Biochemistry (Moscow), Vol. 68, No. 11, 2003, pp. 1171-1180. Translated from Biokhimiya, Vol. 68, No. 11, 2003, pp. 1459-1470.

REVIEW

Non-apoptotic Functions of Caspase-3 in Nervous Tissue

N. V. Gulyaeva

Department of Functional Biochemistry of the Nervous System, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, ul. Butlerova 5a, Moscow 117865, Russia; fax: (7-095) 952-4007; E-mail: nata_gul@pisem.net

Received May 6, 2003

Abstract—Some enzymes that have been recognized as "apoptotic" so far may be involved in important cellular processes not necessarily related to cell death in nervous tissue. The activity of caspase-3, an "apoptotic" enzyme, can be measured in normally functioning neurons. The results reported by several groups point to the possibility that caspases may be involved in nervous tissue function as top enzymes in the regulatory proteolytic cascade. A concept on a new mechanism of synaptic plasticity modulation involving caspase-3 has been formulated postulating a specific role of caspase-3 in normal brain functioning. The idea of synaptic plasticity modulation by caspase-3 is in line with data reported recently. For example, caspase-3 is possibly involved in the long-term potentiation (LTP) phenomenon since proteins that are key players of molecular mechanisms of LTP induction and maintenance are caspase-3 substrates. Experimental results on blocking LTP by a caspase-3 inhibitor confirm this concept.

Key words: caspase, caspase-3, calpain, structural (cytoskeletal) proteins, signal transduction, neuroplasticity, apoptosis, long-term potentiation

1. CASPASES – MORE THAN JUST KILLERS?

Caspases, cysteine-containing proteolytic enzymes that cleave at an aspartate residue, were discovered almost a decade ago and are recognized as essential mediators of apoptotic cell death (see [1-3] for review). Fourteen caspases have been identified so far that play distinct roles in inflammation and apoptosis [4]. Caspases play a role in apoptosis function either as upstream initiators of the proteolytic cascade (caspases-8 and -9), or as downstream effectors (caspases-3, -6, and -7) that cleave cellular proteins. Among the effector caspases, caspase-3 is of particular interest with respect to nervous tissue since it is believed to be closely related to programmed cell death in neurons [5]. Caspase-3 is regarded as an enzyme belonging to the camp of apoptotic caspases that transduce and execute death signals. However, it is becoming clear that certain proteases are not merely degradative enzymes but are highly regulated signaling molecules that control critical biological processes via specific limited proteolysis. The opinion that "caspases are more than just killers" [6] is supported by evidence emerging in recent years implicating the caspases in various non-apoptotic aspects of cellular physiology. Caspases are involved in cytokine processing during inflammation, cell cycle progression, differentiation of progenitor cells during erythropoiesis and of muscle cells, lens fiber development, and proliferation of T lymphocytes, thus attesting to the pleiotropic functions of these proteases [7-9]. In this review we will analyze possible non-apoptotic roles of caspases, in particular caspase-3, in the normal functioning of the nervous system.

2. CASPASE-3 IN THE NERVOUS SYSTEM: BEYOND APOPTOSIS

It seems quite reasonable to suggest that many of the same signals that are involved in neuronal death in both physiological and pathological settings may be also involved in developmental and other forms of neuroplasticity. Indeed, there is growing evidence that in the nervous tissue apoptotic mechanisms are not only involved in apoptosis, but can also regulate synaptic plasticity and growth cone motility [10].

Data reported by Shimonama et al. demonstrating differential expression of rat brain caspase family proteins during development and aging as well as differential subcellular localization of caspase family proteins in the adult rat brain suggest that the expression of caspases is differentially regulated during development and aging and that caspases not only contribute to the regulation of neuronal death, but also to synaptic plasticity [11, 12]. Yan et al. [13] studied expression of active caspase-3 in

0006-2979/03/6811-1171$25.00 ©2003 MAIK "Nauka/Interperiodica"

mitotic and postmitotic cells of the rat forebrain. They demonstrated a wave of active caspase-3-positive cells dividing in the proliferative zones and then migrating to the bulb as they differentiated into neurons. The data suggest that active caspase-3 may play a role in cellular processes such as neuronal differentiation, migration, and plasticity.

In our recent studies caspase-3 activity was shown to specifically reflect different periods of brain maturation [14, 15]. In early postnatal ontogenesis transient decline in CA1 population spike amplitude in the hippocampal slices of rats coincided with a period of caspase-3 activation. There was a clear general trend for caspase-3 activity to decrease before postnatal day 17, while the inverse trend was observed during the next period up to postnatal day 21. Since this period is not related to an increase in apoptosis, these data support the involvement of caspase-3 in synaptic plasticity.

