Roles of Caspases in the Programmed Cell Death of Motoneurons \textit{in vivo}\textsuperscript{*}

Hiroyuki Yaginuma\textsuperscript{1}, Noboru Sato\textsuperscript{1}, Shunsaku Homma\textsuperscript{1} and Ronald W. Oppenheim\textsuperscript{2}

Department of Anatomy\textsuperscript{1}, School of Medicine, Fukushima Medical University, Fukushima, Japan; and Department of Neurobiology and Anatomy and the Neuroscience Program\textsuperscript{2}, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

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\textit{Summary.} Cysteine proteases comprising the caspase family have been considered one of the major executioners of programmed cell death. However, detailed analyses of the programmed cell death of developing motoneurons in mice following the genetic deletion of two key caspases, casp-3 and casp-9, and in the chick embryo following treatment with caspase inhibitors, indicate that normal amounts of cell loss occur although the death process is delayed. Motoneurons undergoing programmed cell death without caspase activities exhibit a nonapoptotic morphology in which nuclear changes such as chromatin condensation are absent or reduced and which exhibit extensive cytoplasmic vacuolization such as is rarely observed in degenerating control neurons. These results suggest that caspases are involved in, but are not indispensable for, the developmental death of motoneurons, and that one function of caspases may be to facilitate the removal of cells that are destined to die. Possible alternative caspase-independent pathways for the programmed death of motoneurons are discussed.

Programmed cell death is a fundamental component of normal development and adult homeostasis in virtually all multicellular organisms (for review, see Glüksmann, 1951; Saunders, 1966; Wyllie et al., 1980; Raff, 1992; Schwartz and Osborne, 1993). In all segments of the developing avian and mammalian spinal cord, developing motoneurons undergo programmed cell death. Approximately 50\% of motoneurons die between embryonic day (E) 6 and E 12 in the chick (Hamburger, 1958; Hollyday and Hamburger, 1976; Chu-Wang and Oppenheim, 1978b; Hamburger and Oppenheim, 1982) and approximately two thirds of lumbar motoneurons die between E13 and E18 in the mouse (Lance-Jones, 1982). The survival of motoneurons at this stage is dependent on their interaction with muscle targets (Hamburger, 1958; Hollyday and Hamburger, 1976; Hamburger and Oppenheim, 1982; Oppenheim, 1991; Caldero et al., 1998). When the limb-bud is removed from the embryo, virtually all the motoneurons that normally innervate the limb muscles die during this period (Hamburger, 1958; Oppenheim et al., 1978; Caldero et al., 1998). Another type of programmed cell death of motoneurons is known to occur only in the non-limb innervating cervical spinal cord of the avian embryo during earlier stages of development (Levi-Montalcini, 1950, 1964; O'Connor and Wyttenbach, 1974; Yaginuma et al., 1996). In the chick, approximately 25–30\% of total motoneurons synchronously die between E4 and E5. These motoneurons can not be rescued by increasing the size of peripheral targets or by treatment with neuromuscular blocking agents, muscle extracts, or defined neurotrophic agents that, all work conversely to rescue motoneurons from the first type of programmed cell death (Yaginuma et al., 1996). Although the mechanisms that induce this type of programmed cell death are still unknown, recent studies have suggested that a certain sub-population of motoneurons is involved (Yaginuma et al., 1996, 2001). A similar early cervical motoneuron death is reported to occur in mammals, although detailed studies remain to be done (Yamamoto and Henderson, 1999). Hereafter, we call these

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two types of programmed cell death in developing motoneurons "later programmed cell death" and "early cervical programmed cell death of motoneurons", respectively. Most of the motoneurons undergoing either type exhibit a morphology featuring a distinct kind of nuclear pyknosis characteristic of an apoptotic type of degeneration, and the dying cells fragment into cytoplasmic/nuclear containing apoptotic body. Debris from dead cells is usually phagocytosed and digested by macrophages or adjacent cells (O'CONNER and WYTTENBACH, 1974; CHU-WANG and OPPENHEIM, 1978a; YAGINUMA et al., 1996).

