review

Role of sphingolipid-mediated cell death in neurodegenerative diseases

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Abstract The metazoan nervous system gives rise intradevelopmentally to many more neurons than ultimately survive in the adult. Such excess cells are eliminated through programmed cell death or apoptosis. As is true for cells of other lineages, neuronal survival is sustained by an array of growth factors, such that withdrawal of neurotrophic support results in apoptotic cell death. Apoptosis is therefore believed to represent a beneficial process essential to normal development of central and peripheral nervous system (CNS and PNS) structures. Although the initiation of neuronal apoptosis in response to numerous extracellular agents has been widely reported, the regulatory mechanisms underlying this mode of cell death remain incompletely understood. In recent years, the contribution of lipid-dependent signaling systems, such as the sphingomyelin pathway, to regulation of cell survival has received considerable attention, leading to the identification of lethal functions for the lipid effectors ceramide and sphingosine in both normal and pathophysiological conditions. Moreover, the apoptotic capacities of several cytotoxic receptor systems (e.g., CD120a, CD95) and many environmental stresses (e.g., ionizing radiation, heat-shock, oxidative stress) are now known to derive from the activation of multiple signaling cascades by ceramide or, under some circumstances, by sphingosine. Inappropriate initiation of apoptosis has been proposed to underlie the progressive neuronal attrition associated with various neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and other neurological disorders that are characterized by the gradual loss of specific populations of neurons. In such pathophysiological states, neuronal cell death can result in specific disorders of movement and diverse impairments of CNS and PNS function. In some autoimmune neurological diseases such as Guillain-Barré syndrome, demyelinating polyneuropathy, and motoneuron disease, persistent immunological attack of microvascular endothelial cells by glycolipid-directed autoantibodies may lead to extensive cellular damages, resulting in increased permeability across brain–nerve barrier (BNB) and/or blood–brain barrier (BBB).—**Ariga, T., W. D. Jarvis, and R. K. Yu.** Role of sphingolipid-mediated cell death in neurodegenerative diseases. *J. Lipid Res.* 1998. **39:** 1–16.

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The normal development of multicellular organisms is dependent on the removal of select populations of cells at specific intervals through the initiation of programmed cell death or apoptosis, an organized, genetically regulated process through which a cell directs its own self-destruction (1). Apoptosis constitutes the subtractive aspect of the physiological regulation of tissue mass, representing a balanced counterpart to mitosis, and thus occurs as an essential feature of normal tissue development and homeostasis. It should be noted that apoptosis is distinct from the passive, energy-independent modes of cell death collectively termed necrosis. The terms apoptosis and necrosis have come into favor over older cytological terms shrinkage (or regressive) necrosis and lytic (or coagulation) necrosis, respectively (1). Apoptotic cell death is an active, energy-dependent event characterized by numerous distinctive cytological alterations, including overall cell shrinkage attendant upon active extrusion of water, condensation of nucleoplasm and cytoplasm, extensive formation of membrane blebs, and karyolytic degeneration of the nu-

Abbreviations: CNS, central nervous system; PNS, peripheral nervous system; AIDS, acquired immunodeficiency syndrome; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; PD, Parkinson's disease; NGF, nerve growth factor; TNF, tumor necrosis factor; CNTF, ciliary neurotrophic factor; GDNF, glial cell-derived neurotrophic factor; IL, interleukin; ICE, interleukin converting enzyme; PKC, protein kinase C; nPKC, group B or 'novel' PKC subfamily; cPKC, group A or 'conventional' PKC subfamily; PKA, cyclic-AMP dependent protein kinase; BNB, brain–nerve barrier; BBB, blood–brain barrier.

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cleus into membrane-bound chromatin fragments (i.e., apoptotic bodies); cellular debris typically is subject to heterophagocytosis by microglia and/or resident macrophages. A fundamental aspect of apoptosis entails parallel activation of cellular proteases and endonucleases (2). Early recruitment of an 'initiator protease' (e.g., granzyme B, FLICE) results in proteolytic activation of 'amplifier' proteases (e.g., ICE or Caspases) (3, 4) which, in turn, activate multiple families of so-called 'machinery' proteases (e.g., CPP32/ICELAP6/Caspase-3); the latter class of enzymes ultimately mediate proteolytic degradation of the cytoskeleton and other substrates (e.g., PARP, lamins) during the final stages of apoptosis (5). Apoptotic proteolysis is accompanied by extensive cleavage of genomic DNA, resulting in double-stranded degradation of genomic DNA into progressively smaller fragments (e.g., ~300-kbp rosettes, ~50-kbp loops, and finally a 'laddered' spectrum of oligonucleosomal fragments in integer-multiples of ~180–200 bp) (2).

Upon the cell's exit from a proliferative state, apoptosis represents an immediate alternative to differentiation, although terminally differentiated cells characteristically are eliminated through apoptotic cell death. Various cytotoxic receptor systems and lethal environmental stresses have been implicated in both positive and negative regulation of apoptosis, whereas receptors for many growth factors can drive proliferation, and delay or prevent apoptosis, thereby extending cell survival (6). While multiple signaling cascades and intranuclear events related to transcriptional/translational control appear to contribute to the apoptotic process, the precise intracellular regulatory mechanisms underlying apoptosis are incompletely understood. Nonetheless, there is a growing awareness that abnormalities in the regulation of apoptosis and other aspects of cell survival may underlie the pathogenesis of several disorders, including neurodegenerative disorders, cancer, acquired immunodeficiency syndrome (AIDS), and a number of autoimmune diseases (7). In this review, we describe the action of several agents in cell death and survival, and the role of sphingolipids and related lipids in neuronal cell death. We also describe the putative relationship between cell death programs and neurodegenerative diseases and related disorders characterized by the gradual loss of neurons.