Another set of data supporting a new, non-apoptotic, role of caspase-3 in neurons was provided by Rossiter et al. [16]. Fractin immunoreactivity was demonstrated in Hirano bodies (inclusions in neuronal processes in the hippocampal CA1 region) suggesting that caspase-like cleavage of actin may play a role in their formation. These data give further evidence for the involvement of caspase-like activity in neuronal processes, distinct from that necessarily associated with acute perikaryal apoptosis.

Furthermore, caspase-3 may be involved in mechanisms of ischemic tolerance of the brain. In an *in vivo* model of ischemic tolerance, McLaughlin et al. [17] were surprised to observe widespread caspase-3 cleavage, without cell death, in preconditioned nervous tissue. Using an *in vitro* model of excitotoxic tolerance (cell culture) they demonstrated that caspase inhibitors blocked ischemia-induced protection against N-methyl-D-aspartate (NMDA). These results are the first evidence for a neuroprotective pathway where caspase-3-mediated events "normally" associated with apoptotic cell death are critical for cell survival.

Caspase-3 belongs to the CPP32-like subfamily that shows preference for aspartate at the P4 position and shares the DXXD consensus site. Caspase-3 holds the power to destroy or modify a huge number of proteins in a cell—over 34 thousands human proteins with a caspase-3 cleavage motif have been sequenced. A variety of the most important substrates cleaved by caspases suggest that these enzymes may play a central role in normal neuronal function. The substrates of caspase-3 that is most active of the executioner caspases in the brain include cytoskeletal and associated proteins, kinases and other proteins involved in signal transduction, members of the Bcl-2 family of apoptosis-related proteins, presenilins and amyloid precursor protein, DNA modulating enzymes, and steroid receptors [18]. Many of the caspase-3 substrates are localized in pre- and/or postsynaptic compartments of neurons. We are not surprised that little by little data are emerging which suggest that, in addition to their involvement in neurodegenerative processes, caspases may play important roles in modulating synaptic plasticity in the absence of cell death. This may include structural remodeling and long-term functional changes.

It has been found that apoptotic biochemical cascades can be activated locally in synaptic terminals and neurites resulting in local functional and morphological alterations. Mattson and Duan [19] were the first to suggest that caspase activation can occur locally in synaptic terminals in response to various stimuli. They proposed that apoptotic cascades function in a continuum in which low levels of activation play roles in adaptive responses to "stressors", whereas higher levels of activation mediate synaptic degeneration and cell death [20]. Apoptotic synaptic cascades that may play roles in neuronal plasticity include activation of caspases that can cleave certain types of ionotropic glutamate-receptor subunits and thereby modify synaptic plasticity. Mattson et al. [19, 20] regard caspases primarily as apoptotic enzymes; this results in a kind of contradiction when the authors are discussing the phenomenon of "synaptic apoptosis" (a term that does not conform to the existing definition of apoptosis, which necessarily includes nuclear events). Indeed, caspases are enzymes functioning both in normal neuron, and in dying neuron, though fulfilling different functions in the two situations.

Mattson's team recently published a series of reports giving additional important evidence for a possible role of caspase-3 in neuroplasticity. They demonstrated direct cleavage of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit GluR1 and suppression of AMPA currents by caspase-3 [19, 21-23]. Caspases may also cleave cytoskeletal protein substrates in growth cones of developing neurons and may thereby regulate neurite outgrowth [10]. These results suggest roles for caspases in the modulation of neuronal excitability in physiological settings.

The above results from the *in vitro* models are very convincing from the perspective of the involvement of caspase-3 in neuroplasticity phenomena in the nervous system. Besides, there are some data of *in vivo* experiments supporting this concept. Dash et al. [24] reported that inhibition of caspase-3 activity in the hippocampus blocked long-term spatial memory. This result falls in the general context suggesting that caspase-mediated cellular event(s) in hippocampal neurons may be critical for long-term spatial memory storage. Recently, we have reported about the seasonal changes of caspase-3 activity in brain regions of ground squirrels [25]. During hibernation, when neuroplastic alterations of neurons take place (e.g., elimination of spines), caspase-3 becomes activated, while its activity decreases during the active period.

3. CASPASE-3 AND NEUROPLASTICITY: POSSIBLE POINTS OF INVOLVEMENT

3.1. Long-term potentiation as a model of neuroplasticity. Though the phenomena of neuroplasticity can be demonstrated *in vivo*, for the elucidation of the molecular mechanisms of neuroplasticity simpler *in vitro* models are being routinely used. Among the variety of different models of neuroplasticity, NMDA receptor-dependent long-term potentiation (LTP) in the CA1 region of the hippocampus is perhaps the most widely studied form of synaptic change. Hippocampal LTP has also become one of the most frequently used models to study cellular mechanisms of learning and memory. Some proteolytic enzymes have been shown to be essential for LTP. Tomimatsu et al. [26] have summarized recent studies on the proteolytic systems that play important roles in LTP. The involvement of neuronal Ca^{2+}-dependent cysteine proteases—calpains, serine proteases such as tissue-type plasminogen activator (tPA), thrombin, and neuropsin, as well as proteases secreted from microglia such as tPA, have been discussed. However, no data on possible involvement of caspases in LTP have been presented.

