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SYMPOSIUM: Neuronal Apoptosis

Neuroprotection by the Inhibition of Apoptosis

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Accumulating evidence strongly suggests that apoptosis contributes to neuronal cell death in a variety of neurodegenerative contexts. Activation of the cysteine protease caspase-3 appears to be a key event in the execution of apoptosis in the central nervous system (CNS). As a result, mice null for caspase-3 display considerable neuronal expansion usually resulting in death by the second week of life. At present, 14 caspase family members have been identified and subdivided into three subgroups on the basis of preference for specific tetrapeptide motifs using a positional scanning combinatorial substrate library. Caspase-3 is a group II member (2, 3, 7) categorized by an absolute substrate requirement for aspartic acid in the P_4 position of the scissile bond. The preferred cleavage motif (DExD) for group II caspases is found in many structural, metabolic and repair proteins essential for cellular homeostasis. Consistent with the proposal that apoptosis plays a central in role human neurodegenerative disease, caspase-3 activation has recently been observed in stroke, spinal cord trauma, head injury and Alzheimer's disease. Indeed, peptide-based caspase inhibitors prevent neuronal loss in animal models of head injury and stroke suggesting that these compounds may be the forerunners of non-peptide small molecules that halt apoptosis processes implicated in these neurodegenerative disorders. A clear link between an hereditary neurodegenerative disorder and failed caspase inhibition has recently been proposed for spinal muscular atrophy (SMA). In severe SMA, the neuronal specific inhibitor of apoptosis (IAP) family member known as NAIP is often dysfunctional due to missense and truncation mutations. IAPs such as NAIP potently block the enzymatic activity of group II caspases (3 and 7) suggesting that NAIP mutations may permit unopposed developmental apoptosis to occur in sensory and motor systems resulting in lethal muscular atrophy. Conversely, adenovirally-mediated overexpression of NAIP or the X-linked IAP called XIAP reduces the loss of CA1 hippocampal neurons following transient forebrain ischemia. Taken together, these findings suggest that anti-apoptotic strategies may some day have utility in the treatment of neurodegenerative disease. The present review will summarize some of the recent evidence suggesting that apoptosis inhibitors may become a practical therapeutic approach for both acute and chronic neurodegenerative conditions.

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Caspases: members and activation hierarchy

Caspases are a family of cysteine proteases with aspartyl protease activity. Phylogenetic analysis indicates that the caspase gene family can be divided into two broad classes on the basis of homology to either ICE (caspase-1) or to mammalian counterparts of CED-3. Additional categorization has been made on whether the proenzymes have short prodomains (caspases-3, -6, -7) or long prodomains (the rest) (33). The use of a positional scanning combinatorial substrate library permitted division of caspase family members into three groups (40, 56). The major specificity determinant is the nature of the amino acid(s) preferred in the P_4 position of the tetrapeptide cleavage motif (for review see, (33)). Group I caspases (1, 4, 5, 13) prefer bulky hydrophobic amino acids such as Tyr or Trp at P_4. This profile is consistent with their known role in cytokine processing but excludes a major function in apoptosis since none of the

Figure 1. Caspase X-ray crystal structure. The caspase tetramer is comprised of two large subunits (outermost left (dark blue) and right (brown) subunits) and two small subunits (inner left (brown) and right (dark blue)). The caspase-3 tetramer is shown with its inhibitor, Ac-DEVD-CHO (red), in each of the two resultingactive sites.

proteins cleaved during apoptosis has hydrophobic residues at P_4. Group II caspases (2, 3, 7) have a near absolute requirement for Asp at P_4, and prefer a cleavage motif DExD found in many proteins cleaved during apoptosis. As a result, group II caspases are considered to be executioners of apoptosis. Group III caspases (6, 8, 9, 10) prefer branched chained aliphatic amino acids in P_4 that are found at the maturation sites of group II and III caspases. Since these bulky amino acids are found at the activation sites for group II caspases, a hierarchy of initiator (caspase III) and executioner caspases is suggested by this amino acid arrangement. Hence, the activation of Group III caspases such as 8 and 9 may be initiation events that led to the activation of Group II caspases. Recently the importance of caspase-9 activation as an early step in neuronal apoptosis has been highlighted by the similar phenotype of caspase-9 and caspase-3 null mice (25). Feedback activation of caspase-9 by caspase-3 is thought to act as a feed forward cycle that once initiated may rapidly result in uncontrolled neuronal apoptosis (51). The development of selective caspase-9 inhibitors will help to determine what the precise role of caspase-9 activation as well as caspase-3 mediated cleavage of caspase-9 plays in neuronal apoptosis in neurodegenerative contexts.