LIPID MESSENGERS AND THE REGULATION OF CELL SURVIVAL

Numerous lines of evidence support the importance of sphingophospholipid- and glycerophospholipid-derived messengers in the regulation of cell survival. The relationship among the various lipid-mediated signaling pathways discussed below is shown in **Fig. 1**. Most notably, generation of ceramide through the sphingomyelin pathway has been associated with the induction of apoptotic cell death in response to cytotoxic humoral factors such as tumor necrosis factor-α (TNF-α) or Fas ligand (FasL) (8, 9). Occupancy of either CD120a (the 55-kDa or 'type-I' receptor for TNF-α, TNFR1) or CD95 (the APO-1 or 'Fas-receptor', FasR) promotes activation of neutral and acidic isoforms of sphingomyelinase (N-SMase and A-SMase), leading to increased formation of ceramide within plasmalemmal and endosomal membranes, respectively (10). Recent observations suggest that the proinflammatory aspects of TNFα are mediated by activation of N-SMase, while the proapoptotic influence of the cytokine is mediated through activation of A-SMase (11). Other receptor systems linked to the sphingomyelin pathway include those for interleukin-1 (IL-1), interferon-γ (IFN-γ), 1α, 25-dihydroxy vitamin D3, and the low-affinity neurotrophin receptor. Ceramide generation is also implicated in the lethal influences of several environmental stresses, including ionizing radiation, heat-shock, and oxidative stress (12), a process that apparently derives from activation of A-SMase (6). Furthermore, de novo synthesis of ceramide from sphingosine through the ceramide synthase pathway has been directly implicated in the apoptotic actions of such antineoplastic agents as anthracyclines and conversion to ceramide has been proposed to underlie some biological actions of sphingoid bases (13).

Given the established apoptotic potential of these diverse lethal stimuli, a conserved role for ceramide as a primary effector in apoptosis has been proposed. In support of this view, experimental manipulations that increase intracellular ceramide levels (e.g., treatment with bacterial sphingomyelinase or exposure to synthetic preparations of ceramide and permeant ceramide analogs) potently induce apoptosis in mammalian cells. The cytotoxic effects of exogenous ceramides entail all of the features of apoptosis associated with responses to primary lethal stimuli (e.g., activation of TNFR1), including cytoarchitectural modifications, suppression of proliferative capacity, and extensive degradation of genomic DNA (14, 15). Ceramide-mediated DNA damage consists of double-stranded breakage of mature DNA, but appears not to involve nascent DNA (15), and has been demonstrated variously by generation of 50-kbp DNA 'loop' fragments, 0.2- to 2-kbp oligonucleosomal fragment ladders, formation and release of small double-stranded DNA fragments, and selective introduction of double-stranded (but not single-stranded) breaks into bulk DNA (16–20).