We recently obtained experimental evidence for the involvement of caspase-3 in the LTP phenomenon. We demonstrated the impairment of LTP by an irreversible and cell permeable specific inhibitor of caspase-3 [27]. Slices from rat hippocampus were incubated with caspase-3 inhibitor Z-DEVD-FMK or with the inactive peptide Z-Phe-Ala-FMK for 30 min. The peptides did not change either input–output curves, or paired-pulse effects at 70 msec interpulse intervals, or amplitudes of population spikes in the CA1 region. However, LTP was significantly suppressed 2.6 h or later after Z-DEVD-FMK treatment compared with the corresponding inactive peptide group. After 3.5 h, LTP was completely blocked. LTP magnitude was dependent on time after Z-DEVD-FMK indicating that caspase-3 substrates or products are involved in the LTP molecular machinery. The results of our study gave the first direct evidence that caspase-3 activity is essential for long-term synaptic plasticity. It is worth mention that caspase-3 inhibitor did not change measures of basal synaptic transmission and short-term plasticity, indicating that caspase-3 is essential for long-term plasticity only.

As mentioned above, potentially there exist over 34 thousands proteins that can serve as caspase-3 substrates. Nevertheless, apoptosis, a process in which caspase-3 is a key player, proceeds in an ordered fashion. We can hypothesize that if caspase-3 is involved in normal functioning of living cells the same should happen—only specific substrates are cleaved at the proper time and in proper cellular compartments. Below we will discuss the involvement in LTP of a few proteins, potential substrates of caspase-3, to speculate about the pathways of caspase-dependent regulation of neuroplasticity and the LTP phenomenon in particular. The candidate substrates are relevant proteins involved in LTP and putative caspase-3-dependent regulators of the LTP machinery.

3.2. Calpain is a protease regulated by caspase-3. Calpains are calcium-dependent cysteine proteinases that are expressed as proenzymes and undergo autocatalytic processing to yield the active forms via a Ca^{2+}-dependent mechanism. The activity of the calpain system of the cell is also regulated by the concentration of its specific inhibitor, calpastatin (see [28] for review). Calpain-mediated proteolysis is believed to be readily driven by synaptic activity and contributes to structural reorganization of synapses [29]. It is now generally considered that calpain activation is necessary for LTP formation in the cleavage of multiple substrates [26]. Indeed, calmodulin antagonists and inhibitors have been demonstrated to block LTP [30, 31]. Lynch and Baudry [32] suggested that tetanic stimulation causes an increase in the postsynaptic Ca^{2+} concentration, which activates calpain. Calpain cleaves fodrin allowing glutamate receptors located deep in the postsynaptic membrane to move to the surface. However, at present several experimental results have been reported that suggest different pathways for calpain participation in the generation of LTP. Several reports have indicated that μ-calpain is involved in limited proteolysis of protein kinase C (PKC) ζ, to yield protein kinase M (PKM), a proteolytic constitutively active fragment of PKC that is active in the absence of calcium, diacylglycerols, or phospholipid cofactors and is necessary for the maintenance of LTP [33, 34]. Calpain directly induces proteolytic cleavage of large N-terminal fragments of all three NR2 subunits and smaller C-terminal fragments of the NR2A subunit of NMDA receptors [35]. Although the basic functional properties of NMDA receptor channels are not influenced by these cleavages, they may regulate localization, modulation by second messenger, or turnover of NMDA receptors. μ-Calpain also degrades the glutamate receptor-interacting protein, an AMPA receptor-targeting and synaptic-stabilizing protein, which is suggested to be involved in the generation of LTP [36].

As mentioned earlier, the endogenous inhibitor, calpastatin, regulates calpain activity. A genetic deficiency in calpastatin in Milan hypertensive strain of rats is associated with enhanced hippocampal LTP [37]. Calpastatin, a substrate of caspase-3, undergoes a caspase-mediated fragmentation once caspase-3 is activated [38-41]. Since calpain has been implicated in LTP and conditions under which calpain activation is increased are associated with a greater degree of synaptic potentiation, proteolysis of calpastatin by caspase-3 may upregulate calpain activity, and thus modulate neuroplasticity, particularly, facilitate LTP.