Generation of active caspase-3 and the active site of this enzyme

Proteolytically active caspase-3 is generated by processing of the caspase-3 precursor into p17 and p12 fragments which form the mature tetramer $(p17/p12)_2$ (Figure 1) (34, 55). Both the large p17 and small p12 subunits contribute residues that form the substrate binding cleft (S_1-S_4), however, the major determinants for substrate specificity (S_4) are contained within the small subunits (Figure 2) (41). In the case of caspase-3, each of the amino acids of the optimal tetrapeptide substrate DEVD (P_1-P_4) fit into one of the four subsites termed S_4-S_1 found in the active site of the active enzyme. The highly precise geometry of the S_4 pocket of caspase-3 is responsible for the strict specificity of this enzyme for Asp in the P_4 binding position. By contrast to the narrow S_4 pocket of caspase-3, the S_4 pocket of caspase-1 is a large shallow depression permitting the binding of large hydrophobic residues. This difference accounts for the broad substrate specificity of caspase-1 relative to caspase-3 that will inevitably permit the development of potent small molecule inhibitors that distinguish between these two caspases.

Neuronal apoptosis and caspase 3 activation following ischemic injury

Within the developing central nervous system (CNS), approximately 50% of the total neuronal population is eliminated by apoptosis (39). Genetically engineered mice which lack caspase-3 fail to display developmental apoptosis in the CNS suggesting that activation of this caspase is crucial to programmed neuronal death (26). Morphological and biochemical characterization of central neurons injured by a brief episode of global or focal ischemia suggests that apoptosis contributes to ischemic cell death (29, 35). Two lines of evidence indicate that caspase-3 activation plays a key role in neuronal apoptosis following transient forebrain ischemia. Immunohistochemical and biochemical studies have shown that caspase-3 activation occurs in susceptible cortical and hippocampal neurons following transient focal and global ischemia, respectively (32, 59). Using an antibody generated against active caspase-3, we have observed that caspase-3 activation occurs in degenerating CA1 neurons following a brief episode of forebrain ischemia (59). These increases occurred before the appearance of fragmented DNA in CA1 neurons suggesting that ischemia-induced caspase-3 activation and DNA fragmentation may be linked. Recently, a caspase-3-activated deoxyribonuclease termed CAD has been identified which causes the degradation of chro-

mosomal DNA into nucleosomal units characteristic of apoptosis (14). In normal cells, CAD activity is suppressed by binding to its inhibitor ICAD. Caspase-3 cleaves ICAD permitting CAD to enter the nucleus and commence DNA fragmentation (44). The second line of evidence comes from studies performed with modified peptide inhibitors that take advantage of the high-affinity binding of a P_4-P_1 tetrapeptide to the caspase active site. A major caveat associated with such an approach is that commonly used inhibitors such as Z-VAD(OMe)-CH2F and DEVD(OMe)-CH_2F are not caspase-3, or for that matter, caspase selective inhibitors. For instance, both of these inhibitors block cathepsins (46). Nevertheless, intracerebral administration of Z-VAD(OMe)-CH_2F or DEVD(OMe)-CH_2F has been shown to reduce cellular and behavioural deficits following transient focal ischemia (15, 19). Moreover, neuroprotection can still be achieved when intracerebral administration of DEVD(OMe)-CH_2F is delayed by 6-9 hr after mild (30 min) transient focal ischemia (15, 16) or cerebral hypoxia (49). This prolonged therapeutic window makes caspase inhibitors particularly attractive for the treatment of stroke.