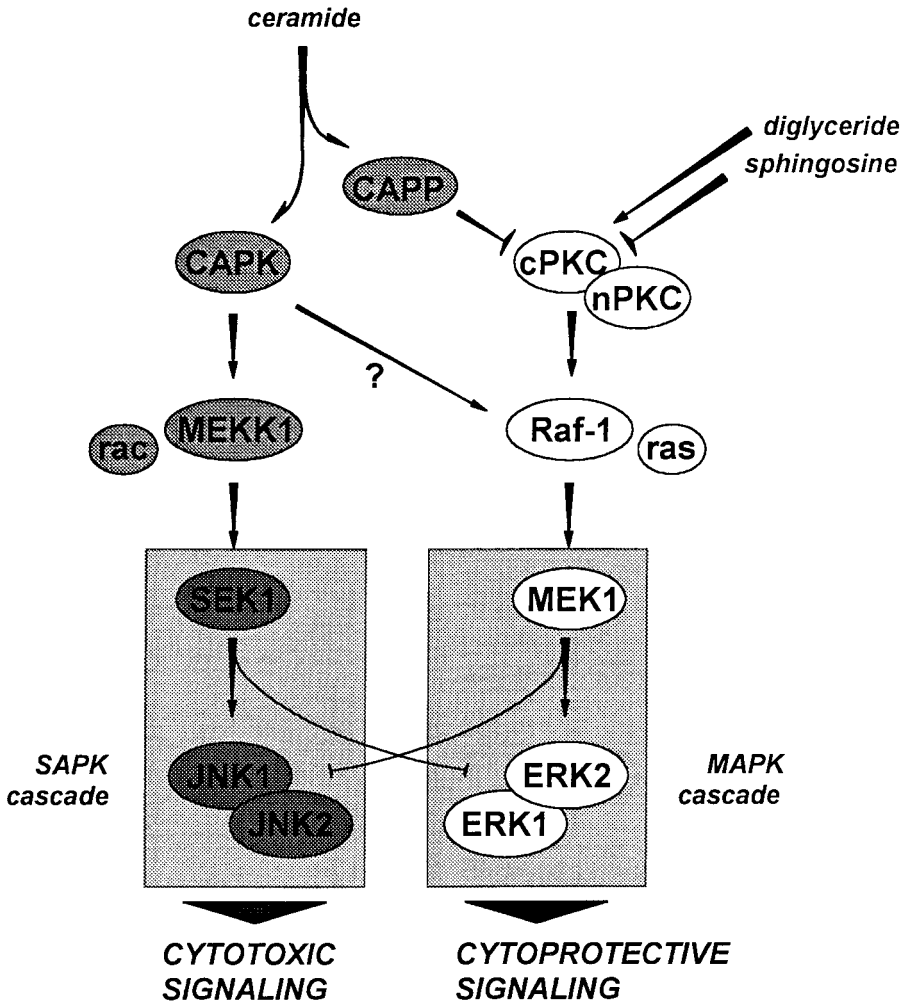

Fig. 1. Sphingolipid-dependent survival signaling. Multiple lipid messengers are implicated in the proximal regulation of cell survival. Opposing cytotoxic and cytoprotective signals are integrated further downstream within the stress-activated protein kinase (SAPK) and mitogen-activated protein kinase (MAPK) cascades. Lethal cellular stresses lead to the formation of ceramide. Proximal targets for ceramide include both a ceramide-activated protein kinase (CAPK) and ceramide-activated protein phosphatase (CAPP). The distinct role for CAPK in the initiation of apoptosis is uncertain, but it possibly involves recruitment of 'MAPK/ERK kinase kinase' (MEKK1). MEKK1 activates SAPK-kinase (SEK1), which, in turn, engages the SAPKs or 'Jun N-terminal kinases' (JNK 1/2); the outflow of stress signaling through the JNKs ultimately triggers the onset of apoptosis. While CAPK also activates Raf-1 kinase under some circumstances (e.g., in inflammatory responses), the contribution of Raf-1 to cell death is ambiguous. The small GTP-binding docking proteins rac and ras are implicated in various aspects of survival signaling. Conversely, the conventional and novel isoforms of protein kinase C (cPKC and nPKC, respectively) promote sequential activation of Raf-1, MAPK-kinase (MEK1), and the MAPKs or 'extracellular signal-regulated kinases' (ERK 1/2). Because the ERKs antagonize lethal signaling, lipid messengers that converge upon cPKC/nPKC are therefore able to modulate the cytoprotective actions of the MAPK cascade. Thus, the apoptotic capacity of ceramide is respectively attenuated or amplified by the influences of diradylglycerols and sphingoid bases over cPKC/nPKC. Several points of crosstalk may exist between these systems. For example, CAPP can reduce stimulated basal and stimulated cPKC activity through dephosphorylation. In addition, the SAPK and MAPK cascades appear to be coordinately regulated, allowing one system to predominate over the other, such that (a) SEK strongly stimulates JNK but moderately inhibits ERK, and (b) MEK1 strongly stimulates ERK but weakly inhibits JNK.

Naturally occurring ceramides consist of a long-chain sphingoid base with an amide-linked fatty acid substituent (typically with acyl chain lengths of 16–24 carbon atoms). Only D-*erythro*-ceramide and D-*erythro*dihydroceramide are present normally in eukaryotes (21); apoptosis is elicited by ceramides, but not dihydroceramides (14, 22), indicating that the *trans*-4 double bond is essential for cytotoxicity. D-*erythro*-ceramide, then, represents the physiologically relevant ceramide in mammalian systems. Detailed structure–activity relationship (SAR) studies have also examined the importance of the two chiral carbons of ceramide with respect to the

lipid's bioactivity; interestingly, although D and L forms of both *erythro* and *threo* ceramide stereoisomers exhibit substantial apoptotic potential, the greatest cytotoxic capacity is associated with the D-*threo* and L-*threo* species rather than the naturally occurring D-*erythro* form (23). Moreover, additional SAR studies have demonstrated that whereas D-*erythro*-dihydroceramide is inactive, as noted above, D-*threo*-dihydroceramide possesses full cytotoxic bioactivity (22).

Numerous signaling systems are associated with ceramide actions, including rapid activation of the stress-activated protein kinase (SAPK) cascade during apoptotic responses (12), and either activation or inactivation of mitogen-activated protein kinase (MAPK) cascade during inflammation or apoptosis, respectively (24, 25). The mechanisms through which ceramide engages these and other downstream pathways are incompletely understood but appear to be directly dependent upon the context within which the ceramide signal is presented. Ceramide interacts with several proximal subcellular targets, and intense efforts have focused on the identification of the element(s) responsible for mediating the lethal aspects of ceramide action. The best characterized is a 97-kDa membranal, proline-directed, serine/threonine kinase (ceramide-activated protein kinase, CAPK) (26–28). CAPK differs from other members of the proline-directed kinase family in its selective recognition of X-Thr-Leu-Pro-X as a minimal substrate peptide sequence (26). Further characterization of this activity demonstrated CAPK to be identical with a previously known 100-kDa membrane protein, kinase suppressor of ras (KSR) (29). KSR/CAPK mediates inflammatory signaling by TNF-α through phosphorylation and activation of Raf-1 (30); Raf-1 may also bind and be activated by ceramide (31). After binding of GTP-ras and translocation to the plasma membrane, Raf-1 initiates sequential activation of the MEK1 and ERK1/ERK2, enzymatic elements comprising the primary MAPK module. Consistent with these observations, ceramide promotes activation of ERK1 (25) and multiple downstream MAPK targets, including phospholipase A_2 (32). In this regard, it is noteworthy that activation of phospholipase A_2 reportedly is required for ceramide-dependent apoptotic and/or inflammatory responses in some settings (33); thus, apart from serving an essential signaling role in normal inflammatory responses, presently it is not clear whether the pathway linking KSR/CAPK to activation of the MAPK cascade (and some downstream elements) also contributes to the induction of apoptosis (34). At present, the corresponding proximal target(s) for ceramide in the initiation of lethal signaling is unknown, although several downstream effector elements essential to the lethal actions of ceramide have been identified. Ceramide engages, through an as yet unidentified coupling mechanism, the protein kinase MEKK1; MEKK1 in turn initiates sequential activation of SEK1 and JNK1/JNK2, which comprise the primary SAPK signaling module. Ceramide rapidly activates JNK1 and JNK2 and multiple downstream SAPK targets, most notably c-Jun (which represents a major component of the AP-1 transcription factor complex) and ATF2 (12, 35–37). Induction of the SAPK cascade is also closely associated with multiple ceramide-dependent proapoptotic stimuli, ranging from activation of cytotoxic receptor systems to the onset of lethal environmental stresses (12). Moreover, interruption of the SAPK cascade (e.g., dominant-negative suppression of SEK1 activity) or interference with outflow from JNK1/JNK2 (e.g., pharmacological inhibition or dominant-negative quenching of c-Jun transactivation potential) abolish the apoptotic responses to ceramide or ceramide-dependent lethal stimuli (12, 20, 37). Ceramide-mediated induction of cell death thus requires acute activation of the SAPK cascade; in addition, activation of this system may be accompanied, in some instances, by a reciprocal inactivation of the MAPK cascade (20), consistent with proposals that the two systems are regulated coordinately.

Other proximal targets for ceramide have been identified. Ceramide stimulates a heterotrimeric cytosolic serine-threonine (class 2A) phosphoprotein phosphatase (ceramide-activated protein phosphatase, CAPP) (38, 39). As ceramide-related apoptosis is reportedly antagonized by okadaic acid and other inhibitors selective for class-2A phosphatases (14), a cytotoxic role for CAPP in ceramide action has been inferred (6). Furthermore, ceramide promotes the okadaic acid-sensitive dephosphorylation of cPKCα, resulting in the loss of phosphorylation on autophosphorylation sites and consequent inactivation of the enzyme (40); because multiple isoforms of cPKC/nPKC lie upstream of Raf-1 in various settings, CAPP-mediated PKC dephosphorylation may represent one mechanism through which ceramide coordinately activates SAPK and inactivates MAPK. Other ceramide target proteins are also known. For example, ceramide additionally stimulates the 'atypical' PKC ζ-isoform (aPKCζ) (41, 42), and stimulates the activity of Vav, a close homolog of Sos selectively expressed in hematopoietic cells (43); neither of these activities has yet been implicated in apoptotic signaling, however.

