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Involvement of Calpain Activation in Neurodegenerative Processes

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ABSTRACT

One of the challenges in the coming years will be to better understand the mechanisms of neuronal cell death with the objective of developing adequate drugs for the treatment of neurodegenerative disorders. Caspases and calpains are among the best-characterized cysteine proteases activated in brain disorders. Likewise, during the last decade, extensive research revealed that the deregulation of calpains activity is a key cytotoxic event in a variety of neurodegenerative disorders. Moreover, interest in the role of calpain in neurodegenerative processes is growing due to implication of the involvement of cdk5 in neurodegenerative diseases. Since calpain inhibitors appear to not only protect brain tissue from ischemia, but also to prevent neurotoxicity caused by such neurotoxins as β-amyloid or 3-nitropropionic acid, the currently available data suggest that calpain and cdk5 play a key role in neuronal cell death. It seems clear that the inappropriate activation of cysteine proteases occurs not only during neuronal cell death, but may also contribute to brain pathology in ischemia and traumatic brain disorders. Pharmacological modulation of calpain activation may, therefore, be useful in the treatment of neurodegenerative disorders. It is possible, although difficult, to develop synthetic inhibitors of cysteine proteases, specifically calpains. The inhibition of calpain activation has recently emerged as a potential therapeutic target for the treatment of neurodegenerative diseases.

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INTRODUCTION

It is well known that calcium ions are intracellular messengers involved in the regulation of important physiological functions, including synaptic activity, membrane excitability, exocytosis, and enzyme activation (32,80). Although neurons possess mechanisms to regulate intracellular calcium levels, a deregulation or dramatic increase in the cytoplasmatic levels of calcium is the first indicator of neuronal cell death (4,49,56,63). Thus, calcium overload could significantly contribute to neuronal injury through activation of several enzymes such as calpains and phospholipase A_2, as well as mitochondrial alterations (3,5,32,33,46). It is widely known that mitochondria have been implicated in neuronal cell death through the release of key proteins such as cytochrome c (Cyt c), the second mitochondria derived activator of caspase (Smac, also known as DIABLO), and apoptosis-inducing factor (AIF) (15,17,25,33). Mitochondria regulate the apoptotic process by a caspase-dependent (via activation of caspase-3) or caspase-independent process. Furthermore, mitochondria could generate an increase in free radical production, which causes DNA damage. In addition, mitochondria can also orchestrate the apoptotic process through the activation of DNA reparatory enzymes such as poly-ADP-ribose polymerase (PARP). Indeed, recent data have demonstrated that the activation of PARP is involved in AIF release. This pathway could constitute a cyclic process involved in neuronal cell death (35,55,61). Apart from mitochondrial activation, recent studies revealed a prominent contribution of cysteine proteases, namely caspases, calpains and cathepsins in neuronal cell death. While, the role of the caspase family in neuronal cell death is probably well characterized, the role of calpains and cathepsins in apoptosis is still unclear. Likewise, during the last decade, extensive research has pointed to the deregulation of calpain activity as a key cytotoxic event in a variety of neurodegenerative disorders (7,8,19). Thus, the aim of the present review is to discuss the contribution of calpains to the process of neuronal cell death.

CALPAINS

Structure and Activation

Calpains were originally identified as calcium-activated proteolytic enzymes located in the cytoplasm of cells (9,36,48,56). As stated in the introduction, these cysteine proteases are activated by calcium. Although up to 15 types of calpains have been identified, two isoforms, namely μ-calpain (calpain I) and m-calpain (calpain II), are widely distributed in mammalian tissues (48,80,82). Calpain is localized in the cytoplasm as an inactive proenzyme and is activated in the presence of high levels of calcium. The difference between the two calpains resides in the calcium levels required to activate them, 2 – 80 μM for calpain I and 0.2 – 0.8 mM for calpain II. In regards to their structure, both calpains are heterodimers of a specific catalytic subunit of about 80 kDa and a common smaller regulatory subunit of 28 kDa. These small subunits are identical within the same tissue and/or animal species, while the large subunits are similar but not identical. The large subunits are divided into four domains: domain I is the site of autolytic cleavage; while domain II contains the interaction site with substrates having the cysteine protease activity; the function of domain III is not known; domain IV features the binding sites for calcium (48).