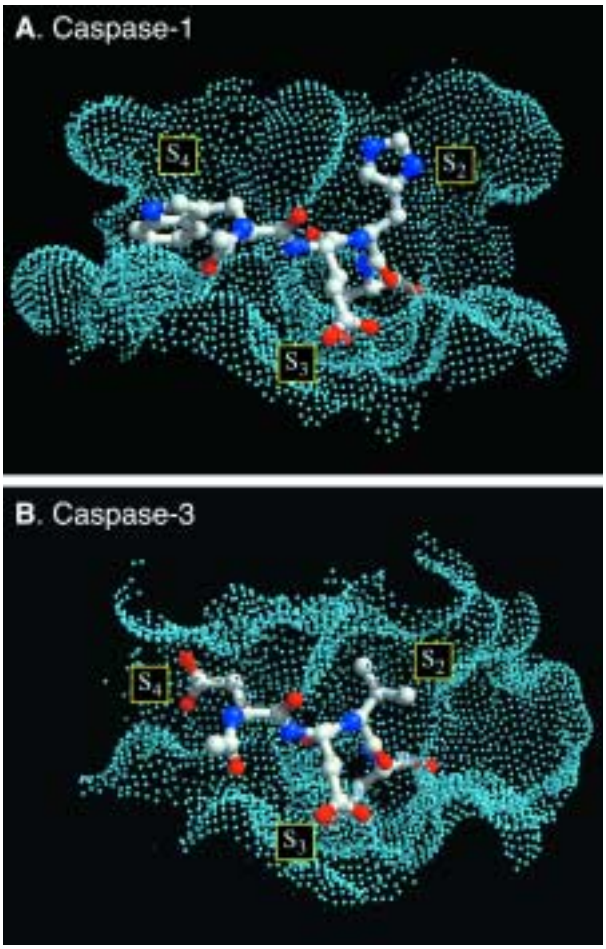

Figure 2. Topology of caspase-1 (**A**) and caspase-3 (**B**) active sites. The solvent-accessible surface is shown in marine blue. Bound inhibitors are shown in white (nitrogens are coloured blue, oxygens are red). Caspase-1 (**A**) is shown with Ac-WEHD-CHO whereas caspase-3 (**B**) is shown with Ac-DEVD-CHO (aldehyde inhibitor versions of the optimal substrate for each enzyme). The major subsites (S_4, S_3, S_2 which bind their respect P_4, P_3, P_2 residues) are indicated. The P_1 Asp penetrates into the plane of the figure and is not visible. Note the major difference in the S_4 subsite which is a large, open depression in caspase-1 versus a smaller, tighter pocket in caspase-3.

The IAP gene family

The importance of caspase-3 activation in apoptosis is highlighted by the discovery of proteins that block apoptosis, at least in part, by inhibiting group II caspases (3 and 7). These proteins, known as inhibitors of apoptosis (IAPs) are encoded by insect viruses which enable viral propagation by blocking defensive apoptosis of the infected cell (1, 5, 6, 8). Subsequent work has revealed that these genes represent a highly conserved anti-apoptotic strategy found in organisms ranging from *C. elegans* to *Drosophila melanogaster* (20) to birds (13) to mammals (28). The first human IAP to be identified was Neuronal Apoptosis Inhibitor Protein (NAIP) which led to the discovery of three other human IAPs by homology screening.

NAIP

A provocative link between developmental apoptosis and a hereditary neurodegenerative disease has been shown for acute spinal muscular atrophy, a fatal childhood neurological disorder (SMA) (43). Linkage analysis of SMA inheritance enabled positioning cloning of the mutated sites which interestingly occurred in a novel anti-apoptotic gene termed *naip* (neuronal apoptosis inhibitor protein). Mutations resulting in loss or truncation of the 150 kD encoded protein are highly correlated with the severity of SMA. Mutations in the gene encoding the Survival of Motor Neurons (SMN) protein clearly play a fundamental role in SMA, likely by disrupting pre-mRNA splicing (27, 37). However, genetic mutations of NAIP may also contribute to this disorder by permitting unrestrained developmental apoptosis in a variety of sensory and motor systems. The molecular mechanisms by which NAIP blocks apoptosis are dependent upon three conserved domains approximately 60-70 amino acids in length initially characterized by Dr. Miller as baculoviral-inhibitor of apoptosis repeats (BIRs) (5). These regions, shared by all members of the