During the last 10 years the molecular mechanisms of programmed cell death have rapidly been clarified. Cysteine proteases comprising the caspase (casp) family are one of the major classes of pro-apoptotic molecules (CRYNS and YUAN, 1998; LI and YUAN, 1999). Among these, casp-3 is the most widely investigated member of the caspase family involved in the execution of cell death in vertebrates. It is known that casp-3 activity is required at the step where a protease cascade pathway converges. Casp-3 can be activated by casp-8 in the process of Fas-induced programmed cell death (NAGATA, 1997), by casp-9 in combination with cytochrome c, released from the mitochondria, and Apaf-1 (LI et al., 1997; KUIDA et al., 1998), or by granzyme B in cytotoxic T-cell induced cell death (DARMON et al., 1995; MARTIN et al., 1996; QUAN et al., 1996) (Fig. 1). Activated casp-3 cleaves ICAD/DFF45 (LIU et al., 1997; SAKAHIRA et al., 1998) leading to the activation of CAD/DFF40 that causes DNA fragmentation in dying cells (ENARI et al., 1998; LIU et al., 1998; SAKAHIRA et al., 1998). Casp-3 also activates casp-6 and acinus which then mediate the shrinkage of nuclei (HIRATA et al., 1998; KAWAHARA et al., 1998) and chromatin condensation (SAHARA et
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Fig. 2. Photomicrographs of the triple staining of the active form of caspase-3 (green), TUNEL (red) and DAPI (blue) in the ventral horn region of the E4.5 chick cervical spinal cord (A) and in the lateral motor column of the E8 chick lumbar spinal cord (B). Arrowheads indicate the active form of caspase-3 in cells whose nuclei have become TUNEL positive. The arrow in A indicates a cell in which activated caspase-3 is expressed but whose nuclei is not TUNEL labeled and which lacks chromatin aggregation by DAPI-staining, thus being apparently intact. Scale bars=10 μm. (A: adapted from YAGINUMA et al., 2001 with permission).

al., 1999), respectively. Developing motoneurons can be rescued by an over-expression of anti-apoptotic Bcl-2 family molecules or by the genetic deletion of pro-apoptotic Bax (DUBOIS-DAUPHIN et al., 1994; MARTINOU et al., 1994; FARLIE et al., 1995; DE BILBAO and DUBOIS-DAUPHIN, 1996; DECKWERTH et al., 1996; SATO et al., 1998; WHITE et al., 1998). Because the target of these molecules is known to be the mitochondria, this organelle appears to be a key upstream step in the cell death signaling of developing motoneurons.

Studies of casp-3 and casp-9 deficient mice have demonstrated that these animals have reduced programmed cell death and lack DNA fragmentation in the early embryonic central nervous system, resulting in an abnormal organization of the cerebral cortex (KUIDA et al., 1996, 1998; HAKEM et al., 1998; KUAN et al., 2000). However, it has also been reported that structures of the brain stem and spinal cord in these animals are apparently normal (KUIDA et al., 1996, 1998; HAKEM et al., 1998). These results suggested that a normal frequency of programmed cell death of motoneurons may occur in the brain stem and spinal cord. Roles for caspases in the programmed cell death of motoneurons have also been investigated using peptide caspase inhibitors. Ac-YVAD-CHO, an inhibitor of the casp-1 like protease, can arrest the later programmed cell death motoneurons in vitro and in vivo, whereas it fails to rescue the motoneurons from the earlier cervical one (MILLIGAN et al., 1995). An inhibitor of casp-3, Ac-DEVD-CHO, can rescue motoneurons from the later programmed cell death in vitro and in vivo (LI et al., 1998), although it failed to arrest this after limb-bud removal (LBR) (CALDERO et al., 1998). In addition to Ac-DEVD-CHO, the pan-caspase inhibitor, BAF, is also ineffective for rescuing motoneurons after LBR (CALDERO et al., 1998). The different results from these studies suggest that the involvement of casp-3 and other caspases in the programmed cell death of motoneurons may
Involvement of casp-3 in programmed cell death of motoneurons

Casp-3 is synthesized as inactive procasp-3, which becomes active only after being cleaved by upstream casp-8, -9 or granzyme B. Activated casp-3 can be detected either by measuring enzymatic activity using a specific substrate for casp-3 or by immunohistochemistry using an antibody against an active form of casp-3. Because many motoneurons synchronously die in the early programmed cell death of cervical motoneuron, we can directly measure the enzymatic activities in cytosolic extracts from the cervical spinal cord. Casp-3-like activity was increased at st 24 (E4.5), when programmed cell death reaches its peak, in the ventral, motoneuron-containing region of the cervical segments (YAGINUMA et al., 2001).