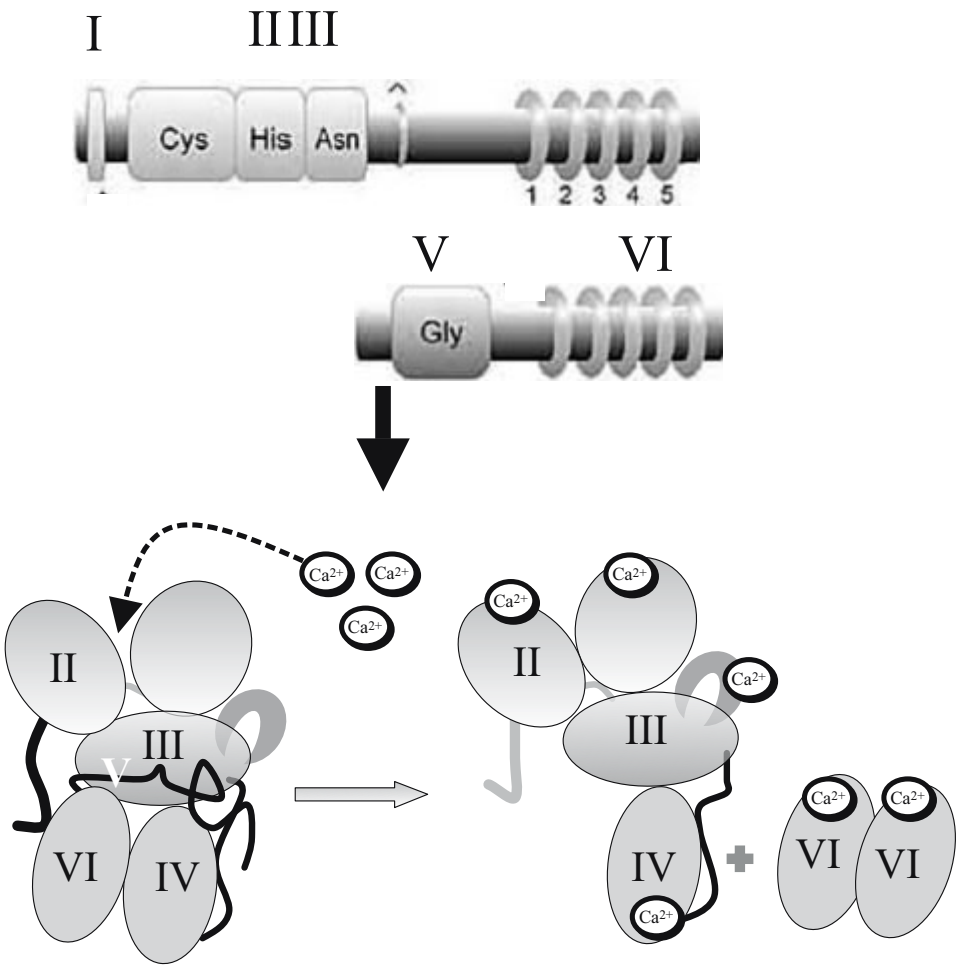

Fig. 1. Schematic representation of the domain architecture of classical calpains. The 80 kDa large subunit contains four domains, plus a short linker that might be important for transducing conformational changes throughout the molecule upon calcium binding.

The smaller regulatory subunit of 28 kDa has two domains, V and VI. The Ca^{2+}-binding region in this small subunit is in domain VI; it is similar to that in the large subunit (82) (Fig. 1).

On the other hand, when calpain is activated it is able to interact with a large number of substrates including cytoskeletal proteins, growth factor receptors, mitochondria, actin-binding proteins (spectrin, actinin, gelsolin, gephyrin, ankyrin, talin) (82), tubulin, microtubules-associated proteins (MAP2, tau), and neurofilaments. These calpain activities are modulated also by interactions with a specific endogenous protein inhibitor of calpains, calpastatin (56). Likewise, of interest is the role played by calpastatin, an endogenous inhibitor of calpain activation. Thus, μ- and m-calpain activity is regulated by calpastatin. Although the latter is the physiological inhibitor of calpains, it has not been proven useful in the treatment for neurodegenerative disorders (56).