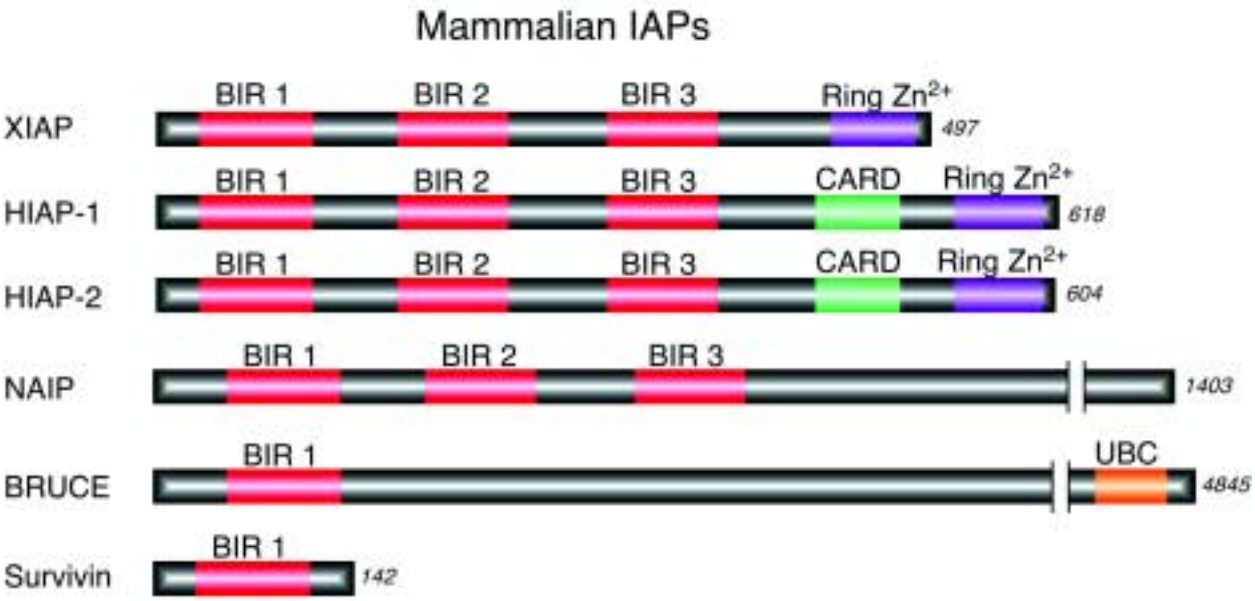

Figure 3. Structural features of mammalian BIR domain-containing proteins. IAPs are defined by the presence of at least one BIR domain. The relative locations of BIR, RING-zinc finger (XIAP, HIAP-1, HIAP-2), caspase recruitment domain (CARD) (HIAP-1, HIAP-2) and UBC (ubiquitin-conjugating) (BRUCE) domains are shown for each IAP. BRUCE (BIR repeat containing ubiquitin-conjugating enzyme) is a giant (528-kD) protein that requires a UBC domain for ubiquitin-conjugating activity. Survivin contains a single BIR and has been shown to be up-regulated in many forms of cancer. The BIR 1 on survivin is most homologous with the caspase inhibitor BIR 2 of NAIP, XIAP, HIAP-1 and HIAP-2. Amino acid length is shown to the right of each protein.

IAP (inhibitor of apoptosis proteins) family (NAIP, XIAP, HIAP-1, HIAP-2, Survivin and Bruce), play an essential role in their ability to block caspase mediated cell death (11, 12, 42). It is therefore noteworthy that over two-thirds of patients with acute SMA exhibit deletions of *naip* which result in loss of all of the first BIR and most of the second BIR domains (43). Thus, it appears that the BIR domains are essential to the function of NAIP in maintaining motor neuron survival. Although initially not thought to be an inhibitor of the group II caspases-3 and -7 (42), recent evidence indicates that NAIP is an excellent group II caspase inhibitor demonstrating that this IAP may reduce apoptosis, at least in part, by blocking the activities of caspases-3 and -7 (unpublished observations).

X-chromosome-liked Inhibitor of Apoptosis Protein (XIAP)

The isolation of NAIP prompted the search for other human genes that might encode IAP-like proteins. First to be identified was a gene located on the X chromosome at Xq24-25 which encodes a protein termed XIAP (for X-linked IAP) that contains three BIR motifs and a RING zinc-finger (28) (Figure 1). The BIR domains of XIAP are sufficient for both suppression of apoptosis and inhibition of caspase-3 and caspase-7 (12, 42). The ability of XIAP to inhibit caspases-3 and -7 has been localized to BIR-2 of XIAP (54).