Sphingoid bases also represent potent cytotoxic effectors in mammalian cells (44, 45). In fact, the initiation of apoptosis by TNF in some cell types appears to derive not from N-SMase-mediated generation of ceramide, but rather from the subsequent deacylation of ceramide to sphingosine (46, 47). This has led to speculation that in some systems sphingosine, rather than

ceramide, represents the primary lethal effector derived from sphingomyelin hydrolysis (46); on the other hand, some investigators have noted that the levels of sphingoid bases sufficient to initiate apoptosis may not be attained under physiological conditions in most cell types (48, 49), but the combined presence of both ceramide and sphingosine may represent a physiologically significant apoptotic signaling mechanism (see below). In any case, sphingosine and dihydrosphingosine (sphinganine) comparably induce apoptosis in many cell types (19, 20, 50, 51). These responses are not mitigated by competitive inhibition of ceramide synthase (e.g., by fumonisin B_1) (19, 20), indicating that the cytotoxicity of sphingosine does not derive from acylation to form ceramide. Myeloid leukemia cells driven to terminal differentiation (becoming monocytes or, ultimately, macrophages) by tumor-promoting phorboids eventually undergo apoptotic cell death (52); this process has been shown to derive from a progressive, maturation-dependent increase in the capacity of differentiated cells to generate sphingosine via deacylation of ceramide (50).

As noted for ceramide, multiple target proteins for sphingosine and sphinganine have been identified. Sphingoid bases inhibit both 'conventional' and 'novel' isoforms of PKC (cPKC, nPKC) through the displacement of diglyceride from the lipid-binding site within the enzymes' regulatory domain (44). This inhibitory influence is associated with both sphingosine and sphinganine, indicating that the *trans*-4 double bond is not essential to confer this aspect of the bioactivity of sphingoid bases; moreover, D and L isomers of *erythro*sphingosine and *threo*sphingosine are comparably effective in the inhibition of cPKC/nPKC, and a complete lack of stereoselectivity in the biological action of sphingosine provides a reliable index of the involvement of cPKC/nPKC. Thus, the apoptotic properties of sphingosine and sphinganine almost certainly derive from acute cPKC/nPKC inhibition, given that the responses *a*) are completely non-stereoselective, and *b*) are closely mimicked by pharmacological agents that inhibit cPKC/nPKC through interaction with the enzymes' regulatory domain (e.g., calphostin C).

Although the cPKC and nPKC isoenzyme subfamilies collectively represent the principal intracellular target for sphingoid base-mediated lethality, there is ample evidence to indicate that the bioeffector properties of these lipids may involve the modulation of additional regulatory systems. Other studies have demonstrated the existence of a novel family of sphingosine-activated protein kinases (53, 54); these isoenzymes are *a*) stimulated by sphingosine in a highly stereospecific manner (with a marked preference for the D-*erythro* species), but *b*) completely insensitive to sphinganine, indicating an absolute requirement for the *trans*-4 double bond in the sphingolipid backbone. Similarly, pronounced stereoselectivity is associated with modulation of other biological targets for sphingosine, including inhibition of the c-src/v-src protein kinases (55) and a variety of enzymatic activities that require calmodulin (CaM) for optimal function (e.g., the multifunctional Ca^{2+}/CaM-dependent protein kinase) (56); the extent to which alterations in these activities contribute to sphingosine-related cell death is presently uncertain.

Among several signaling systems that are implicated in the induction of apoptosis, both ceramide and sphingosine (57–60) have been shown to promote dephosphorylation of the retinoblastoma gene product (pRb), which presumably represents a critical step in cell cycle ejection; ceramide and sphingosine produce G_0/G_1 cell cycle arrest profiles, suggesting that the respective signaling pathways that subserve the lethal effects of each lipid converge within one or more downstream cassettes of cellular activity.

Complex interactions between glycerophospholipid- and sphingophospholipid-derived effectors have been noted with respect to the regulation of cell survival. The capacity of ceramide to promote apoptosis is limited by lysophosphatidate (61, 62) and sphingosine-1-phosphate (24), both of which represent lipid messengers that underwrite proliferative signaling in many cell lines. The downstream systems mediating these anti-apoptotic responses are presently uncertain, but may entail downstream (PKC-independent) activation of the MAPK cascade (63). On the other hand, a detailed study of lipid-sensitive cPKC/nPKC modulation has revealed that these enzymes clearly oppose the apoptotic influence of ceramide. Ceramide-mediated apoptosis is subject to reciprocal modulation by diglyceride and sphingosine in HL-60 and U937 human leukemia cells. Under physiological conditions, chronic elevation of diglyceride promotes differentiation along a monocytic lineage in myeloid cells (64). Interestingly, the lethal actions of ceramide are attenuated or abolished acutely by endogenous and exogenous diglycerides without any evidence of the initiation of a differentiation program (16, 65). In studies performed with synthetic lipids, these responses are exclusively associated with *sn*-1,2-substituted diglyceride isomers (*sn*-2,3-, and 1,3-*rac*-substituted species are ineffective), confirming the direct involvement of cPKC/nPKC in the cytoprotective response. Qualitatively similar cytoprotective influences are associated with pharmacological PKC activators, including both stage-1 and stage-2 tumor-promoting phorboids (e.g., phorbol dibytyrate, mezerein) as well as non-tumor-promoting compounds (e.g., bryostatin 1). Similarly, the apoptotic capacity of ionizing radiation is drastically reduced by phorboids through both

limitation of ceramide generation as well as by antagonism of ceramide-mediated DNA damage (66). Conversely, ceramide-related cytotoxicity is sharply potentiated by sphingoid bases at sub-lethal concentrations (19). Comparable augmentation of the ceramide response is produced by sphingosine and sphinganine; moreover, the potentiative capacity of sphingosine is evenly associated with both D and L forms of *erythro*-sphingosine and *threo*sphingosine. Collectively, these properties demonstrate that enhancement of ceramide action by sphingoid bases derives from inhibition of basal cPKC/nPKC activity rather than from modulation of other subcellular targets such as src or CaM kinase. Ceramide action is also enhanced by highly selective pharmacological inhibitors of PKC (e.g., calphostin C, chelerythrine) at sub-lethal concentrations. These findings have collectively fostered the view that cPKC/nPKC mediates a central cytoprotective influence that counters the cytotoxic outflow of ceramide-dependent signaling systems; the downstream protective system(s) governed by cPKC/nPKC remain to be elucidated, but are likely to include elements known to sustain proliferative activity such as the MAPK cascade (20, 63, 67).