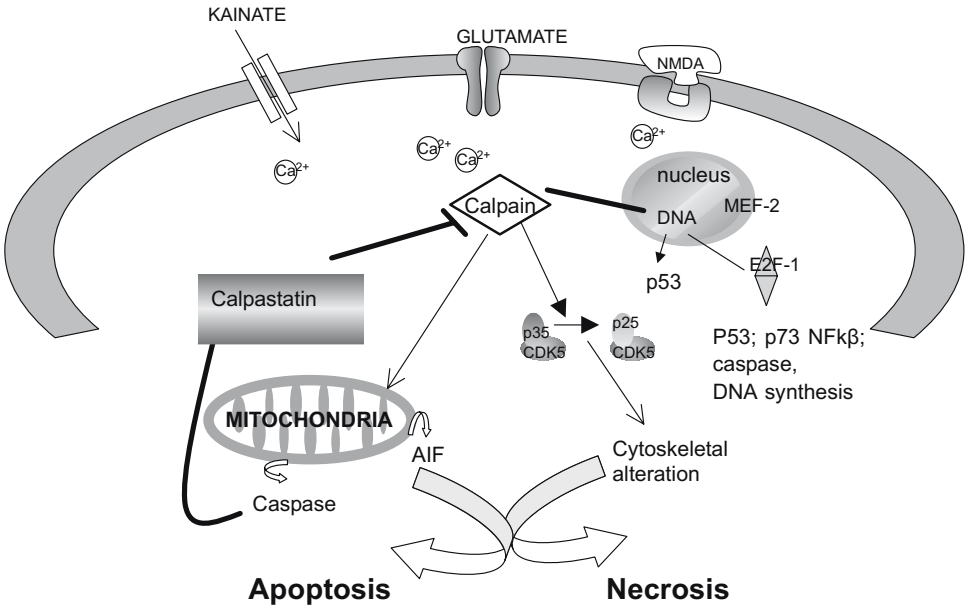

Fig. 2. Some of the mechanism involved in the regulation of calpain activity leading to apoptosis or necrosis.

Role of Calpain Activation in Neuronal Cell Death

Although calpain activation was initially implicated in the necrotic process, recent studies have indicated that these cysteine proteases play a prominent role in the apoptotic process (36) (Fig. 2). This hypothesis is supported by evidence that apoptosis occurs in knockout models of caspase-3, which indicates that, in addition to caspase-3, other cysteine proteases should be implicated in the apoptotic process (39). Furthermore, experimental data suggest that calpain and caspase inhibitors have additive neuroprotective effects (53,57). Increasing evidence suggests cross-talk between the two proteolytic systems. Calpains can cleave pro-caspase 3, thereby leading to the activation of this protease. However, Neumar and colleagues demonstrated that the inhibition of calpain activation by overexpressing calpastatin in human neuroblastoma cells (SH-SY5Y) increased caspase-3 activity and accelerated appearance of apoptotic nuclear morphology (47). Caspases are also involved in calpastatin degradation and thereby they facilitate activation of calpains. These findings are in agreement with excitotoxic studies, where in the presence of calpain inhibitors, NMDA-induced apoptosis has been found to be mediated by activation of the intrinsic apoptotic pathway, specifically of caspase-3 (42). Likewise, using ultraviolet radiation as an apoptotic stimulus in cortical neurons, McCollum and co-workers demonstrated that calpain activity is necessary for caspase-3 activation, although only calpain is important in neuronal cell death (40). Indeed, all these data indicate that the two-cysteine proteases are involved in neuronal cell death. Furthermore, it appears that an autoregulatory mechanism exists between the two systems (64). Thus, the inhibition or activation of one of them could modulate the other system. In a recent attempt to understand the cross-talk occurring between these two systems during excitotoxicity, human calpastatin was generated from mice which were overexpressing the physiological calpain inhibitor

(70). The main conclusion of this study is that overexpression of calpastatin offers partial neuroprotection against excitotoxicity, and, therefore, modulation of the balance between calpains and calpastatin activities offers a potential therapeutic target for the treatment of neurodegenerative pathologies. Other recent advances in elucidating the role calpains play in apoptosis identified AIF as a novel calpain substrate (55). These studies suggest an interaction between calpains and mitochondria, and indicate that calpain activation regulates the release of proteins from the mitochondrial inner membrane to the cytosol. These data suggest that these cysteine proteases play a novel role in the regulation of caspase-independent apoptosis. Indeed, calpain is implicated in the degradation of apoptotic proteins such as Bid (35,51). Bid plays a key role in inducing oligomerization of the proapoptotic Bcl-2 family members Bak and/or Bax, which in turn leads to Cyt c release. Cleavage of Bid to tBid is important in the apoptosis induced by many neurotoxins. Moreover, it has been reported that calpain inhibitors such as calpeptin and PD150606 [3-(4-iodophenyl)-2-mercapto(Z)-2-propenoic acid] both blocked Bid cleavage (35,51).