Human Inhibitor of Apoptosis Protein-1 and -2 (HIAP-1 and HIAP-2)

Next to be identified were two new human cDNAs *hiap*-1 and *hiap*-2 (28). Both of these genes encode proteins that contain three BIR motifs and a RING zinc-finger and map to chromosome 11. The high degree of conservation (72% for the overall amino acid sequence) between HIAP-1 and HIAP-2, as well as their location on chromosome 11 is suggestive of a duplication event. In contrast, XIAP exhibits only 44% and 42% conservation with HIAP-1 and HIAP-2, respectively. NAIP is much more distantly related to the other IAPs, with only 25-30% conservation observed. Moreover, NAIP lacks the RING zinc-finger present in baculovirus and the other human IAPs. Nevertheless, there is a high degree of homology through the BIR domains, especially with HIAP-2, with which NAIP exhibits 58% identity. As is the caspase for other members of the IAP family, the BIR domains of both HIAP–1 and HIAP-2 are excellent inhibitors of caspase-3 and caspase-7 (42). HIAP-1 and HIAP-2 are unique among the IAPs in that they each have a caspase recruitment domain (CARD) motif (21). Although CARD motifs have been identified in several

caspase pro-domains and adapter proteins where they mediate interaction with other CARD containing proteins, a role for this domain in the IAPs has not been established. The structural organization of these proteins is summarized in Figure 3.

NAIP induction is associated with neuroprotection

Immunohistochemical studies have shown that there is a striking overlap between neuronal populations lost in SMA and the distribution of NAIP immunoreactivity in the rodent CNS (61). This work also revealed a relative enrichment of NAIP levels in hind and forebrain neuronal groups known to be relatively resistant to excitotoxic and anoxic damage. For instance, high levels of NAIP immunoreactivity were observed in mesencephalic trigeminal neurons; these sensory neurons are known to be selectively resistant to the injurious effects of kainic acid (7). In the striatum, NAIP immunoreactivity was confined to cholinergic interneurons and up-regulated in these interneurons by a brief episode of transient forebrain ischemia (60). It is well known that cholinergic interneurons in the striatum are considerably more resistant to excitotoxic and ischemic insults than medium spiny neurons which comprise about 95% of the total population of neurons in this structure (2, 4). Consequently, the rapid increase in NAIP levels which occurs in cholinergic neurons after transient global ischemia may contribute to the relative resistance of these neurons to ischemic and excitotoxic injury. In contrast, neurons of the reticular nucleus of the thalamus, known to be exquisitely sensitive to excitotoxic or ischemic injury, display weak NAIP immunoreactivity. Unlike the majority of thalamic neurons, NAIP levels were not elevated in neurons of the reticular nucleus by a brief period of global ischemia. These findings suggest that there is a positive relationship between the ability of neurons to increase NAIP expression and resistance to excitotoxic/ischemic injury. These findings suggested that treatments capable of increasing NAIP expression should be neuroprotective.

The small molecule alkaloid K252a, related in structure to staurosporine, has been shown to exert neuroprotective actions in both *in vitro* and *in vivo* models of neurodegeneration (3, 18, 23, 52). The mechanism by which K252a is able to reduce neuronal injury is unclear but may involve inhibition of c-Jun N-terminal kinase activity or modulation of neurotrophin receptor signaling (30, 38). Interestingly, K252a has been shown to have trophic influences on primary cultures of cholinergic neurons from the spinal cord or basal forebrain (22). Since a loss of NAIP function has been implicated in the death of motor (cholinergic) neurons in SMA, we reasoned that the neurotrophic effects of K252a on these neuronal populations might be mediated by an increase in NAIP expression. In support of this hypothesis, NAIP levels were elevated in the thalamus, striatum, hippocampus and spinal cord 3 hours after a single injection of K252a (0.1 mg/kg, s.c.)(60). These findings suggested that K252a may be able to protect hippocampal neurons from excitotoxic/ischemic injury by elevating NAIP levels. Although a single injection of 0.1 mg/kg, s.c. of the bacterial alkaloid K252a had facilitatory effects on the levels of NAIP mRNA and protein in the hippocampus there was not a reduction in CA1 neuron loss 5 days following 10 minutes of global ischemia (60). However, repeated injections of K252a (0.1 mg/kg/day for 1 week) produced a significantly greater increase in NAIP expression relative to that seen after one injection (60). In a fashion consistent with this NAIP induction profile, neuroprotection was evident after a chronic injection paradigm starting 5 days before the ischemic insult (60). The development of small molecules that selectively up-regulate IAPs such as NAIP may therefore have therapeutic potential for the treatment of stroke. The recent demonstration that expression of ITA, an avian IAP highly homologous to HIAP-1, is dramatically upregulated by NGF in primary cultures of sympathetic and dorsal root ganglionic neurons is consistent with the idea that K252a may induce NAIP by modulating Trk signaling (57).