Clarifying the relative roles of calpains and caspases in synaptic plasticity is further complicated by the growing body of evidence for cross-talk between the two proteolytic systems. For example, calpain-mediated cleavage

of caspase-3 has been reported with varying functional consequences [42]. However, since in the proteolytic cascade regulating LTP caspase-3 is upstream of calpain, we suggest that, modulating calpain activity, caspase-3 may control calpain-mediated mechanisms of signal transduction and structural changes relevant for LTP.

3.3. Modification of neuronal receptors by caspase-3. Caspase-3 modulates functioning of glutamate receptors that are essential for synaptic plasticity. It has been mentioned above that AMPA receptor subunit GluR1 is a substrate for caspase-3 (section 2; [19, 21-23]). Indirectly, activating calpain by cleaving its inhibitor, calpastatin, caspase-3 can modulate NMDA receptors (section 3.3; [35, 36]).

Inositol-1,4,5-trisphosphate (IP3) is an important second messenger for releasing Ca^{2+} from internal stores. The coupling mechanism between endoplasmic reticulum Ca^{2+} stores and plasma membrane store-operated channels is crucial to Ca^{2+} signaling. IP3 receptor (IP3R) works as an IP3 induced Ca^{2+}-release channel, requires IP3 and Ca^{2+} as co-agonist, and is involved in neuronal plasticity [43]. To evaluate the role of the type 1 IP3R (IP3R1) in hippocampal synaptic plasticity, LTP in the CA1 neurons of mice lacking the IP3R1 was studied. In IP3R1-deficient mice, LTP was enhanced while depotentiation and LTP suppression were reduced [44]. These results suggest that, in hippocampal CA1 neurons, IP3R1 is involved in LTP. The facilitation of LTP induction seen in mice lacking IP3R1 indicates that this receptor plays an important role in blocking synaptic potentiation in hippocampal CA1 neurons. The magnitude of tetanus-induced LTP at mossy fiber-CA3 synapses was significantly greater in IP3R1-deficient mice, supporting the suppressive effect IP3R1 on LTP induction [45]. Of all types of IP3R of rat brain studied, only IP3R1 was shown recently to be a potential substrate for caspase-3 [46]. The cleavage of IP3R1 results in a decrease in the channel activity as IP3R1 is digested [47], indicating that caspase-3 can inactivate IP3R1 channel functions and remove suppression of LTP induction by IP3R1.

3.4. Signal transduction proteins are substrates of caspase-3. Activation of the NMDA-receptors, the first step in the induction of LTP, is followed by a chain of biochemical events involving signal transduction processes, with a central role of identified kinases and their pre- and postsynaptic substrates [48, 49]. Substrates of proteases, in particular, of caspase-3, are key components of these signal transduction systems and synaptic proteins involved in the plastic changes. Here we briefly discuss possible consequences of caspase-3 mediated cleavage of some signal transduction proteins involved in LTP phenomenon.

Phospholipases are enzymes that modify membrane phospholipids and participate in signal transduction. Phospholipase A2 (PLA2) is the name for the class of lipolytic enzymes that hydrolyze the acyl group from the sn-2 position of glycerophospholipids, generating free fatty acids and lysophospholipids. The products of the PLA2-catalyzed reaction can potentially act as second messengers themselves, or be further metabolized to eicosanoids, platelet-activating factor, and lysophosphatidic acid. Under physiological conditions, PLA2 may be involved in phospholipid turnover, membrane remodeling, exocytosis, detoxification of phospholipid peroxides, and neurotransmitter release [50]. Several different PLA2 enzymes exist in brain, and, based on their catalytic dependence on Ca^{2+}, they are classified into two subtypes, Ca^{2+}-dependent (cPLA2) and Ca^{2+}-independent (iPLA2). The predominant PLA2 activity in rat hippocampus is iPLA2 (at least an order of magnitude higher than that of cPLA2).

Recent data indicate that iPLA2, particularly iPLA2β, with highest expression in testis and brain, contains a caspase-3 cleavage motif [51]. Caspase-mediated cleavage of iPLA2 results in increased iPLA2 functions [52, 53]. Caspase-3 also cleaves cPLA2, an essential initiator of stimulus-dependent arachidonic acid metabolism, however, specifically inhibiting its activity. Thus, caspase-3 may differentially modulate activities of PLA2 isoforms, depending on their Ca^{2+}-dependence. An essential role of iPLA2 in synaptic plasticity has been suggested and involvement of iPLA2 in the development of LTP has been demonstrated [54-58]. Activation of PLA2 evokes both an enhanced glutamate release and an increased sensitivity of AMPA receptor, two events that may support synaptic facilitation and LTP formation [59, 60]. Since LTP requires activation of iPLA2, caspase-3 can facilitate the induction of LTP by activating iPLA2, while caspase-3 inhibition may have an opposite effect.