In addition to the connection between the two cysteine proteases and the mitochondria link, recent reports suggest that calpains could be activated by DNA damage. Using camptothecin, a topoisomerase 1 inhibitor that damages DNA, Sedarous et al. demonstrated that calpains modulate the activation of p53 mediated by this compound (61). Indeed, calpain inhibitors reduce the activation of p53, suggesting that calpains are modulators of apoptosis stemming from DNA damage upstream of p53. Likewise, recent findings suggest that the transcription factor, E2F-1, is a key modulator of neuronal apoptosis in several neurodegenerative diseases (69). In fact, the existence of a relationship between E2F-1 and calpain activation has recently been demonstrated. This transcription factor is involved in the regulation of neuronal cell death by activating p53 and p73, decreasing the pro-survival action of the caspase pathway's $NF_{\kappa B}$ activation, and by synthesizing DNA. The most recent apoptotic pathway that was shown to be governed by calpains is the activation of cyclin-dependent kinase 5 (cdk5) by cleaving cdk5/p35 to cdk5/p25 (2,19,32, 38,39) (Fig. 2).

An additional mechanism by which calpains activation contributes to cell loss is, for example, by the cleavage of several essential cytoskeletal proteins of neuronal axons (30) (Fig. 2). Among them, calpain-mediated proteolysis of α-spectrin has been observed during retinal cell death induced by hypoxia. Indeed, calpain is involved in the proteolysis of tau during retinal cell death, since SJA6017, a calpain inhibitor [N-(4-fluorophenylsulfonyl)-L-valyl-L-leucine] partially attenuates tau degradation (8). In cerebellar granule neurons, glutamate induces a cleavage of myosin Va by calpains, while calpain inhibitors improve neuronal viability by preventing myosin Va proteolysis (1). Another mechanism by which calpains contribute to cell death is through the cleavage of the NMDA receptor under exocytosis. A recent report provides insight into the molecular mechanisms by which calpains contribute to excitotoxic cell death. During excitotoxicity, the influx of Ca^{2+} through glutamate receptors is followed by a second, delayed, and uncontrolled Ca^{2+} increase leading to neuronal death. Bano et al. showed that calpains cleave the plasma membrane Na^{+}/Ca^{2+} exchanger during brain ischemia in neurons undergoing excitotoxicity (5). The proteolytic inactivation Na^{+}/Ca^{2+} exchanger is responsible for the delayed excitotoxic upregulation of Ca^{2+} and the consequent death of neurons. In this model, the overexpression of calpastatin protects neurons from excitotoxic death by decreasing secondary Ca^{2+} overload (5).

CALPAINS IN NEURODEGENERATIVE DISORDERS

At present, calpains are widely implicated in a broad range of pathologies such as Alzheimer's, Parkinson's, and Huntington's diseases, cerebral ischemia, excitotoxicity, traumatic brain injury, and cataracts. The mechanism by which calpains regulate or modulate neuronal cell death is not yet well understood, although the main pathways regulated by calpains in neurodegenerative diseases are known and are discussed below.