Double labeling with TUNEL staining and immunocytochemistry with an antibody against active casp-3 revealed that activated casp-3-like immunoreactivity is expressed in dying motoneurons in the ventral horn from st 23+ to st 26 (Fig. 2A) (YAGINUMA et al., 2001). Immunoreactivity was also observed in cells whose nuclei were TUNEL-negative and in which the cellular morphology was apparently normal (Fig. 2A arrow), suggesting that the activation of casp-3 precedes DNA fragmentation. In vivo activation of casp-3 during the later programmed cell death period was also confirmed by immunocytochemistry in the mouse and chick (Fig. 2B) (OPPENHEIM et al., 2001a).

Programmed cell death of motoneurons in chick embryos treated with caspase inhibitors and in casp-3 deficient mice

To examine the role of activated casp-3 in the programmed cell death of motoneurons, the effects of a specific peptide inhibitor treatment on the early programmed cell death of cervical motoneuron were examined in the chick embryo (YAGINUMA et al., 2001). The later programmed cell death of motoneurons was also examined in mice in which casp-3 had been genetically deleted (OPPENHEIM et al., 2001a).

Early programmed cell death of cervical motoneurons in the chick embryo

In sections stained with hematoxylin and eosin, the morphology of dying motoneurons in cervical segments at E4.5 treated with Ac-DEVD-CHO for 12 h was distinctly different from controls (Fig. 3A, B). In the controls, the nucleus of dying cells was round in shape, and the chromatin was condensed and darkly stained by hematoxylin (nuclear pyknosis) to subsequently become fragmented (Fig. 3A). By contrast, the nuclei of cells from Ac-DEVD-CHO treated embryos exhibited only moderate chromatin condensation and pyknosis and had a crenulated shape (Fig. 3B). Although TUNEL staining revealed that DNA fragmentation, one of the hallmarks of apoptosis, does not occur in these cells (Fig. 3D, E), they express an apoptosis specific antigen (ASP) (Fig. 3G, H). Expression of the motoneuron marker, Islet-1, is lost in these cells (Fig. 3G, H).

Electron microscopic observations revealed that the soma/cytoplasm of these moderately pyknotic motoneurons exhibited degenerative changes characterized by a shrinkage of the cell size, condensation, an increased electron density, and the accumulation of clear vacuoles and aggregated ribosomes (Fig. 4A–C). By contrast, the nuclei of these same cells appeared relatively normal; although most nuclei exhibited a slight increase in electron density and an irregular shape as well as a slight aggregation of chromatin, the extreme shrinkage and fragmentation of nuclei seen in degenerating control cells was rarely recognizable (Fig. 4A–C). These aberrantly degenerating cells were often already engulfed by processes of presumptive macrophages (Fig. 4D), indicating that the cells were actually dead or dying.

When the number of dying (pyknotic) cells in hematoxylin-eosin stained sections was counted — including the atypical, moderately pyknotic cells, there was no significant difference in the number of pyknotic cells between controls and Ac-DEVD-CHO treated embryos. The number of surviving motoneurons was also similar to control values (Fig. 5) (YAGINUMA et al., 2001).