There are several lines of evidence indicating that pre- and postsynaptic kinases and their specific substrates are involved in molecular mechanisms underlying LTP. During induction of LTP, depolarization of the postsynaptic membrane allows Ca^{2+} into the postsynaptic spine through the NMDA receptor channel. The resultant Ca^{2+} increase triggers LTP by activating protein kinases such as calcium/calmodulin-dependent protein kinase II (CaMKII) and PKC.

Many studies focus on the involvement of PKC in LTP [61, 62]. Although most kinases are activated briefly by second messengers, an autonomously active kinase could persistently potentiate synapses to maintain LTP [63, 64]. Persistently active forms exist for several serine/threonine protein kinases, including CaMKII, cAMP-dependent protein kinase (PKA), and PKC, a family of conventional, novel, and atypical isozymes [65]. All these kinases are important in induction, but only some of them are required for maintenance of LTP [66, 67]. The constitutively active, independent catalytic domain of an atypical isozyme PKC, PKMζ, specifically increases in LTP maintenance [68, 69]. PKMζ, the predominant form of PKM in brain, has high levels in hip-

pocampus. It is suggested that the compartmentalization of PKC isoforms in neurons may contribute to their function, with the location of PKMζ prominent in areas notable for long-term synaptic plasticity [70]. PKMζ has been shown to be necessary and sufficient for LTP maintenance [71]. During maintenance of LTP, the AMPA receptors remain phosphorylated by persistent PKM activity, leading to increased conductance of AMPA receptor channels [68]. Atypical PKC is turned into a persistently active form via Ca^{2+}-activated proteolytic cleavage by calpain [68]. However, Frutos et al. [72] reported recently about the cleavage of PKCζ by caspase-3. Thus, like many other protease-regulated components involved in LTP, PKCζ may be regulated by both caspase-3 and calpain, the latter being regulated by caspase-3 through the cleavage of calpastatin.

CaMKIV is another LTP-related signal transduction protein that can be cleaved by either caspase-3 or calpain. The importance of well-characterized CaMKII in hippocampal LTP is well established; however, several CaMKs other than CaMKII are not yet clearly characterized and understood. Kasahara et al. [17] reported about the transient activation of CaMKIV, which is localized predominantly in neuronal nuclei and functions as a cyclic AMP-responsive element-binding protein (CREB) kinase, in high frequency stimulation-induced LTP in the rat hippocampal CA1 region. Phosphorylation of CREB, a CaMKIV substrate, and expression of c-Fos protein, which is regulated by CREB, are increased during LTP. During LTP, the activation of CaMKII is sustained for a long period, whereas that of CaMKIV is transient suggesting that CaMKII is involved in LTP induction, while CaMKIV is rather involved in LTP maintenance [73]. The data from other groups confirm that not only CaMKII but also CaMKI and CaMKIV contribute to synaptic plasticity formed in LTP [74, 75]. From all CaMK isoforms in the brain, CaMKIV is a substrate for caspase-3. In primary cultures of cerebellar neurons *in vitro* CaMKIV was demonstrated to be cleaved by caspase-3 [76], while in SH-SY5Y neuroblastoma cells CaMKIV is cleaved by both caspase-3 and calpain [77].

Regulation of synaptic efficacy involves signaling via tyrosine kinases. Induction of LTP in area CA1 of rat hippocampal slices enhanced association of the Src-family kinases Fyn and c-Src kinases with an approximately 120 kD tyrosine phosphorylated component containing the focal adhesion kinase (FAK) and its homolog PYK2. These results suggest that Fyn and c-Src are involved in distinct signaling pathways and provide evidence for activation of FAK and PYK2 following synaptic stimulation inducing LTP *in vitro* [78]. Fyn, as a Src family kinase, regulates the NMDA channel by phosphorylation of NR2A and NR2B subunits of the NMDA receptor. A Fyn-dependent phosphorylation of one of the sites on NR2B (Tyr1472) is elevated upon the LTP induction of hippocampal CA1 neurons [79]. Blocking the tyrosine kinase Src can prevent induction of LTP in CA1 pyramidal cells of rats, and Src activity is increased by stimulation producing LTP. Directly activating Src in the postsynaptic neuron enhances excitatory synaptic responses, occluding LTP. Thus, Src activation is necessary and sufficient for inducing LTP and may function by up-regulating NMDA receptors [80]. The impairment of LTP observed in adult Fyn-deficient mice suggests that Fyn contributes, at least in part, to the molecular mechanisms of LTP induction [81, 82]. Grant et al. [83] reported that in mice with Fyn mutation LTP was blunted even though synaptic transmission and short-term forms of synaptic plasticity were normal.