Alzheimer's Disease

Several etiologic factors or molecular events are known to contribute to neuronal cell death in Alzheimer's disease (AD). Among them, intracellular calcium and oxidative stress are probably the most important factors that initiate the intracellular process of cell death (68). Studies performed in neuronal cell cultures of laboratory animals and in brain samples of AD patients suggest that calpains play a prominent role in the activation of AD pathology (7,37,49). Initial immunohistochemical studies of AD brains demonstrated accumulation of calpains immunoreactivity in neuritic plaques and neurofibrillary tangles (49,59). Recent studies suggest that in AD calpains activate neuronal cdk5 and mitogen-activated protein kinase (MAPK) pathway. They are also involved in the phosphorylation of tau, which in turn causes neuronal death. Furthermore, in AD brain calpain I activation may play a role in neurodegeneration through the breakdown and activation of calcineurin. In neuronal cell preparations, β-amyloid is widely used as a neurotoxin to study the potential pathways involved in neurodegenerative disorders (2). β-Amyloid in cortical neurons activates calpains, thereby mediating the cleavage of PARP, an enzyme that is thought to contribute to the pathophysiology of AD by inducing neuronal apoptosis (7,71). The involvement of calpains in β-amyloid toxicity is supported by the fact that cell-permeable calpain inhibitors such as MDL 28170 have neuroprotective properties (32). Calpain also regulates the cleavage of the cdk5 activator p35 (19,52). Cdk5 is a serine/threonine protein kinase, although it is not activated by cyclins. Instead, cdk5 activity requires association with one of the two brain-specific regulatory subunits called p35 and p39. Unlike other cyclin-dependent kinases, cdk5 is not involved in cell cycle regulation and is localized in the central nervous system (24,27,28,32). Using an antisense strategy, Alvarez et al. were first to describe the role of cdk5 in an experimental model of AD (2). They also found that in rat hippocampal cell cultures a cdk5 inhibitor significantly attenuates neurotoxicity of β-amyloid (2). How does abnormal cdk5 activity contribute to Alzheimer's disease? An obvious substrate for hyperphosphorylation is tau. Hyperphosphorylated tau forms the paired helical filaments found in neurofibrillary tangles (NFT) which, along with amyloid plaques, are a major hallmark of AD. Tau becomes hyperphosphorylated in p25-overexpressing transgenic mice which exhibit AD lesions (29,38). Overexpression of p25 causes tau hyperphosphorylation and triggers neuritic dystrophy in cultured neurons (84). An association between calpain activation and increased levels of p25 and NFT formation has been demonstrated in AD brain, but not in normal brain tissue (39). Moreover, p25 can be induced by Aβ peptide treatment of primary culture neurons, indicating that p35 to p25 cleavage may be a downstream event of β-amyloid toxicity mediated by calpain activation (19,24,27,71). Therefore, not only is p25 neurotoxic, but its introduction into neurons leads to hyperphosphorylation of tau, disruption of the cytoskeleton, and neuronal cell death. Thus, synthetic calpain inhibitors could exert neuroprotect-

ive effects by inhibiting p25 formation. In this way, hyperactivation of cdk5 not only contributes to tau hyperphosphorylation and cytoskeletal collapse, but also to neuronal loss. Since cdk5 has important physiological functions, another strategy involves CIP, a cdk5 inhibitory peptide. In $A\beta_{1-42}$–treated neurons CIP specifically inhibits the activated p25/cdk5 complex and consequently the neuronal apoptosis (84). These findings suggest a new therapeutic AD strategy, prevention of abnormal phosphorylation of neuronal cytoskeletal proteins by p25/cdk5. Therefore, as discussed above, cdk5 inhibition could constitute a potential target for potential anti-Alzheimer drugs. Another potential target is the myocyte enhancer factor 2 (MEF-2). MEF-2 is localized in the nuclei and promotes neuronal cell death (19,24,67).

Still another strategy for the development of new drugs to treat AD could be the overexpression of calpastatin, which might prevent tau phosphorylation (70). The downregulation of calpain activity should attenuate degradation as well as phosphorylation of tau. The phosphorylation of tau substantially reduces the affinity of tau for microtubules, resulting in the instability of microtubules and increasing microtubule-unbound tau, which is prone to self-aggregation. Accordingly, calpain inhibition may be helpful in protecting neurons against disruption of the neuritic cytoskeleton and formation of abnormal tau fibrils.