Later programmed cell death of motoneurons in casp-3 deficient mice

Similar results to that described above for the chick embryo were also obtained by detailed observations of the later programmed cell death in casp-3 deficient mice (OPPENHEIM et al., 2001a). In contrast to the typical pyknotic, apoptotic morphology of dying control neurons, degenerating neurons in casp-3 deficient embryos were intact, but were reduced in
Fig. 3. Photomicrographs showing the morphology, DNA fragmentation, expression of a motoneuron specific marker (Islet), and expression of the apoptosis specific protein (ASP) by motoneurons in the cervical cord at st 24 following treatment with Ac-DEVD-CHO and BAF for 12 h. Bar in A = 10 μm for A–F; bar in G = 10 μm for G–I. C: Semi-thin sections (2 μm-thick) stained with hematoxylin and eosin; D–F: TUNEL reaction; G–I: triple staining of ASP (green), Islet-1 (red) and DAPI (blue). A, D and G: controls; B, E and H: treated with 400 μg of Ac-DEVD-CHO; C, F and I: treated with 200 μg of BAF. Arrows in A, B indicate examples of pyknotic neurons. The insets in A–C show a higher magnification of the morphologies of typical pyknotic neurons (A), modified degenerating neurons (B), and darkly stained (presumptive degenerating) neurons (C). DNA fragmentation was inhibited in the embryos treated with Ac-DEVD-CHO (E) and BAF (F). Note that, following BAF treatment, most of the cells in the ventral horn retain Islet-1, and that ASP-positive cells are localized to the lateral portion of the ventral horn (I). (Adapted from YAGINUMA et al., 2001 with permission).
Fig. 4. Electron micrographs showing the ultrastructural morphology of degenerating cells in the ventral horn of the cervical spinal cord of the chick embryo following treatment with Ac-DEVD-CHO and BAP for 12 h. A. Typical example of the type of degeneration found in a st 24 normal embryo. The condensation of chromatin (n), aggregated ribosomes (arrows) and increased electron density of the cytoplasm are distinct. B–D. st 24 embryos following 12 h of treatment with Ac-DEVD-CHO. n Nucleus. The arrow in C indicates a degenerating cell with lumpy chromatin condensation. Note that a degenerating neuron is engulfed by a macrophage-like cell (m) in D (arrows). Bars=2 μm. (Adapted from YAGINUMA et al., 2001 with permission).
size, exhibited less nuclear condensation, and were darker. Under the electron microscope, neurons in casp-3 deficient embryos exhibited cytoplasmic changes (e.g., vacuoles, dilated mitochondria) rarely observed in degenerating neurons from the controls (Fig. 6) (Oppenheim et al., 2001a). Although there was a reduction in the number of TUNEL-positive cells in the spinal cord, there were no significant differences in the number of degenerating cells or of surviving motoneurons between the controls and casp-3 deficient mice (Oppenheim et al., 2001a).

These observations suggest that, despite the absence or reduction in TUNEL labeling, normal numbers of these neurons undergo cell death. The morphology of degenerating neurons, however, is non-apoptotic and ultrastructurally distinct from degenerating neurons in control animals. These observations also indicate that casp-3 activity plays a major role in the apoptotic nuclear changes. It has been reported that, during the later programmed cell death of chick embryo motoneurons, casp-3 like activity is involved and that peptide inhibitors of casp-3 can reduce the number of pyknotic motoneurons in vivo (Li et al., 1998). This apparent discrepancy may be explained by differences in the criteria used for counting degenerating cells. In the previous study, the kind of atypical pyknotic degenerating motoneurons following inhibitor treatment described above may not have been counted as degenerating cells. This issue needs to be re-examined because the programmed cell death of motoneurons examined in caspase deficient mice is temporally analogous to the chicken motoneuron death studied by Li et al. (1998).

Casp-3 activity facilitate kinetics of programmed cell death

The effects of prolonged treatment with Ac-DEVD-CHO in the early programmed cell death of the cervical motoneuron suggest that one role of casp-3 in vivo is to facilitate the removal of cells that are destined to die by accelerating the degeneration of cell nuclei, the fragmentation of DNA, and the phagocytosis and digestion by other cells (Yaginuma et al., 2001). Following treatment with caspase inhibitors for 24 h, there were significantly more pyknotic cells in the cervical ventral horn, whereas in these same embryos the number of healthy motoneurons was comparable to that of the controls. This suggests that the time required for the programmed death of each cell was retarded rather than that more motoneurons underwent programmed cell death.

