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# Molecular Pathways in Cerebral Ischemia

## *Cues to Novel Therapeutic Strategies*

***Brigitte Onténiente,* Sowmyalakshmí Rasika, Alexandra Benchoua, and Christelle Guégan***

*INSERM U421, Université Paris XII-Val-de-Marne, Faculté de Médecine, F-94010 Créteil cedex, France*

## Abstract

Stroke is one of the leading causes of death and severe disability in most industrialized countries. Despite the extensive research efforts of both academic and industrial laboratories during the last few decades, no changes have been brought about by the design of neuroprotective therapies. The progressive decrease of stroke-induced death and disability is entirely attributable to improvements in the identification and reduction of risk factors. Over the past few years, experimental research has led to the emergence of a wealth of information regarding the complex and interrelated processes of neuronal degeneration and death triggered by ischemia. This unprecedented insight has led to new theories on the mechanisms of ischemic damage, and has suggested new targets and strategies for therapeutic intervention designed to reduce the clinical consequences of stroke. Among current developments, three strategies seem particularly appealing—namely, the limitation of initial or secondary neuronal death by inhibition of apoptotic mechanisms, the enhancement of the endogenous capacity of nervous structures to restore lost function, and the replacement of lost cells by transplantation therapy.

**Index Entries:** Apoptosis; caspases; clinical research; therapy; stroke.

## Introduction

Stroke is a major cause of morbidity and mortality, and accounts for a large proportion of the health care costs of industrialized countries. Approximately one-half of the patients who are hospitalized for neurological diseases are stroke patients, and ischemic brain insults are common in hospitalized patients who are undergoing surgery. Because the incidence of stroke increases with age *(1)*, the absolute number of patients with stroke is likely to reach new heights in the coming decades because of the aging of the population. The

*Author to whom all correspondence and reprint requests should be addressed. E-mail: ontenien@im3.inserm.fr

premature newborn population is also at high risk. Ischemic brain injury is a major cause of mortality and severe neurodevelopmental disability (cerebral palsy, mental retardation, epilepsy, neurological handicap, and learning disability) in 40% of newborns with intraventricular or germinal matrix hemorrhage *(2).*

Despite substantial research into neuroprotection, and a remarkable number of positive results from laboratories, no neuroprotective agent has been conclusively shown to be clinically effective in acute stroke to date *(3).* Except for thrombolysis therapy, which is applicable in the case of a very limited proportion of patients *(4),* current clinical management is limited to supportive measures *(5),* and stroke therapy is at the same stage of neuroprotection as 20 years ago. The failure of neuroprotective drugs in the clinic has been tentatively attributed to several factors that include: i) difficulty in finding a clinically relevant delivery system to administer compounds intracerebrally over a long period of time; ii) difficulty in transposing standardized experimental settings to human situations, which are characterized by extreme heterogeneity in the etiology, location, and severity of ischemic stroke, and often associated with comorbidity in elderly patients; and iii) the lack of experimental evidence for long-term protection.

The development of new therapeutic approaches thus remains a crucial challenge. It depends upon the elucidation of the molecular mechanisms that mediate neural cell death and degeneration in ischemic insults. Several new strategies are currently emerging, based on recent advances in our understanding of molecular pathways that could be considered as potential therapeutic targets. Among these strategies, the most extensively studied are: i) the restriction of cell death, particularly in view of the finding that apoptosis occurs in these pathologies, ii) the stimulation of intrinsic autoprotective and repair mechanisms, and iii) the reconstruction of damaged tissue by transplantation of multipotent cells. This article summarizes the biochemical basis of ischemic degeneration, and the development of the first clinically targeted compounds based on this knowledge. It also describes more recent advances regarding the discrete molecular events of apoptosis and the resulting novel therapeutic approaches.

## Necrosis vs Apoptosis: the Quest for Definitions

The characterization of cell-death pathways in pathological settings is of primary importance to therapeutics. Driven by the compelling desire to cure stroke, the definition of the types of cell death in ischemia has been a topic of intense investigation during the last decade. During the course of these studies, scientific controversies and semantic debates have appeared that have made this topic even more confusing. Today, it appears that ischemic cell death cannot truly be confined to specific categories, but instead proceeds by switching from one form to another, according to the specific adaptive capacities of each cell type and the regional evolution of environmental perturbations.