Role of Calpains in Parkinson's Disease

Parkinson's disease (PD) is a progressive disease with a mean age at onset of 55, the incidence markedly increasing with age (39). Clinically this disease is characterized by a loss of nigrostriatal dopaminergic neurons and by the presence of intraneuronal protein cytoplasmic inclusions, termed Lewy Bodies (13,43,45). Following the discovery that several neurotoxins such as MPTP and rotenone could be used as models of PD, enormous efforts have been made to understand the potential pathways involved in neuronal cell death (16,18). To this end, the main target of these neurotoxins has been shown to be the mitochondrial complex I. The inhibition of this complex led to free radical production as well as a mitochondrial alteration that induced the release of proapoptotic proteins (e.g., Cyt c and AIF) from the mitochondria and the activation of the caspase route (25,33,35). Although it has been postulated that neuronal cell death mediated by Parkinsonian neurotoxins could be prevented by caspase inhibitors, increasing evidence implicates other cysteine proteases such as calpains (16,18). Previous studies demonstrated an increase in the expression of m-calpain in the substantia nigra and locus coeruleus of patients with PD. Likewise, experimental studies demonstrated that calpain inhibitor MDL-28170 significantly attenuated the loss of nigral dopamine neurons in rats teated with MPTP (67). These data indicate that calpain activation is involved in the process of neuronal cell death in experimental PD. Moreover, studies performed in neuronal cell cultures using Parkinsonian neurotoxins supported this hypothesis (33). Furthermore, the inhibition of mitochondrial complex I by MPP^+ and rotenone activated calpain and seems to constitute an early event in the neurodegenerative process prior to caspase activation (16).

The intial hypothesis postulated that calpain contributes to PD by activating the intrinsic apoptotic pathway. As mentioned above, calpain is involved in the cleavage of the apoptotic protein Bax. It is well known that translocation of Bax from the cytosol to the mitochondria is a critical event in neuronal apoptosis. Indeed, it has been demonstrated that in cerebellar granule cells MPP^+-induced apoptosis occurs through caspase-3 activation (25). The second potential pathway regulated by calpain activation could be the

caspase-independent pathway (35). Thus, MPP^+ has been shown to induce calpain activation and AIF release in PC12 cells that had been prevented by calpain inhibitors. Collectively, these studies suggest that calpain could modulate caspase-dependent and -independent apoptotic neuronal cell death in PD. The third hypothesis is that DNA damage could be an activator of calpains, with calpain activation modulating activation of additional targets. This hypothesis is essentially in agreement with the evidence that calpains activate phosphorylated p53. Moreover, it is well known that this oncogene is implicated in neuronal apoptosis (61). Calpain inhibitors exert an effective neuroprotective role in the p53-mediated death paradigm. The last hypothesis also suggests the involvement of cdk5 in the pathogenesis of PD (45). Cdk5 has been co-localized with Lewy bodies in brains of patients with PD. Therefore, it has been hypothesized that cdk5 might be a kinase involved in the phosphorylation of Lewy body components. In rats MPTP induces a dramatic increase in p25 expression in the substantia nigra, a process that is calpain-dependent (66). Moreover, mice deficient in p35 dopaminergic neurons are resistant to MPTP-induced cell death and flavopiridol ameliorates the neurotoxic effects of this neurotoxin (66,67). Likewise, calpain-mediated regulation of cdk5/p35 increases phosphorylation activity, and consequently the inactivation of the pro-survival factor, myocyte enhancer factor 2. These studies implicate the calpain/cdk5/MEF2 pathway in dopaminergic neuron loss associated with PD (67). Thus, calpain inhibition could be a suitable strategy for the treatment of PD.

Role of Calpains in Huntington's Disease

Huntington's disease (HD) is a neurodegenerative disorder caused by a trinucleotide CAG expansion, resulting in elongation of the polyglutamine tract at the N terminus of huntingtin (Htt), which is a mutation in this protein (23). Abnormal proteolytic processing of mutant Htt has been implicated as a critical step in the initiation of HD. In an HD mouse model expressing full-length expanded human Htt, resting Ca^{2+} levels increased in CA1 pyramidal neurons (82). To further investigate the role of calpains in HD, one study demonstrated increased calpain activity in the brains of human HD tissues, but not in human controls samples. On the other hand, the excitatory amino acid glutamate could be implicated in this disease since a transgenic mice expressing full-length mutant Htt showed significantly reduced synaptic vesicular uptake of glutamate (48). Thus, NMDA receptor activation could be an important target in glutamate-induced neuronal cell death through calpain activation (53). Since Ca^{2+} deregulation is altered in HD patients and mouse models of this disease (46,48), several studies have implicated calpains in the apoptotic/necrotic process of neuronal cell death. Furthermore, administration of the mitochondrial neurotoxin 3-nitropropionic acid (3NP) induces a vicious cycle in which mitochondrial defects in HD could amplify the toxic effects of mutated Htt. The mitochondrial toxin 3NP, an irreversible inhibitor of succinate dehydrogenase complex II, constitutes a useful tool for reproducing the disease in laboratory animals and neuronal cell cultures (53). Chronic systemic administration of 3NP to rats and non-human primates produces a blockade of complex II in the brain mitochondria, leading to preferential striatal degeneration associated with behavioral abnormalities that are similar to HD. Furthermore, experimental data suggest that calpain activation plays a role in the striatum of this neurodegenerative model, which is associated with a calpain-dependent cleavage of huntingtin (23). Likewise, calpain inhibition prevents the processing of huntingtin, re-