In a normal situation, dying or dead cervical motoneurons are rapidly phagocytosed and digested,
and by st 26 (E5) virtually all pyknotic cells have disappeared (YAGINUMA et al., 1996). Although further studies are needed to identify which step of this process is affected by caspase inhibitors, and whether phagocytes are involved when caspases are inhibited, these findings provide the first in vivo evidence that the significance of caspase activity in at least some forms of neuronal programmed cell death is normally to facilitate the processing of dying cells (MCCARTHY et al., 1997; SAKAHIRA et al., 1998) without being required for the programmed cell death itself.

It is known that casp-3 deficient mice have reduced programmed cell death in the early embryonic brain resulting in an abnormal organization (exencephaly) of the cerebral cortex (KUIDA et al., 1996). As has been described, however, following the period of cell death there are normal numbers of motoneurons in postnatal and embryonic casp-3 deficient mice, and during the cell death period there are normal numbers of degenerating motoneurons, but decreased numbers of TUNEL positive cells (OPPENHEIM et al., 2001a). The numbers of dorsal root ganglion cells, spinal interneurons, and sympathetic neurons are also normal (OPPENHEIM et al., 2001a). One possible explanation for the apparent differential involvement of caspase activity is that, in early developmental stages when prominent and rapid proliferation and death of precursor cells occurs in the neural tube, a delay in the process of programmed cell death owing to a lack of casp-3 activity may result in increasing numbers of neuronal precursors. These increased neuronal precursors may yield increased numbers of neurons and cause an irreversible disorganization of the neural tissues. By contrast, at later developmental stages, because neuronal cell death occurs over a relatively longer period and involves postmitotic cells, most neurons that lose trophic support finally die and disappear without any disorganization of tissues even though the entire process of degeneration is less efficient in the absence of caspase activity.

Fig. 6. Electron micrographs of degenerating spinal cord neurons from casp-3 (+/−) (A) and casp-3 (−/−) (B) E14.5 mouse embryos showing the distinct morphology of dying neurons in the caspase KO. These cells exhibit reduced chromatin condensation and nuclear pyknosis and marked cytoplasmic vacuolization compared with neurons from the casp-3 (+/−) or casp-3 (+/+) embryos. c Cytoplasm/soma, N nuclei, asterisks indicate normal neurons, arrowheads in B indicate the cell boundary of this degenerating motoneuron; note the numerous vacuoles and abnormal organelles in the cytoplasm of this cell. (Adapted from OPPENHEIM et al., 2001a with permission).
Involvement of upstream caspases

The results presented to this point suggest that there are other signaling cascades that occur before, and are independent of, the activation of casp-3 that is responsible for the degenerative changes that occur in the cytoplasm of dying motoneurons in both types of programmed cell death. To address this issue, the later programmed cell death of motoneurons in casp-9 deficient mice has also been examined (Oppenheim et al., 2001a). The morphology of dying motoneurons and the number of surviving motoneurons in casp-9 deficient mice were identical to those in casp-3 deficient mice. Furthermore, a recent study has reported that the magnitude of programmed cell death is normal in Apaf-1 deficient embryos compared with wild-type controls (Oppenheim et al., 2001b). Therefore, it seems that neither casp-9 nor Apaf-1 is responsible for the cytoplasmic degenerative changes in motoneurons.

Additional information on this issue comes from the examination of chick embryos treated with the pan-caspase inhibitor BAF (Yaginuma et al., 2001). After treatment with BAF for 12 h, the number of pyknotic cervical motoneurons was significantly smaller than that of the controls. Instead, there were many abnormal cells that were smaller in size and stained darker (Fig. 3C). These abnormal cells lacked DNA fragmentation (Fig. 3F) and the immunoreactivity for the active form of casp-3, but expressed ASP (Fig. 3I). When quantified, the number of these abnormal cells was similar to the number of pyknotic motoneurons in control embryos. Because these abnormal cells retained motoneuron specific marker, Islet-1, the total number of Islet-1 positive motoneurons increased significantly (Fig. 5). Electron microscopic observations confirmed that there were many smaller cells with irregularly shaped small nuclei and a slightly electron dense cytoplasm, but with apparently normal organelles and nuclear chromatin (Fig. 7A). Taken together, the aberrant morphology, ASP immunolabeling, and the cell counts indicate that these abnormal cells are likely presumptive degenerating motoneurons in which the morphological changes have been delayed. Following treatment with BAF for 24 h, the total number of surviving motoneurons was not different from that of the control embryos (Fig. 5). Thus, it was concluded that the extent of programmed cell death is comparable to that of the controls although its kinetics are delayed. Some of these aberrantly degenerating cells exhibited morphology very similar to that observed following 12 h treatment with Ac-DEVD-CHO. However, we also noted that autophagic vacuoles were conspicuous in many of the degenerating cells (Fig. 7B).

