Festoff BW (2002) Protein cross-linking, tissue transglutaminase, alternative splicing and neurodegeneration. Neurochem Int 40: 69–78

Condorelli DF, Dell'Albani P, Amico C, Kaczmarek L, Nicoletti F, Lukasiuk K, Stella AM (1993) Induction of primary response genes by excitatory amino acid receptor agonists in primary astroglial cultures. J Neurochem 60: 877–885

Condorelli DF, Conti F, Gallo V, Kirchhoff F, Seifert G, Steinhauser C, Verkhratsky A, Yuan X (1999) Expression and functional analysis of glutamate receptors in glial cells. Adv Exp Med Biol 468: 49–67

Cooper AJL, Sheu KF, Burke JR, Strittmatter WJ, Gentile V, Peluso G, Blass JP (1999) Pathogenesis of inclusion bodies in $(CAG)_n/Q_n$-expansion diseases with special reference to the role of tissue transglutaminase and to selective vulnerability. J Neurochem 72: 889–899

Cooper AJL, Jeitner TM, Gentile V, Blass JP (2002) Cross-linking of polyglutamine domains catalyzed by tissue transglutaminase is

greatly favored with pathological-length repeats: does transglutaminase activity play a role in $(CAG)_n/Q_n$-expansion diseases? Neurochem Int 40: 53–67

Cox JA, Felder CC, Henneberry RC (1990) Differential expression of excitatory amino acid receptor subtypes in cultured cerebellar neurons. Neuron 4: 941–947

De Laurenzi V, Melino G (2001) Gene disruption of tissue transglutaminase. Mol Cell Biol 21: 148–155

Doble A (1999) The role of excitotoxicity in neurodegenerative disease: implications for therapy. Pharmacol Ther 81: 163–221

Fesus L (1998) Transglutaminase-catalyzed protein cross-linking in the molecular program of apoptosis and its relationship to neuronal processes. Cell Mol Neurobiol 18: 683–694

Gentile V, Sepe C, Calvani M, Melone MA, Cotrufo R, Cooper AJ, Blass JP, Peluso G (1998) Tissue transglutaminase-catalyzed formation of high-molecular-weight aggregates *in vitro* is favoured with long polyglutamine domains: a possible mechanism contributing to CAG-triplet diseases. Arch Biochem Biophys 352: 314–321

Grènard P, Bates MK, Aeschlimann D (2001) Evolution of transglutaminase genes: identification of a transglutaminase gene cluster on human chromosome 15q15. J Biol Chem 276: 33066–33078

Griffin M, Casadio R, Bergamini CM (2002) Transglutaminases: Nature's biological glues. Biochem J 368: 377–396

Griffiths R, Ritchie L, Lidwell K, Grieve A, Malcolm CS, Frandsen A, Scott M, Meredith C (1998) Calcium influx via L-type voltage-gated channels mediates the delayed, elevated increases in steady-state *c-fos* mRNA levels in cerebellar granule cells exposed to excitotoxic levels of glutamate. J Neurosci Res 52: 641–652

Ientile R, Caccamo D, Macaione V, Torre V, Macaione S (2002) NMDA-evoked excitotoxicity increases tissue transglutaminase in cerebellar granule cells. Neuroscience 115: 723–729

Ientile R, Campisi A, Raciti G, Caccamo D, Currò M, Cannavò G, Li Volti G, Macaione S, Vanella A (2003) Cystamine inhibits transglutaminase and caspase-3 cleavage in glutamate-exposed astroglial cells. J Neurosci Res 74: 52–59

Igarashi S, Koide R, Shimohata T, Yamada M, Hayashi Y, Takano H, Date H, Oyake M, Sato T, Sato A, Egawa S, Ikeuchi T, Tanaka H, Nakano R, Tanaka K, Hozumi I, Inuzuka T, Takahashi H, Tsuji S (1998) Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. Nat Genet 18: 111–117

Jeitner TM, Bogdanov MB, Matson WR, Daikhin Y, Yudkoff M, Folk JE, Steinman L, Browne SE, Beal MF, Blass JP, Cooper AJ (2001) N(epsilon)-(gamma-L-glutamyl)-L-lysine (GGEL) is increased in cerebrospinal fluid of patients with Huntington's disease. J Neurochem 79: 1109–1112

Lai TS, Tucker T, Burke JR, Strittmatter WJ, Greenberg CS (2004) Effect of tissue transglutaminase on the solubility of proteins containing expanded polyglutamine repeats. J Neurochem 88: 1253–1260