duces the size of the striatal lesions, and almost completely abolishes 3-NP-induced DNA fragmentation in striatal cells (10,11). These studies suggest that calpain may play an important role in HD pathogenesis and could be a potential therapeutic target for slowing disease progression (81). The inactivation of caspase-9 by calpain may be responsible for inhibition of the regulation of caspase-3 in the 3NP model (11). Studies using neuronal preparations also suggest the importance of calpain activation in 3NP-induced neurotoxicity. It has been postulated that calpain activity precedes caspase-3 activation and contributes to the necrotic process.

Calpain Activation in Other Pathological Conditions

Prion protein (PrP) triggers a rise in intracellular calcium through the release of mitochondrial calcium stores. In addition, the cytosolic localization of PrP may be involved in the pathogenesis of prion disease (26). Thus, calpain activation in tandem with caspase activation leads to apoptosis since a combination of caspase and calpain inhibitors is necessary to inhibit prion protein-mediated apoptosis (50). Furthermore, it seems that calpain may play a role in removing cytosolic prion protein (79). Therefore, calpains could contribute to the neurodegenerative process in prion disease.

Excessive activation of glutamate has been implicated in a large number of neurological disorders, including amyotrophic lateral sclerosis (ALS), spinal cord injury (SCI), and traumatic brain injury (TBI). Furthermore, stimulation of inotropic glutamate receptors causes Ca^{2+} influx with the activation of Ca^{2+}-dependent proteases, including calpains. It is, therefore, reasonable to assume that calpains may be involved in the pathogenesis of brain disorders in which glutamate plays a prominent role.

Calpain activation is also involved in the death of motoneurons and thus could be implicated in ALS (20). Various studies have showed that glutamate is responsible for facilitation of motoneurons death. It has been postulated that non-NMDA receptors, in tandem with intracellular calcium increases and reactive oxygen species (ROS), are implicated in this disease. Thus, exposure of primary rat motoneurons to α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) has been established as an *in vitro* model of excitotoxic motoneuron death (72). Along this line, treatment with leupeptin, a calpain inhibitor, protects motoneurons and improves their function (29). Neuroprotection by calpeptin, an inhibitor of calpain, would indicate an involvement of calpain in neuronal apoptosis, as it occurs in ALS. Therefore, inhibition of calpains may rescue motoneurons from cell death (34,41). Pathological activation of calpains may be responsible for the neuronal pathology associated with TBI (54,56). This is based on studies addressing intracellular Ca^{2+} increases in TBI, which induce calpain activation and subsequent cytoskeletal degradation. Although calpains were rapidly activated in a study involving rats, calpain mRNA expression increased, reaching its highest levels at 72 h after TBI. A calpain inhibitor SJA6017 had a beneficial effect in mice, although spectrin breakdown was not attenuated by this drug in the brain samples. Studies with calpain inhibitors demonstrated their neuroprotective effect suggesting the importance of calpain in TBI (14,30,58).

Cerebral ischemia triggers activation of apoptotic pathways in neurons. In fact, the activation of several cysteine proteases, including calpain, has been implicated in this process (57). Direct inhibition of calpain has been shown to reduce neuronal cell death in ischemic models (6). Calpain inhibitors exert neuroprotective effects in ischemic neuronal models involving laboratory animals. In addition, in neuronal cell cultures they inhibit

hippocampal calpain activity and exert neuroprotective effects (36). Bartus et al. demonstrated that calpain inhibitor, AK295, [Z-Leu-aminobutyric acid-CONH$(CH_2)_3$-morpholine], affords substantial neuroprotection in a rat model of cerebral artery occlusion (6). These data support the assumption that calpain plays a role in brain degeneration associated with cerebral ischemia.