Fyn, similar to another member of Src-family tyrosine kinases, c-Src, is processed by caspase-3 [84, 85]. Several groups reported that during apoptosis, FAK is preferentially cleaved by caspase-3 [86, 87]. During the cleavage, the level of FAK phosphorylation is rapidly decreased, and the phosphorylation pattern of FAK-associated proteins is dramatically modified, showing significant yet divergent changes in signal transduction [86, 88, 89]. Thus, caspase-3-dependent cleavage may represent a new mechanism for the regulation of FAK and Src kinases with important functional and physiological consequences for synaptic plasticity.

In regulating synaptic plasticity, protein kinases and phosphatases play antagonistic roles. Both kinase inhibition and phosphatase activation impair hippocampal LTP. The late phase of LTP requires activation of cAMP-dependent protein kinase (PKA) for its full expression. Relieving an inhibitory constraint is one way in which PKA may critically modulate LTP by protein phosphatases. Genetic and pharmacological experiments support the role for PKA-mediated suppression of protein phosphatases in gating the expression of late LTP. Genetic inhibition of PKA impairs LTP by reducing PKA-mediated suppression of protein phosphatase (PP) 1 and 2A [90]. Inhibitors of PP1 and PP2A block "silent" metaplasticity of the late phase of LTP [91]. Using autophosphorylated CaM kinase as substrate, Fukunaga et al. [92] have demonstrated that LTP induction and maintenance are also associated with a significant decrease in calyculin A-sensitive protein phosphatase (PP2A) activity. These results suggest that the decreased activity of PP2A following LTP induction contributes to the maintenance of constitutively active CaMKII and to the long-lasting increase in phosphorylation of synaptic components implicated in LTP. The regulatory subunit Aα of PP2A was found to be a substrate for caspase-3. This suggest that caspase-3-dependent change in the activation state of PP2A results in a change in the phosphorylation state of the cell providing another link between the caspase-3 and signal transduction pathways involved in plasticity of the neuron [93].

3.5. Neuronal structural proteins are substrates of caspase-3. Postsynaptic densities from excitatory synaps-

es are enriched in many cytoskeletal proteins including actin, spectrin, tubulin, and microtubule-associated proteins [94] that are substrates of caspases and/or calpains. Among those, actin and spectrin, whose interactions play major roles in regulating growth cone behaviors in developing neurons and synaptic structure (e.g., dendritic spine formation) in mature neuronal circuits, are substrates for both caspases and calpains.

Cleavage of actin and spectrin by caspases and calpains is believed to serve the function of modulating neuronal calcium homeostasis [18]. The cytoskeleton, and actin filaments in particular, can modulate calcium influx through plasma membrane ligand- and voltage-gated channels. In cultured hippocampal neurons, calcium release from IP3- and ryanodine-sensitive endoplasmic reticulum stores is modulated by polymerization and depolymerization of actin filaments [95].

Actin cleavage at a caspase-sensitive site is believed to disrupt actin filaments [16, 96, 97]; however, the turnover of these filaments may be important for normal synaptic plasticity. There is evidence that reorganization of the neuronal actin cytoskeleton is important for consolidation of LTP. Dynamic actin filaments are suggested to participate in specific aspects of synaptic plasticity. Krucker et al. [98] demonstrated on the Schaffer-collateral-CA1 pyramidal cell synapse of mouse hippocampal slices that compounds inhibiting actin filament assembly specifically impaired the maintenance of LTP. The results are consistent with a model in which dynamic actin filaments play an essential role in the molecular mechanisms underlying the early maintenance phase of LTP, such as growth of new synaptic connections or conversion of silent synapses.

In the CA1 region of rat hippocampus postsynaptic actin filaments are involved in a dynamic process required to maintain AMPA receptor-mediated transmission and to enhance it during LTP [99]. Skeberdis et al. [100] suggested a role for cytoskeletal protein(s), primarily actin, in the mGluR-mediated potentiation of NMDA-receptor activity that is relevant for synaptic transmission and plasticity including LTP. Thus, caspase-3-dependent proteolysis of actin, either direct or indirect (by calpain), can affect plasticity via different mechanisms.