IAP overexpression *in vivo* (dorsal hippocampus) reduces both CA1 neuron and spatial memory losses following ischemic injury

As a direct test of the *in vivo* neuroprotective effects of NAIP, we used an adenoviral expression system to elevate NAIP levels in the CA1 region (60). This expression system contained a 6-Myc tag that was used to confirm overexpression of the NAIP-Myc tagged construct. Cell counts performed on coronal tissue sections immunohistochemically processed to visualize myc-labeled CA1 pyramidal neurons suggested that approximately the same number of neurons were infected as survived the anoxic insult. NAIP overexpression suppressed DNA fragmentation normally evoked by an episode of transient forebrain ischemia suggesting that NAIP overexpression protected against ischemic injury by blocking apoptosis. In support of this hypothesis, adenovirally-mediated overexpression of the potent caspase-3 inhibitor XIAP (12) blocks ischemia-induced increases in conformationally-active caspase-3 and DNA fragmentation in CA1 neurons (59).

Comparisons of the relative neuroprotective efficacy of XIAP, NAIP, HIAP-1 and HIAP-2 in both *in vitro* and *in vivo* settings suggest that XIAP is the most effective of the four IAPs examined (50, 59, 60). A major concern following cerebral ischemia studies is that blockade of apoptosis may not be sufficient to prevent the loss of neurophysiological processes responsible for movement and cognition. Spatial memory is sensitive to the loss of CA1 pyramidal neurons following a short period of global cerebral ischemia. The Morris Water maze was therefore employed to determine whether XIAP overexpression could maintain long-term spatial memory. As a further control for the health of CA1 hippocampal neurons, we examined expression of the neuronal activity marker NGFI-A by immunohistochemistry. Basal expression of NGFI-A in the hippocampus of normal animals is high and driven by natural synaptic activity. Administration of the NMDA receptor inhibitor MK-801, markedly reduces basal levels of NGFI-A immunoreactivity in CA1 neurons suggesting that expression of this immediate-early gene is driven by depolarization (58). Consistent with this line of reasoning, NGFI-A expression in the contralateral visual cortex is reduce by prolonged blockade of action potentials in retinal ganglion neurons. With respect to ischemic injury, basal expression of NGFI-A in the CA1 region of the hippocampus is markedly reduced 48 hours after a 20 minute episode of global ischemia (31). This decrease appeared to be a function of reduces neuronal activity rather than a general reduction in cellular protein synthesis because the expression of Jun and FosB are maintained 48 hours after transient forebrain ischemia ischemia (31). These findings suggest that NGFI-A immunoreactivity can be used to gauge the status of excitatory gene signaling in CA1 neurons following global ischemia.

Analysis of cell survival 14 days following a 12 min episode of cerebral ischemia revealed a 90% loss of CA1 neurons in the dorsal hippocampus of animals injected bilaterally with control virus encoding the bacterial reported LacZ. The XIAP adenovrius increased cell survival by 5-6 fold relative to the LacZ controls. These animals also failed to display loss of spatial memory in the water maze. Cell counts of NGFI-A positive neurons revealed that CA1 neurons rendered more resistant to ischemic injury by the XIAP adenovirus displayed high basal levels of NGFI-A immunoreactivity (59). Hence, XIAP overexpression maintains the homeostasis of CA1 neurons following transient forebrain ischemia.

Adenovirally-mediated IAP overexpression *in vitro* prevents neuronal apoptosis by inhibition of caspases.