Cell death in ischemic lesions has traditionally been considered necrotic on the basis of location, time elapsed after the insult, loss of basophilia, and the presence of karyorhexis *(6,7).* However, necrosis is not a form of cell death, but rather a morphological pattern resulting from intracellular modifications, which for the most part must still be qualified. In addition, the term "necrotic cell death" has been applied in ischemia to two different forms of decay—one in which the cell swells until the membrane ruptures, which concurs with the original description of necrosis but is rarely observed, and another in which the cell shrinks and becomes very electron-dense. From the mid 1990s, our knowledge of the apoptotic process has been fostered by the development of techniques intended to define the molecular cues of apoptosis, and the participation of this form of cell death in injuries previously known as "necrotic," which include ischemia, has become obvious *(8–10).* Although this was accepted

rather widely, the controversy arose from the fact that some of the molecular hallmarks of apoptosis present in most ischemic cells did not find the expected ultrastructural correlates. This was particularly evident in infarct cores or in damaged areas after prolonged global ischemia. Apoptosis is an energy-requiring mechanism that involves active proteolytic activity, leading to nuclear and cytoplasmic condensation, internucleosomal DNA cleavage, and cell compartmentalization into apoptotic bodies that are engulfed by neighboring macrophages *(11).* In ischemic tissues, electron microscopy reveals mixed features, characteristic of both apoptosis and necrosis, in single cells, or co-existence of neurons with either apoptotic or necrotic features in the same area *(12).* The main characteristics of necrosis—cell and organelle swelling and rupture *(13)*—have rarely been observed in neurons of the core. As a result, there is significant confusion regarding which morphological features accurately reflect necrosis or apoptosis in acute insults *(14).*

To reconcile the available biochemical and morphological evidence, two main hypotheses have been proposed. The first is that ischemic cell death may proceed via a number of hybrid pathways with similar operative mechanisms, along a continuum between necrosis and apoptosis *(15,16).* Such common mechanisms include excitatory amino acid release and alterations in ionic homeostasis, which contribute to both necrosis and apoptosis. An alternative hypothesis is based on the attractive concept that, under anoxic/ischemic conditions, apoptosis may be masked by necrosis *(17).* In vitro studies have provided several examples of shifts from apoptosis to necrosis, and *vice versa,* following environmental perturbations *(18)* or modifications of intrinsic determinants of cell death such as adenosine triphosphate (ATP) *(19)* or nitric oxide *(20).* A new term, necrapoptosis, has also been suggested to reflect possible changes in the course of cell death *(21).* In cerebral ischemia, energy levels in the infarct core approach zero *(22),* but are maintained in the penumbra because of retrograde perfusion from adjacent arteries via anastomoses, providing an opportunity for neuronal apoptosis to fully develop.

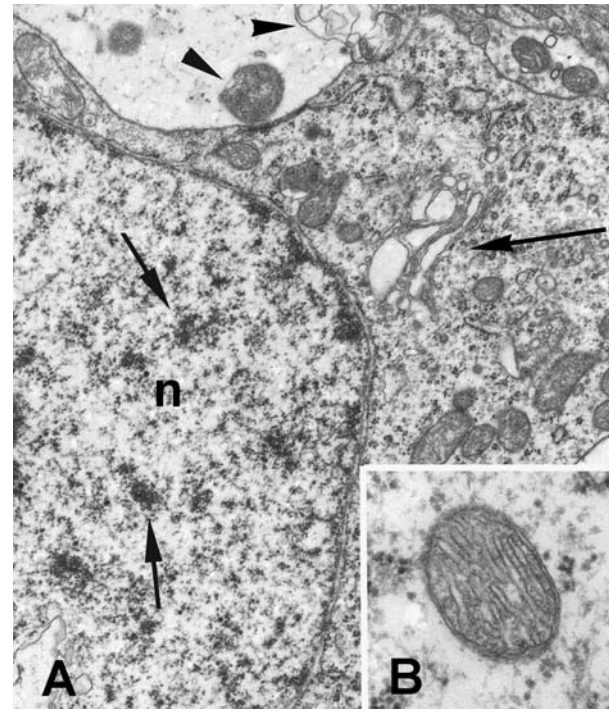


Fig. 1. Examples of the early morphological changes associated with ischemic cell death in the core of a focal infarct after 1 h occlusion of the middle cerebral artery in mice). **(A)** Conventional necrosis (arrow) is featured by rupture of protoplasmic and mitochondrial membranes (arrow-heads). **(B)** Early signs of apoptosis include swelling of the ER and light compaction of chromatin (arrows), with intact mitochondrial structure. n, nucleus.

Confusion has also arisen because most of the observations on the morphology and biochemistry of dying cells have been made during late stages of infarction. In the early stages of cerebral infarction, neurons of the so-called "necrotic" core display a number of morphological features of early apoptosis, such as dilation of the endoplasmic reticulum (ER) without rupture of the membrane (Fig. 1). Thus, apoptosis is indeed being triggered by acute ischemia, but as the availability of cellular energy declines, apoptotic degenerative

processes are replaced to varying degrees by necrotic processes.