During LTP induction, activation of NMDA receptors is accompanied by Ca^{2+}-sensitive proteolysis of nonerythroid αII-spectrin (α-fodrin), a major dendritic cytoskeletal protein [30, 32]. The processing of brain spectrin (fodrin) by Ca^{2+}-dependent proteases at the postsynaptic membrane has been postulated to be one of the central molecular mechanisms underlying LTP [101]. Spectrin is a substrate of both calpain and caspase-3 cysteine proteases. Independent cleavage of αII-spectrin by calpain and caspase-3 results in accumulation of protease-specific spectrin breakdown products (SBDPs) [102-104]. These SBDPs, together with spectrin, are found in postsynaptic densities. Moreover, there is substantial evidence that spectrin regulates the surface chemistry and morphology of cells and thus its partial degradation would be expected to produce pronounced and persistent modifications in synapses [32]. Vanderklish et al. [105] demonstrated that slices in which Θ-burst stimulation was applied to the Schaffer collateral fibers had pronounced accumulation of SBDP that were restricted to field CA1, the zone innervated by the stimulated axons. The caspase cleavage site of spectrin was identified that yields the distinctive 120 kD SBDP120, while the calpain-mediated SBDP is a 145 kD protein [106, 107]. Thus, caspase-3, directly or through calpain, may control the involvement of spectrin cleavage in synaptic plasticity.

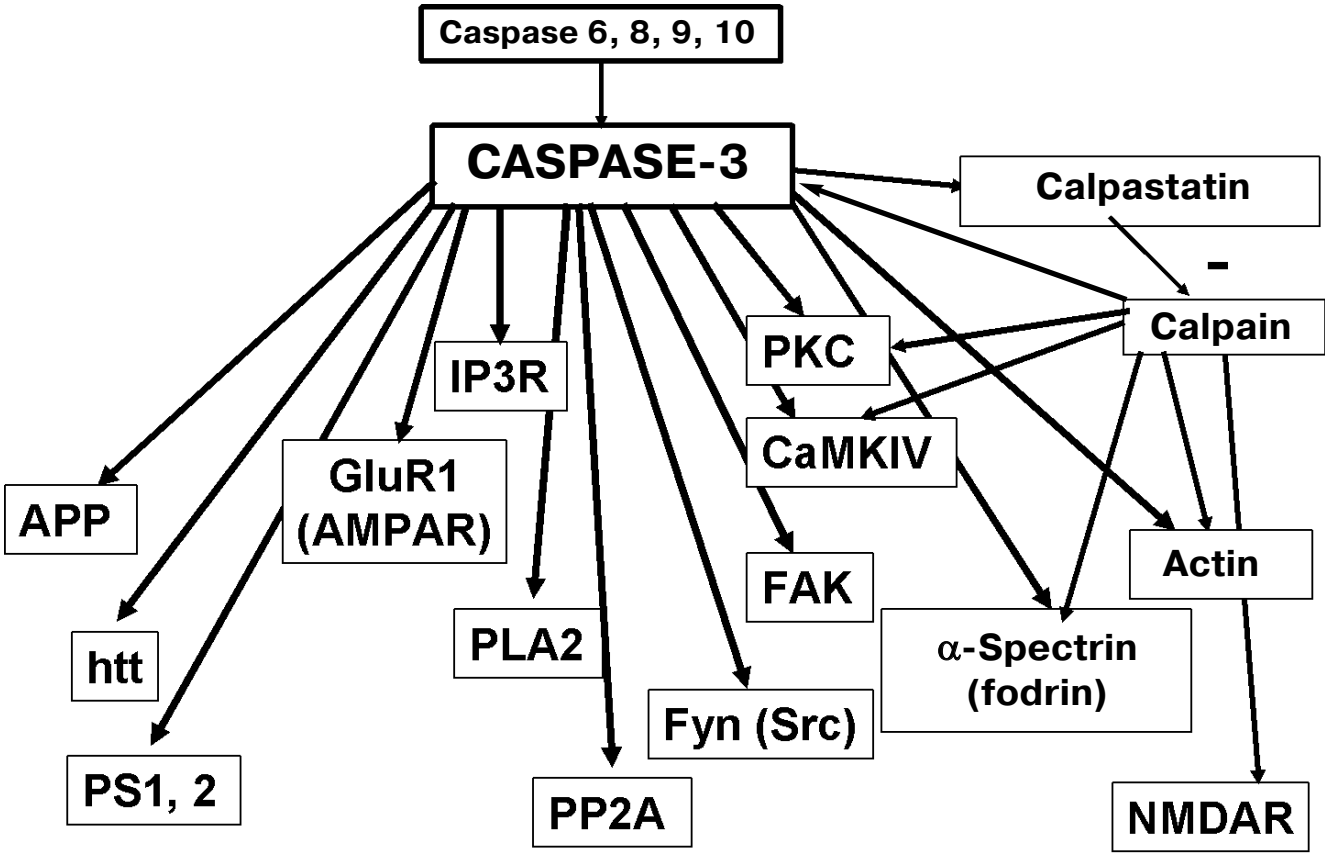
3.6. Other substrates of caspase-3 involved in neuroplasticity. In addition to plasticity relevant substrates of caspase-3 discussed above, there are a number of neuronal proteins that are cleaved by caspase-3 and have been shown to be involved in synaptic changes in neurodegenerative diseases (Alzheimer's disease, Huntington's disease, etc.). Their role in normal brain function is suggested but poorly understood [18]; however, there are results that may be relevant for our analysis on the involvement of caspase-3 in neuroplasticity. Amyloid precursor protein (APP), a caspase-3 substrate [108], is involved on the morphogenetic level in synaptic changes, on one hand, and signal transduction effects on the other [94, 109]. Both in APP-null mice and in transgenic mice that over-express mutant APP, LTP in the CA1 region of the hippocampus is reduced [110, 111]. Presenilins (PSs), polytopic transmembrane proteins intimately involved in proteolysis of APP, are cleaved by caspase-3 to alternative N-terminal and C-terminal fragments [112-115]. Disturbances in hippocampal LTP have been demonstrated in genetically modified mice that underexpress PS1 (PS1(+/–) mice) [116] as well as in transgenic mice with familial Alzheimer's disease (FAD)-linked mutations in PS1 (A246E PS1 variant) [117]. Huntingtin (htt), a protein with its normal function at present not well understood, was the first neuronal protein shown to be a caspase-3 substrate [118-120]. Mice engineered to carry the human Huntington's disease mutation in the endogenous *htt* gene show a significant reduction in LTP [121]. Caspase-3 substrates, proteins related to neurodegenerative diseases (APP, PSs, and htt), may be involved in synaptic plasticity, including LTP, and their cleavage with caspase-3 may be of importance in this respect.