OTHER LIPIDS IN APOPTOSIS

Lysosphingolipids

Extensive cellular attrition is associated with inappropriate accumulation of various lysosphingolipids in sphingolipidoses. Cytotoxicity under these conditions appears to derive directly from lipid-related inhibition of cPKC/nPKC (45). Globoid cell leukodystrophy (GLD), otherwise known as Krabbe's disease, evolves from deficiency in the expression of galactocerebroside β-galactosidase (EC 3.2.1.23); this condition evolves from accumulation of the deacylated derivative of galactocerebroside (GalCer), galactopsychosine (galactosylsphingosine; GalSph). Abundant evidence suggests that the lysolipid GalSph, rather than the parental species GalCer, represents the primary pathogenic metabolite in GLD, however (review, 68). An analogous disparity exists between the bioactivity profiles of these lipids with respect to cPKC/nPKC, inasmuch as GalSph has been demonstrated to inhibit cPKC/nPKC activity potently in vitro (44), whereas GalCer is variously reported to be without effect on these enzymes (45) or to exert a mildly stimulatory influence (69). In addition, homologous disorders of sphingolipid metabolism exhibit similar, if less intensively studied, pathologic bases in that *a*) the deacylated species represents the primary disease pathogen, and *b*) direct inhibition of PKC is implicated in the cytotoxicity of the deacylated species (68). Deficient expression of glycoside β-glucosidase (EC 3.2.1.21) leads to Gaucher's disease, a related condition associated with parallel accumulation of glucocerebroside (glucosylceramide; GlcCer) and glucopsychosine (glucosylsphingosine; GlcSph). GlcSph, which is the primary pathogen in Gaucher's disease, also potently inhibits cPKC/nPKC; in fact, GalSph and GlcSph reportedly are equipotent in the inhibition of cPKC/nPKC activity in vitro (44). Similarly, inadequate expression of sulfatide arylsulfatase A (EC 3.1.6.1) in metachromatic leukodystrophy (MLD), results in excessive accumulation of sulfatide (sulfogalactosylceramide; SO_4GalCer) and lysosulfatide (sulfogalactosylsphingosine; SO_4GalCer). The cytotoxicity that characterizes this disease is selectively associated with SO_4GalSph (but not SO_4GalCer); this attains greater significance in light of the fact that SO_4GalSph (but not SO_4GalCer) inhibits PKC activity in vitro.

Formal consideration of conserved functional relationships between psychosines and cerebrosides was outlined by Suzuki in 1972 in the form of a so-called 'psychosine hypothesis' in which it was proposed that the lipid-mediated pathogenicity in sphingolipidotic disorders is exclusively manifested by the deacylated species (e.g., galactopsychosine) rather than from the acylated parental form (e.g., galactocerebroside) (review, 68). The idea that PKC inhibition was involved in such disease processes as a specific signaling mechanism was subsequently advanced during the mid-1980's by Hannun and Bell (45). In an attempt to explain the broad similarities between many sphingolipidoses, these investigators proposed that the inhibitory influence of sphingosine on cPKC/nPKC associated with the sphingosyl moieties of multiple pathogenic lysosphingolipids derives from prolonged suppression of an undefined cytoprotective system governed by cPKC/nPKC. In each of these sphingolipidotic conditions, prolonged suppression of cPKC/nPKC activity produces extensive loss of glia (e.g., oligodendrocytes, Schwann cells) in both CNS and PNS structures; it is unlikely that the cytotoxicity of lysosphingolipids is unique to glia, but may also be generalized to cell types of nonglial lineage. The idea that acute inhibition of PKC constitutes a pro-apoptotic signal strongly supports the participation of sphingoid bases and other lysosphingolipids as biologically relevant effectors in the apoptotic component of cell survival. It is presently uncertain, however, whether the cytotoxicity associated with GalSph and related lipid pathogens actually represents an unrecognized form of apoptosis.

Bacterial lipopolysaccharides and fatty acids

Joseph et al. (70) have reported that TNF-α, IL-1β, and bacterial lipopolysaccharides (LPS), stimulate