These results suggest that other caspases or signaling molecules that are inhibited by BAF but not inhibited by Ac-DEVD-CHO play key roles in the apoptotic morphological changes in the cytoplasm. It is also suggested that there are caspase-independent pathways that result in programmed cell death, as has been suggested by previous in vitro studies (Xiang et al., 1996; McCarthy et al., 1997; Janicke et al., 1998; Sakahira et al., 1998; Woo et al., 1998;
ZHENG et al., 1998; CREGAN et al., 1999; STEFANIS et al., 1999; SUSIM et al., 1999; XUE et al., 1999; LEIST and JÄÄTTELÄ, 2001).

Possible alternative caspase-independent mechanisms

Apoptosis-inducing factor (AIF)

What mechanisms mediate programmed cell death under caspase inhibition? One possible candidate is the apoptosis-inducing factor (AIF) released from mitochondria (Fig. 1) (SUSIM et al., 1999). AIF is a flavoprotein and is normally confined to mitochondria but translocates to the nucleus when apoptosis is induced. A microinjection of AIF into intact cells induces chromatin condensation, a large scale fragmentation of DNA, dissipation of the mitochondrial transmembrane potential, and exposure of phosphatidylserine in the plasma membrane. A pancaspase inhibitor, z-VAD-fmk prevents none of these AIF-induced effects. However, an over-expression of Bcl-2 prevents the release of AIF from the mitochondria (SUSIM et al., 1999). AIF induces characteristic lumpy chromatin condensation (SUSIM et al., 1999; LEIST and JÄÄTTELÄ, 2001), which is similar to the kind of chromatin condensation often observed in motoneurons of casp-3 deficient mice and Ac-DEVD-treated chick embryos (Figs. 4C, 6) (OPPENHEIM et al., 2001a; YAGINUMA et al., 2001). The finding that the phagocytosis of motoneurons by macrophages occurs in Ac-DEVD-treated chick embryos (YAGINUMA et al., 2001) is also consistent with the AIF-induced exposure of phosphatidylserine in the plasma membrane which is known as an ‘eat me’ signal for macrophages (MESSMER and PFELLSCHUPPER, 2000; SCHLEGEL et al., 2000). These facts suggest that AIF may be involved in the casp-3 independent cell death of motoneurons. However, more studies are needed to elucidate the role of AIF in degenerative changes in the cytoplasm and to confirm the release of AIF in dying motoneurons.

Autophagy

Another possible caspase-independent pathway is autophagy. Many studies have demonstrated that autophagy occurs in various kinds of normal cell death as well as following treatment with cell death-inducing stimuli (CLARKE, 1990; NITATORI et al., 1995; JIA et al., 1997; OHSAWA et al., 1998; ISAHARA et al., 1999; XUE, 1999, for review see UCHIYAMA, 2001). For example, when caspase activity is inhibited, autophagic mechanisms and the activity of cathepsin D, a lysosomal enzyme, can mediate the programmed cell death of sympathetic neurons and PC12 cells following NGF or serum deprivation in vitro (ISAHARA et al., 1999; XUE et al., 1999). Interestingly, 3-methyl adenine, which can inhibit autophagy, can also inhibit cytochrome c release from the mitochondria and prevent the activation of casp-3 following various kinds of apoptosis-inducing stimuli (XUE et al., 1999). This indicates that the initiation of autophagy occurs upstream of the mitochondrial step of programmed cell death signaling and suggests that there is a common pathway which promotes both mitochondrial dysfunction and autophagic activation (XUE et al., 1999).