Cultured cerebellar granule neurons are a useful and widely used model to study molecular and biochemical pathways of neuronal apoptosis. After differentiation of cerebellar granule neurons in culture and the development of an extensive neuronal network, potassium withdrawal leads to a synchronous apoptotic cell death characterized by chromatin chondensation, pyknosis, and nucleosomal size DNA fragmentation (9, 47, 48, 63). Following potassium withdrawal new mRNA and protein synthesis, the release of cytochrome c and the activation of caspases are sequential and essential events to induce apoptosis (17, 48). There is no regulation of mRNA or protein expression of Bcl-2 family members in this model (17). This model system was used to study the effects of virally mediated overexpression of NAIP, HIAP-1, HIAP-2 and XIAP (50). Cerebellar granule neurons express significant levels of rat IAP (RIAP-2) mRNA and protein, but endogenous expression of RIAP-1, NAIP, and XIAP was not detected. RIAP-2 mRNA content and protein levels did not change after potassium withdrawal. In culture more than 90% of cerebellar granule neurons are successfully infected by adenovirus-mediated gene transfer and express the reporter gene product. Overexpression of NAIP, HIAP-1, HIAP-2 and XIAP in cerebellar granule neurons blocks activation of caspase-3, DNA fragmentation, TUNEL labelling and apoptotic cell death after potassium withdrawal (50). Of the IAPs tested, XIAP had the greatest efficacy while NAIP was least. Since the expression of XIAP blocked not only caspase-3 activity but also the activation of caspase-3 (50), we asked whether XIAP interferes with upstream initiator caspases. In fact, we found inhibition of caspase-9 by overexpression of XIAP after potassium withdrawal (Gerhardt and Schulz, manuscript in preparation). This finding is consistent with recent results obtained using recombinant proteins that show the BIR-3-RING zinc finger of XIAP is a specific inhibitor of caspase-9 whereas the BIR-1 to BIR-2 portion is specific for caspases-3 and -7 (10). Therefore XIAP possesses two different caspase inhibitory activities which can be attributed to distinct domains within XIAP. These data may provide an explanation for why IAPs have evolved with multiple BIR domains.

To study whether the protective effects of IAPs are specific for caspase mediated cell death or whether they may also be protective in other cell death paradigms we tested whether adenoviral mediated overexpression of

XIAP, HIAP-1, HIAP-2 and NAIP would also protect against NMDA-induced excitotoxicity. At NMDA concentrations that did not induce caspase-3 activity, no protection was observed, suggesting that IAP overexpression may selectively block apoptosis mediated by caspases but not excitotoxicity (50).

Neurotransplantation as a treatment for Parkinson's disease: Caspase-3 inhibitors improve graft survival

L-DOPA therapy is currently the most effective treatment for PD, however, the long-term utility of this therapeutic approach is limited because L-DOPA does not abate the disease process, but rather, temporarily ameliorates the symptoms of PD. Hence, the efficacy of L-DOPA therapy is reduced over time as the disease advances and there are fewer surviving nigral neurons to supply the necessary dopamine to the striatum. More recently, functional recovery through embryonic neural tissue grafts into the area of the substantia nigra or the denervated striatum have shown encouraging potential for a long-term treatment for PD (24). While this surgical approach has shown promise, complications such as host-graft rejection, profound graft cell death and transplant regulation require further study in order to maximize the clinical efficacy of this treatment. Whereas current treatment regimes suffer limitations in technique or length of treatment efficacy, caspase inhibition holds tremendous potential as a possible adjunct to human embryonic neurotransplantation treatments.

The utility of neural grafting as a treatment for PD is limited by the shortage of human donor tissue and the poor survival of dopaminergic neurons grafted into patients, which is estimated at 5-10% (36). Caspase-3 activation occurs in embryonic nigral neurons following isolation as well as grafting into the host striatum. Treatment of embryonic nigral cell suspension with the caspase inhibitor Ac-YVAD-cmk increases survival of dopaminergic neurons grafted to hemiparkinsonian rats and thereby improved functional recovery (45). These findings suggest that caspase inhibitors may be able to increase the survival of embryonic dopaminergic neurons transplanted into the striatum of PD patients.

Clinical development of caspase-3 inhibitors for the treatment of neurodegeneration

The demonstration that caspase activity increases in damaged neurons following acute neurodegenerative insults such as head injury, spinal cord trauma and cerebral ischemia coupled with the ability of peptide-based, non-selective caspase inhibitors (Z-VAD(OMe)-CH_2F and DEVD(OMe)-CH_2F) to reduce traumatic and ischemic injury strongly suggest that small molecule inhibitors may have utility in the treatment of these disorders (32, 53, 62). Several obstacles will have to be overcome to make such an approach practical, i.e. improved blood-brain-barrier penetration, caspase selectivity, potency and pharmacokinetic properties. In order to be neuroprotective, currently available inhibitors must be injected directly into the brain in large quantity compromising interpretation of the experimental results. In this regard, the neuroprotective features of the IAPs are particularly instructive since these proteins selectively block group II caspases (3 and 7). Accordingly, group II selective inhibitors should be neuroprotective. The next and most important test will be to establish at the behavioural level whether such compounds can maintain neuronal function in animal models of neurodegeneration.

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