similar cellular responses in myeloid cells. In contrast to TNF-α and IL-1, LPS does not cause sphingomyelin hydrolysis and thus stimulates KSR-CAPK without generation of ceramide. Strong structural similarity between ceramide and lipid A (i.e., the biologically active core of LPS) has been suggested (71). The effect of LPS is markedly enhanced by LPS-binding proteins, and additionally may require the LPS receptor CD14. Thus, LPS provokes cellular responses by mimicking the second messenger function of ceramide. Recently, Yasugi et al. (72) have reported that exposure of rat glioma C6 cells to dolichylphosphate results in cell shrinkage followed by nuclear fragmentation and internucleosomal cleavage of genomic DNA, all of which are classical characteristics of apoptosis. Dolichylphosphate-induced apoptosis is associated with activation of MAP kinase, and inhibition of tyrosine phosphorylation of MAPK by herbimycin A results in inhibition of DNA fragmentation (73). On the other hand, Moore and Matlashewski (74) have shown that lipophosphoglycan (LPG) inhibits apoptosis in macrophages. Jayadev, Linardic, and Hannun (75) have reported that TNF-α stimulates the rapid release of arachidonic acid in HL-60 cells and promotes arachidonic acid-dependent sphingomyelin hydrolysis. These investigators have also found that exogenous addition of arachidonic acid induces sphingomyelin hydrolysis and reproduces effects of ceramide on cell growth. Acute addition of synthetic diacylglycerol, a candidate mediator of TNF-α responses, is without effect on either sphingomyelin turnover or cell growth. Given that the TNF-α activates the sphingomyelin pathway, resulting in the generation of ceramide by stimulation of neutral sphingomyelinase, arachidonic acid has been suggested to serve as a mediator of the TNF-α effects on sphingomyelin turnover. In HL-60 cells, TNF-α reportedly stimulates a rapid release of arachidonic acid; in turn, arachidonic acid stimulates sphingomyelin hydrolysis through the activation of phospholipase A_2 and concomitant ceramide generation. This effect can be mimicked by oleate; the methyl ester and alcohol derivatives of fatty acids are inactive (75). Arachidonic acid generated as a result of phospholipase A_2 activation, ceramide released following activation of sphingomyelinase, and formation of free radicals all have been implicated in a signal transduction pathway resulting in cell death (14, 76). 1,1-Dichloroethylene may initiate apoptosis-like cell degradation in selected parenchymal cells in the liver (77). Macrophage phagocytosis of apoptotic lymphocytes is inhibited by liposomes containing phosphatidyl-L-serine, but not phosphatidyl-D-serine (78, 79). Ether-linked glycerophospholipids such as 1-octadecyl-2-methyl-*rac*-glycero-3-phosphocholine induce apoptosis in human leukemic HL-60 cells (80, 81). Lipid hydroperoxides such as 15-hydroperoxy eicosatetraenoic acid induce cell death in 8E5 cells, a chronically human immunodeficiency virus (HIV)-infected human T cell line (82). The effect is apparently due to a marked reduction of glutathione peroxidase activity. This suggests that the effect of these lipids can contribute to the depletion of CD4 T cells that occurs in AIDS. Retinoids are potential therapeutic agents for apoptosis in Kaposi's sarcoma (83). A synthetic phospholipid, 4-(hexadecyloxy)-3-(*S*)-methoxybutyl phophatidic acid, and naturally occurring phophatidic acid and lysophosphatidic acid are potent and specific inhibitors of phosphatidyl inositol-3-kinase. Inhibition of this kinase after metabolic degradation of ether lipids by phospholipase D may contribute to the cytotoxicity (84).

GANGLIOSIDES AND GLYCOLIPIDS IN APOPTOSIS

Gangliosides and other complex sphingolipids play important roles in cell-to-cell interaction, modulation of cell growth, and cellular differentiation. The biological actions of these lipids are thought to be mediated in part through tyrosine kinases associated with growth factor receptors (85–88) as well as PKC (69, 89, 90). GM1, which has been shown to prevent apoptotic death in PC12 cells, may act by facilitating NGF-induced TrkA dimerization (86–88). Mangeney et al. (91) reported that CD77 and globotriaosyl ceramide (Gb3) may be involved in B cell differentiation. $CD77^+$ B lymphocytes present a morphology similar to that of cells undergoing programmed cell death. Treatment of $CD77^+$ cells with recombinant IL-4 and anti-CD40 antibody prevents apoptosis (91). Lipophosphoglycan, the major surface molecule of *Leishmania promastigotes*, inhibits apoptosis in bone marrow-derived macrophages (92). Koike et al. (93) have reported that mixed bovine brain gangliosides inhibit TNF-α-mediated apoptosis in the murine fibrosarcoma cell line L929 in a dose-dependent manner, resulting in suppression of DNA-cleaving endonucleases. The autoantibody against b-series gangliosides in acute sensory neuropathy induces cell death in rat dorsal root ganglion (DRG) (92). Saito and Saito (94) have also shown that Schwanoma cells cultured in high density in the absence of serum for 5 days start to die after the addition of 100 μM of gangliosides; greater than 91% of the cells die within 4 days, while the control cells show no decrease in the cell number. The effects of gangliosides on cell survival were observed at a concentration as little as 1 μM. Among the gangliosides tested, GT1b showed the strongest effect followed by GD1b and GM1.

GROWTH FACTORS AND NEUROTROPHIC FACTORS IN APOPTOSIS

Many trophic factors can influence axonal growth, elaboration of the dendritic arbor, and neuronal survival during nervous system development, as well as in regeneration of neural structures within the CNS and PNS (94, 95). The sensitivity of neurons to growth factor deprivation, ischemia, excitatory amino acids, etc., may result in progressive neurodegeneration and loss of cognitive function through the deregulation of apoptosis (96). Recent studies have indicated that multiple neurotrophic factors are released from astrocytes, oligodendrocytes, and microglia, suggesting that these neurotrophic factors can prevent neuronal death and promote tissue repair (97, 98). Prakas-Kramarski et al. (99) have reported that rat brain neurons and glial cells express Neu differentiation factor (heregulin) which represents a survival factor for astrocytes. Both ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) reportedly promote the survival and differentiation of developing motoneurons and oligodendrocytes (100). The leukemia inhibitory factor (LIF) and CNTF induce apoptosis in cultured sympathetic neurons (101, 102). BDNF can prevent apoptosis of rat cerebellar granule neurons in culture (103).

Itoh and Horigome (104) have recently shown that NGF promotes ceramide generation through activation of the low-affinity NGF receptor (LNGFR); activation of the LNGFR by NGF prevents apoptosis in some cell lines (review, 105), however, suggesting that the sphingomyelin pathway may, under some circumstances, mediate the cytoprotective (i.e., anti-apoptotic) actions of NGF. This is supported by the additional finding that exogenous ceramides mimic the protective effect of NGF on cell growth inhibition and process formation in glioma cell lines. Overexpression of LNGFR in neural cell lines increases the rate of cell loss due to apoptosis, whereas binding of NGF reverses this process (104). IL-1β is released in the primary cultures of DRG neurons, resulting in apoptosis; this effect can be prevented by NGF. Mayer and Noble (106) have reported that *N*-acetyl-L-cysteine is a pluripotent protector against cell death and an enhancer of trophic factor-mediated cell survival. Dobrowsky, Jenkins, and Hannun (107) have recently examined neurotrophin-induced sphingomyelin hydrolysis and demonstrated the requirement for the expression of the low affinity neurotrophin receptor, $p75^{NTR}$, or the co-expression of $p75^{NTR}$ and tyrosine kinase (Trk) receptors in the cell. These authors have additionally described close physical associations between $p75^{NTR}$ and neutral sphingomyelinase with the microdomain scaffolding protein caveolin (108), strongly suggesting that the proximal signaling apparatus for neurotrophin-induced ceramide generation is co-localized within caveolae or an analogous microdomain structure. These studies support the notion that $p75^{NTR}$ serves as a common signaling receptor for neurotrophins through induction of sphingomyelin hydrolysis and that cross talk exists between the $p75^{NTR}$ and Trk pathways.