Two lines of evidence suggest that autophagy may also be involved in the early programmed cell death of cervical motoneurons. First, ASP, a homologue of APG5 is expressed by degenerating cervical motoneurons (YAGINUMA et al., 2001). APG5 is known to be involved in autophagy in yeast (HAMMOND et al., 1998; TERWEL and VAN DE BERG, 2000). Although the specific function of ASP in vertebrates is still unknown, ASP is expressed by many kinds of dying neurons, and this expression can occur in caspase-dependent and caspase-independent situations (HAMMOND et al., 1998; AYALA et al., 1999; FERRER, 1999; YAGINUMA et al., 2001). Second, autophagic vacuoles in dying motoneurons were observed following caspase inhibitor treatment (YAGINUMA et al., 2001). Although many of these vacuoles appeared devoid of lysosomal material, some of them, especially those observed in the degenerating cells following BAF treatment for 24 h (Fig. 7B), appeared to be autophagic vacuoles, defined as vacuoles that are limited by double membranes and that contain cytoplasmic organelles (DUNN, 1990).

Morphology of dying cells and caspases

The evidence revealed above suggests that some of the morphological diversity of developmental programmed cell death can be explained by the extent to which caspases are involved in the cell death process. CLARKE (1990) has classified the morphology of dying cells during development into three types: type 1 apoptosis; type 2 autophagic degeneration; and type 3 non-lysosomal vesiculate degeneration. Type 3 can be further divided into type 3A non-lysosomal disintegration, and type 3B the cytoplasmic type of degeneration. More recently, LEIST and JÄÄTTELÄ (2001) have classified cell death into four patterns: apoptosis, apoptosis-like programmed cell death, necrosis-like programmed cell death, and accidental necrosis/ cell lysis. Apoptosis-like programmed cell death involves forms of cell death with a chromatin condensation that is less compact than in apoptosis. Necrosis-like programmed cell death defines cell
death in the absence of chromatin condensation, or with chromatin clustering appearing as speckles. Clarke's type 2 and type 3 seem to correspond to apoptosis-like programmed cell death. Leist and Jaätela (2001) have concluded that these differential morphologies reflect a different cell death execution machinery downstream of mitochondrial dysfunction. As the result of various kinds of cell death inducing signals converging on the mitochondria, three different independent signals emanate from mitochondria: cytochrome c, AIF, and reactive oxygen species (ROS) and Ca++ (Fig. 1). Activated caspases triggered by cytochrome c induce classical apoptosis. When caspases can not work owing to genetic depletion, treatment with inhibitors or ATP depletion, then caspase-independent AIF signaling results in an apoptosis-like programmed cell death. If neither caspases nor AIF are involved, then ROS and Ca++ induce a necrosis-like programmed cell death. Anti-apoptotic Bcl-2 family molecules can rescue most kinds of cell death by inhibiting release of these signaling molecules. The evidence reviewed here for programmed cell death of developing motoneurons are consistent with this proposal. The morphology of dying motoneurons has been classified as typical type 1, apoptotic cell death (O'Connor and Wyttenbach, 1974; Chu-Wang and Oppenheim, 1978a; Clarke, 1990; Yaginuma et al., 1996). The morphology of motoneurons in the chick embryos treated with caspase inhibitor and in the casp-3 deficient mice more closely resembles Clarke's type 3B and type 2 cell death or apoptotic-like programmed cell death (Oppenheim et al., 2001a; Yaginuma et al., 2001).

CONCLUSIONS

The present results indicate that casp-3 and casp-9 are involved in — though dispensable for — development motoneuron death. The inhibition of caspases (chick) or genetic deletion of either casp-3 or casp-9 (mice) does not prevent the normal loss of developing motoneurons. One function of caspases may be to facilitate the removal of cells that are destined to die by accelerating the degeneration of cell nuclei, fragmentation of DNA, and phagocytosis and digestion by other cells. Because neither the results from the chick embryo nor the analysis of casp-3 or casp-9 deficient mice can exclude the possibility that other caspases not affected by these perturbations may mediate the programmed cell death of motoneurons, we cannot determine whether cell death in these situations is truly caspase-independent. However, the normal occurrence of programmed cell death in the chick embryo following treatment with the pan-caspase inhibitor BAF and similar results in cultured sympathetic neurons (Xue et al., 1999) are consistent with the possibility of caspase-independent pathways.

REFERENCES