4. CASPASE-3 IN THE PROTEOLYTIC CASCADE INVOLVED IN NEUROPLASTICITY

The results presented suggest the existence of a proteolytic cascade that regulates the activity of proteins—main players in the molecular mechanisms of neuroplas-

ticity (figure). Caspase-3 plays a central role in this regulatory cascade. Caspase-3 directly modifies the structure and activity of the components of synaptic signal transduction as well as the structure and functioning of most important cytoskeletal proteins. In other words, we postulate a multi site involvement of caspase-3 in regulatory signaling cascades underlying phenomena of synaptic plasticity. There is no doubt now that calpain-mediated cleavage of several synaptic substrates is essential for long-term neuroplasticity. However, calpain activity is caspase-3 dependent. Thus, caspase-3 controls signal transduction and the status of neuronal cytoskeleton both directly by cleavage of its respective substrates and indirectly through the regulation of calpain activity as upstream initiator of the proteolytic cascade.

Is caspase-3 on the very top of the proteolytic cascade involved in neuroplasticity? The answer is "No". First, the upstream caspases 6, 8, 9, and 10 activate caspase-3 and, though indirectly, are involved in all caspase-3-dependent mechanisms (figure). Second, it is theoretically possible that these upstream caspases may have substrates among plasticity relevant proteins. At present, a least one study confirms this suggestion. Meyer et al. [122] examined the modulatory role of nicotine in primary mixed cortical neuronal-glial cultures on activity-dependent caspase cleavage of a glutamate receptor, GluR1 (caspase-3 substrate, see section 2). GluR1 was found to be a substrate for agonist (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid)-initiated rapid proteolytic cleavage at aspartic acid 865 through the activation of caspase-8-like activity that is not coincident with apoptosis. Dose-dependent nicotine preconditioning for 24 h antagonized agonist-initiated caspase cleavage of GluR1 through a mechanism that was coincident with desensitization of both nAChRα4β2 and nAChRα7 receptors and the delayed activation of a caspase-8-like activity. The modulation of GluR1 agonist-initiated caspase-mediated cleavage by nicotine preconditioning



Schematic representation of the multi site involvement of caspase-3 in regulatory signaling mechanisms underlying phenomena of neural plasticity. Caspase-3 substrates, key components of neuroplasticity phenomena, are presented. APP) amyloid precursor protein; CaMKIV) calcium/calmodulin-dependent protein kinase type IV; FAK) focal adhesion kinase; Fyn (Src)) Fyn (Src) tyrosine kinase; GluR1) glutamate receptor GluR1; htt) huntingtin; IP3R) inositol 1,4,5-trisphosphate receptor type I; NMDAR) NMDA glutamate receptor; AMPA) AMPA glutamate receptor; PKC) protein kinase C; PLA2) phospholipase A2; PP2A) protein phosphatase 2A; PS1, 2) presenilins 1 and 2.

offers a novel insight into the involvement of caspases in neuroplasticity. Thus, a much more complicated scheme (as compared with one presented in this paper) for the involvement of different caspases in phenomena of neuroplasticity can be suggested. The results of future studies will hopefully provide us with more experimental data and give further evidence for this suggestion.

This study was partially supported by the Russian Foundation for Basic Research, grant Nos. 01-04-49476 and 03-04-48479.

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