APOPTOSIS IN NEURODEGENERATIVE DISEASE

Alzheimer's disease

Alzheimer's disease (AD), the most common form of dementia in late life, represents a primary neurodegenerative disorder, but may also involve aberrant regenerative processes (review, 109). The major clinical symptom of AD is the progressive loss of cognitive function. Neurofibrillary tangles, attendant upon inappropriate sprouting, are related to extensive neuronal loss in AD brain; senile plaques appear to be directly involved in the pathogenesis of AD (110). Pathologically, the major hallmarks consist of the loss of neurons and the appearance of neuritic (or 'senile') plaques and neurofibrillary tangles in surviving neurons throughout the neuropil. Paired helical filaments are found in neurons of patients with AD. Massive somato-dendritic sprouting of neurons is frequently observed in AD brains (111). Neurons in AD brains express fetal antigens, including the fetal type of tau antigen (112), the A68 protein recognized by the monoclonal antibody ALZ-50 (113), gangliosides recognized by the monoclonal antibody A2B5 (114), and a fetal form of α-tubulin. The exact role of cell death in the pathogenesis of AD is presently unknown. The accumulated β-amyloid in the brain may prevent neurons from receiving trophic factors generated at synapses or by adjacent glial cells. Alternatively, exposure to β-amyloid may render neurons more susceptible to the cytotoxic effects of excitatory amino acids (115). Pike et al. (116) have reported that aggregated β-amyloid impairs neurite outgrowth in hippocampal neurons, whereas un-aggregated β-amyloid facilitates neurite outgrowth. Aggregated, but not the monomeric, β-amyloid peptides can induce cell dysfunction and death. Of particular therapeutic interest are attempts to attenuate secretion of β-amyloid peptides from neuronal and glial cells, a strategy that may prove to be of benefit to patients with AD (117). Recent studies have provided strong evidence that abnormal phosphorylation of tau proteins in the affected neurons in AD may play an important role in cell death, possibly by the mechanism of premature apoptosis in-

duced by pro-β-amyloid peptide (118). Tau protein phosphorylation can be induced in human neuroblastoma cells by a hyper-stimulating mixture, consisting of NGF, dbcAMP, gangliosides and sodium butyrate (119, 120); sodium butyrate alone also induces aberrant tau protein phosphorylation (121).

There have been reports on several AD-associated genes such as ALG-1 (presenilin-1, chromosome 14), ALG-2 (presenilin-2, chromosome 1), APP (amyloid precursor protein, chromosome 21), and apoE (apolipoprotein E, chromosome 19), which are involved in AD pathogenesis (122). The ALG-2, Ca^{2+}-binding protein, consists of a 435-base pair cDNA insert and may mediate Ca^{2+}-dependent apoptotic signals. Apoptosis may also play a role in AD-related T-cell receptor-, Fas-, and glucocorticoid-induced cell death (122). In addition, the mechanism of β-amyloid neurotoxicity may be mediated via the phosphorylation of tau and other substrates by tau protein kinase I (TPK-1), resulting in a disruption of axonal transport, cytoplasmic accumulation of amyloid β-protein precursor and its degradation products, synaptic dysfunction, and eventual cell death (121). Aurintricarboxylic acid, a general inhibitor of apoptotic endonucleases, prevents DNA fragmentation and delays cell death (118). Vitamin E, an antioxidant and free radical scavenger, inhibits amyloid β protein induced cell death (123). Pike et al. (124) recently reported that thrombin pretreatment significantly attenuates neurotoxicity mediated by fibrillar aggregates of β 1–42 and β 25–35 amyloid, and it has been shown in the numerous clinical trials that cholinesterase inhibitors (e.g., donapezil hydrochloride; 'aricept') can have beneficial effects on maintenance of cognition and memory in AD patients (125).

Several reports have indicated that the monoclonal antibody A2B5 specifically stains neurons undergoing neurofibrillary degeneration and neuritic processes with senile plaques in AD brains (114, 126, 127). The A2B5 antibody recognizes 'c-series' gangliosides, including GQ1c (128), GT3, and *O*-acetylated derivatives of GT3 (129), which are abundantly expressed during embryonic stages of the brain development (130, 131). Studies on the ganglioside distribution in AD brains have demonstrated altered ganglioside composition in some brain regions (132, 133) and accumulation of 'c-series' gangliosides (GQ1c, GP1c) in neurofibrillary tangles in distinct brain regions. The presence of fetal antigens such as the 'c-series' gangliosides and microtubule-associate protein 5 in AD brain may suggest that regeneration or sprouting of neurons is an ongoing process associated with re-induction of gene expression characteristic in early stages of CNS development (134). The frontal cortex and hippocampus of AD cases exhibit high levels of A2B5 immunoreactivity within those neurons exhibiting neurofibrillary degeneration (135). Collectively, these findings suggest that embryonic gangliosides may be 're-expressed' in AD neurons that have regenerative potential. In fact, this latter observation has prompted a clinical trial using GM1 ganglioside as a therapeutic agent for promoting nerve regeneration in AD (136, 137). It is therefore significant that beneficial effects of GM1 ganglioside have been documented in the treatment of stroke and spinal cord injuries, particularly when treatment has been initiated within a few hours of the acute event. Continuous intraventricular infusion of GM1 has recently been shown to be significantly beneficial in AD of early onset (Type I) (137). Given the established involvement of gangliosides in neuronal survival and/or regeneration (review, 138), abnormalities in ganglioside metabolism may contribute to the process of neuronal degeneration and regeneration in AD.