Another interesting point is the role or possible activation of calpains in an aging process (12). Experimental studies demonstrated that the levels of intracellular calcium increase with age (4,48,49). Another plausible explanation regarding calpain activity in aging could stem from decreased calpastatin levels, although more studies are necessary to confirm this hypothesis (48).

In rats methamphetamine-induced neurotoxicity is mediated by calpain activation. Moreover, methamphetamine induces changes in tau in the cortex and hippocampus of rats (76). These findings suggest a potential mechanism involving cytoskeleton disruption, which has been implicated in the neurotoxicity of this commonly abused drug.

Kainic acid is a neurotoxin involved in the stimulation of non-NMDA receptors (72). Experiments performed in hippocampal neurons suggest that calpains are involved in the cell death mechanisms triggered by activation of AMPA/kainate receptors (32). In this neurotoxic model caspases have been shown to be involved in excitotoxic damage caused by glutamate receptor activation, but they do not appear to be involved in the activation of KA receptors (32,42). Slight caspase activation has been demonstrated in this model, although caspase inhibitors offered no neuroprotection (33).

Potential Use of Calpain Inhibitors in Neurodegenerative Disorders

Because calpain activation exerts important upstream regulatory control over the pathway of neuronal cell death in neurodegenerative processes, enormous efforts have been made in recent years to develop new calpain inhibitors as a therapeutic strategy for neurodegenerative diseases. Although these compounds were initially of microbial origin (i.e., leupeptin, which was isolated from actinomycetes) (21), today most compounds are synthetic. Potent peptide-based reversible (aldehyde and α-ketocarbonyl) and irreversible (halomethyl ketone, diazomethyl ketone, epoxysuccinate, and acyloxymethyl ketone) inhibitors of calpain have been reported. Most of them are derived from peptides, in particular dipeptide aldehydes such as Z-Val-Phe-H (MDL 28,170). However, MDL 28,170 did not show a specific selectivity against calpains. This poses a major problem in the quest to develop new calpain inhibitors. One potential clinical application of calpain inhibitors is in the treatment of cataract. In fact, calpain inhibitors such as SJA6017 (a reversible and cell-permeable calpain inhibitor) and E-64-d, an epoxysuccinyl derivative and irreversible, general inhibitor of cysteine proteases [(L-3-trans-ethoxycarbonyloxirane-2-carbonyl)-L-leucine-(3-methylbutyl) amide] have shown anti-cataract efficacy in lens culture models (22,31,44). Transcorneal permeability of SJA6017 has been improved by introduction of cyclic hemiacetal into the chemical structure of SJA6017. The α-mercaptoacrylic acid derivatives PD150606 and PD151746 [3-(5-fluoro-3-indolyl)-2-mercapto-(Z)-2-propenoic acid] are non-peptide, cell-permeable, uncompetitive and selective calpain inhibitors that bind to the Ca^{2+}–binding domains of calpains 1 and 2 with high affinity (74). Numerous studies have demonstrated that PD150606 inhibits calpain activation in neuronal cell preparations, thereby providing neuroprotection. Recently it has been suggested that PD150606 blocks the AMPA receptor in motoneurons (72). Other pep-

tides oxoamide inhibitor molecules, such as AK275 [Z-Leu-aminobutiric acic-$CONH(CH_2)_3OH$] and AK295 [Z-Leu-aminobutyric acid-$CONH(CH_2)_3$-morpholine], have been shown to provide neuroprotection in the central nervous system. AK295 reduced the degree of axonal degeneration in a rodent model of Taxol neuropathy (75). Since this compound improved cognitive deficits following brain trauma, calpains have been implicated in the post-traumatic memory events and neuromotor dysfunction.

CONCLUSIONS

Although treatments for neurodegenerative diseases and brain pathologies remain limited, calpain activation may offer a potential therapeutic target in the neurodegenerative process. The major limitation to the clinical use of calpain inhibitors is their lack of specificity among cysteine proteases and other proteolyitc enzymes. As we suggested in this manuscript, development of selective synthetic calpain inhibitors could constitute a potential strategy in the coming years for the prevention and treatment of brain pathologies. Of interest is also the increase in calpain activity during the aging process. The currently available experimental findings suggest potential importance of calpain inhibitors in the treatment of neurodegenerative disorders and their potential use in the treatment of cataract.

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