Lewen A, Matz P, Chan PH (2000) Free radical pathways in CNS injury. J Neurotrauma 17: 871–890

Lipton P (1999) Ischemic cell death in brain neurons. Physiol Rev 79: 1431–1536

Liu HN, Giasson BI, Mushynski WE, Almazan G (2002) AMPA receptor-mediated toxicity in oligodendrocyte progenitors involves free radical generation and activation of JNK, calpain and caspase 3. J Neurochem 82: 398–409

Lok J, Martin LJ (2002) Rapid subcellular redistribution of Bax precedes caspase-3 and endonuclease activation during excitotoxic neuronal apoptosis in rat brain. J Neurotrauma 19: 815–828

Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, Portera-Cailliau C (1998) Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: A perspective on the contributions of apoptosis and necrosis. Brain Res Bull 46: 281–309

Matute C, Alberdi E, Ibarretxe G, Sanchez-Gomez MV (2002) Excitotoxicity in glial cells. Eur J Pharmacol 447: 239–246

McNaughton LA, Hunt SP (1992) Regulation of gene expression in astrocytes by excitatory amino acids. Brain Res Mol Brain Res 16: 261–266

Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 130: 1007–1015

Melino G, Piacentini M (1998) Tissue transglutaminase in cell death: a downstream or a multifunctional upstream effector? FEBS Lett 430: 59–63

Nanda N, Iismaa SE, Owens WA, Husain A, Mackay F, Graham RM (2001) Targeted inactivation of Gh/tissue transglutaminase II. J Biol Chem 276: 20673–20678

Nicholas B, Smethurst P, Verderio E, Jones R, Griffin M (2003) Cross-linking of cellular proteins by tissue transglutaminase during necrotic cell death: a mechanism for maintaining tissue integrity. Biochem J 371: 413–422

Olney JW (1986) Inciting excitotoxic cytocide among central neurons. Adv Exp Med Biol 203: 631–645

Paschen W, Röhn G, Schmidt-Kastner R (1990) Transglutaminase activity in reversible cerebral ischemia in the rat. Neurosci Lett 110: 232–236

Perry MJ, Mahoney SA, Haynes LW (1995) Transglutaminase C in cerebellar granule neurons: regulation and localization of substrate cross-linking. Neuroscience 65: 1063–1076

Piacentini M, Davies PJA, Fesus L (1994) Tissue transglutaminase in cells undergoing apoptosis. In: Tomei LD, Cope FO (eds) Apoptosis II: The molecular basis of apoptosis in disease. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 143–163

Piacentini M, Farrace MG, Piredda L, Matarrese P, Ciccosanti F, Falasco L, Rodolfo C, Giammarioli AM, Verderio E, Griffin M, Malorni W (2002) Transglutaminase overexpression sensitizes neuronal cell lines to apoptosis by increasing mitochondrial membrane potential and cellular oxidative stress. J Neurochem 81: 1062–1072

Polakowska RR, Graf BA, Falciano V, LaCelle P (1999) Transcription regulatory elements of the first intron control human transglutaminase type I gene expression in epidermal keratinocytes. J Cell Biochem 73: 355–369

Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. Science 298: 789–791

Snider BJ, Du C, Wei L, Choi DW (2001) Cycloheximide reduces infarct volume when administered up to 6 h after mild focal ischemia in rats. Brain Res 917: 147–157

Tenneti L, Lipton SA (2000) Involvement of activated caspase-3-like proteases in N-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. J Neurochem 74: 134–142

Tucholski J, Johnson GVW (2002) Tissue transglutaminase differentially modulates apoptosis in a stimuli-dependent manner. J Neurochem 81: 780–791

Wiessner C, Sauer D, Alaimo D, Allegrini PR (2000) Protective effect of a caspase inhibitor in models for cerebral ischemia *in vitro* and *in vivo*. Cell Mol Biol 46: 53–62

Xia Y, Ragan RE, Seah EE, Michaelis ML, Michaelis EK (1995) Developmental expression of N-methyl-D-aspartate (NMDA)-induced neurotoxicity, NMDA receptor function, and the NMDA-R1 and glutamate-binding protein subunits in cerebellar granule cells in primary cultures. Neurochem Res 20: 617–629

Yu AC, Wong HK, Yung HW, Lau LT (2001) Ischemia-induced apoptosis in primary cultures of astrocytes. Glia 35: 121–130

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