Although amyloid β protein is secreted into the extracellular space, Yanagisawa et al. (139) have recently noted the presence of membrane-bound amyloid β-protein that tightly binds GM1 ganglioside in AD brains, suggesting that this novel species of amyloid β-protein is eventually shed into extracellular space and may act as a 'seed' for β-amyloid fibril formation. This protein is most likely derived from plasma membranes including synaptic membranes, and/or the trans-Golgi network inasmuch as GM1 is abundant in caveolae, small membranous invaginations that are associated with the cell surface and are functionally linked to the trans-Golgi network in the protein and lipid sorting (140). The recently identified familial Alzheimer's disease (FAD) gene S182, which has been directly linked to AD, encodes a Golgi-derived membranous protein involved in the export of other proteins (141). Therefore, extracellular accumulation of this novel amyloid β-protein may reflect preexisting abnormalities in membrane transport at the stage of amyloid formation (139). In normal brain, GM1-bound amyloid β-protein is absent, supporting the notion that inappropriate accumulation of amyloid β-protein reflects abnormal protein sorting and/or transport in AD patients. Immunolocalization of GD1a ganglioside observed in dystrophic neurons in AD brains suggests that neurites accumulate a novel glycolipid membrane component. GD1a ganglioside accumulates in some senile plaques, and thus has been suggested to contribute to formation of senile plaques (142). Significantly, increased levels of both GM1 and GD1a are evident in cerebrospinal fluid from AD patients as compared with age-matched controls (143).

Parkinson's disease

TNF-α receptors have been observed on the dopaminergic neurons that degenerate in Parkinson's dis-

ease (PD) and TNF-α-immunoreactive glia have been detected in close proximity to these neurons in PD patients. Activation of the sphingomyelin pathway by TNF-α may thus mediate neurodegeneration in PD (144). Recently Brugg et al. (145) have reported that dopaminergic and other neurons in primary cultures derived from the mesencephalon, a primary region of neuronal degeneration in PD, undergo apoptosis through a ceramide-dependent mechanism that may be identical to the cytokine-stimulated signaling pathway in the immune system. The conserved presence of a ceramide-dependent apoptotic system in mesencephalic dopaminergic neurons suggests that inappropriate activation of such a cell death mechanism contributes to PD (145).

Huntington's disease

Kordower et al. (146) have reported that intravenous administration of transferrin receptor antibody-NGF conjugates prevents degeneration of cholinergic striatal neurons in a model of Huntington's disease (HD). This approach may also prove useful in the treatment of AD and other neurological disorders that are amenable to treatment by proteins that cannot readily cross the blood–brain barrier (147). Gliosis characteristic of cortical deterioration associated with demyelinating disease such as multiple sclerosis or Guillain-Barré syndrome is thought to be secondary to changes in neuronal death; thus, glial changes may be involved in the release of glial cell-derived neurotrophic factors (GDNF) that are important in maintaining dopaminergic neurons in PD and motor neurons in ALS (148). Thus GDNF may be a useful therapeutic agent for neurodegenerative disorders, including Alzheimer's disease (149). Recently, it has been demonstrated that GDNF signals through the receptor tyrosine kinase Ret (product of the c-*ret* protooncogene) in a GDNF-responsive motoneuron cell line (150, 151).

Immune-mediated neurological disorders

In the case of demyelinating diseases of the peripheral nerve such as acute ataxic neuropathy and acute sensory-motor neuropathy, it has been reported that IgM antibodies against peripheral nerve glycosphingolipids attack myelin (152–154). Antibodies against various glycosphingolipids have been observed in patients with demyelinating polyneuropathy and several related disorders (152). The mechanisms of how autoantibodies directed against glycolipids cause nervous tissue damage are not fully understood, however. Ohsawa et al. (92) have reported that autoantibodies against b-series gangliosides in acute sensory neuropathy underlie neuronal cell death in rat DRG; these authors speculate that high titers of the autoantibodies against the carbohydrate structure of glycoconjugates on the surface of DRG neurons cause the damage in large myelinated fibers, subsequently resulting in sensory polyneuropathy. We have reported the presence of sulfated glucuronic acid-containing glycolipids (SGGLs) bearing the HNK-1 epitope in bovine brain microvascular endothelial cells (BMEC) (155); we have also presented evidence suggesting that SGGLs play critical roles in the immunopathogenesis of such neurological disorders as peripheral polyneuropathy with IgM paraproteinemia, Guillain-Barré syndrome, and chronic inflammatory demyelinating disorders (152, 156). More recently, we have demonstrated that immunological insults against BMEC-bound glycolipid antigens can induce the destruction or malfunction of the blood–brain barrier (BBB) and blood–nerve barrier (BNB), resulting in the penetration of circulating immunoglobulins into the nerve parenchyma (155). These observations suggest that immunological block of the SGGL epitope by IgM antibody results in disruption of essential cellular interconnections among BMECs, and that loss of such structural organization results in eventual cell death. In this regard, the presence of various antiglycolipid autoantibodies has been reported in several neurologic disorders, including demyelinating peripheral polyneuropathy (156, 157), motoneuron disease (158), polyneuropathy with multifocal conduction block (158), and Guillain-Barré syndrome (160). The pathogenicity of such antibodies has been widely postulated in these disorders, but the mechanism through which a macromolecule the size of an immunoglobulin can traverse the BBB/BNB remains obscure (152). Our study has revealed that BMECs and the peripheral nervous tissues share many glycolipids including SGGLs. Hence, the presumed cascade of pathological processes involves the following essential steps: immunological attack of microvascular endothelial cells, malfunction or destruction of BBB/BNB, increased permeability across BBB/BNB, leakage of immunoglobulins into the endoneurial space, and the subsequent immunological attack of peripheral myelin and axons leading to their destruction (155).

CONCLUSION

Research into sphingolipids has entered into a new and exciting era in recent years owing to the discovery of their modulatory functions in several receptor-mediated signaling pathways and the participation of several sphingolipid metabolites as lipid second messengers in a variety of cellular systems. The physiological effects can be diverse, ranging from their regulatory effects on cel-

lular differentiation and proliferation, as well as on apoptosis. The use of different cell systems at different differentiation states further contributes to the complexity in reaching an understanding of their precise functional roles. Evidence for their involvement in the pathogenetic mechanisms of a variety of neurodegenerative diseases is emerging. The present review, which brings together our current knowledge in several cellular systems as well as the potential role of these sphingolipid metabolites in the nervous system, may stimulate new avenues of research in this exciting area.

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