Despite semantics, necrosis and apoptosis can no longer be categorized as two exclusive pathways for ischemic cell death, clearing the way for the development of neuroprotective therapeutic strategies for stroke that focus on the effects rather than the causes. Considering the lack of potential "anti-necrotic" factors, cell death observed in the core of the infarct was believed to be beyond the reach of therapeutics until now *(18),* while factors interfering with apoptotic cascades were being actively developed for clinical purposes *(23).* It is now time to take advantage of the versatility of cell death pathways to define broad-spectrum strategies.

## Molecular Pathways of Ischemic Cell Death

### *The First Events: Beyond Therapeutic Reach*

A rise in cytosolic $Na^+$, extrusion of $K^+$, fall in ATP, and anoxic depolarization are the earliest events in global ischemia and in the core of a focal ischemic infarct *(24).* The massive influx of $Na^+$ follows the inhibition of the electron transport chain, and thus of oxidative phosphorylation. The decrease in ATP, which reaches 90% after 5 min of arterial occlusion in the core of a focal infarct, leads to inhibition of $Na^+$- and $K^+$-ATPase activity in both the plasmatic and ER membrane. The resulting increase of intracellular $Na^+$ levels and the concomitant extrusion of $K^+$ ions induces a profound membrane depolarization, and its sum of causes a macroscopic phenomenon known as anoxic depolarization *(25).* This correlates with the transient opening of the voltage-dependent $Ca^{2+}$ channel and increased $Ca^{2+}$ entry into the cells. Changes in the $Na^+/K^+$ ratio and ATP levels are much less dramatic in the penumbra, where anoxic depolarization is replaced by intra-ischemic depolarization, characterized by a shorter duration and rapid repolarization of the cell.

Theoretically, the rapidity with which these initial events occur—within minutes after the insult—places them beyond therapeutic reach. However, modifications of ionic concentrations are maintained in the secondary phase of degeneration and during reperfusion (*see* ref. *7*). As a result, a number of $Na^+$-channel blockers—namely lidocaine, tetrodotoxin, BW-1003C87, BW-619C89, and lamotrigene—have been demonstrated to counteract the primary events in stroke. All drugs efficiently reduce glutamate release, decrease the rate of ATP decrease, and provide very strong protection against experimental focal *(26,27)* and global ischemic damage in animals *(28–30).* Notably, these drugs are also effective when administered 2 h after the end of a global ischemic event *(31),* or in the penumbra of a focal event, suggesting that the delayed rather than the initial entry of $Na^+$ is involved. Thus, the core of a focal insult is never protected by blockade of $Na^+$ entry.

Unfortunately, the clinical transposition of these results has been unsuccessful, apparently because of the lack of specificity of these compounds for their discrete targets, or the difficulty in administering them in sufficient quantities specifically at the lesion site without potential side effects on a widespread range of tissues. Clinical trials (*see* Table 1) performed to date include the anticonvulsant phenytoin (Fosphenytoin, phenytoin pro-drug), which also blocks voltage-dependent $Na^+$-channels and effectively protects against focal ischemia in rodents *(32),* Sipatrigine (BW619C89), still in phase II, and Lubeluzole (Prosynap®). All three compounds have failed to lead to satisfactory functional improvement, although a reduction in mortality was observed in subgroups of patients *(33).*

### *Glutamate and Calcium, the Main Targets*

Coincident with massive changes in ion gradients, ischemia induces increases in the extracellular concentration of excitatory amino acids and in intracellular $Ca^{2+}$ concentrations that reach neurotoxic levels. Because of their

Table 1
Clinical Trials with Neuroprotective Agents in Stroke

| Compound | Trial name | Phase | Results | Published in (year) | refs. |
|---|---|---|---|---|---|
| | | | **$Na^+$-channel blockers** | | |
| Fosphenytoin (Cerebix®) | Phosphenytoin Phase 3 | III | Enrollment halted, no effect | – | – |
| Lubeluzole (Prosynap®) | LUB-INT-13 | III | No effect | 1999 | *(33)* |
| | Lub | III | No effect | 1997 | |
| | LUB-USA-6 | II | Ongoing | | |
| | | | **Potassium-channel opener** | | |
| BMS-204352 (MaxiPost®) | POST-010 | III | Ongoing | 2001 | – |
| | | | **NMDA glutamate-receptor antagonists** | | |
| CGS19755 (Selfotel®) | – | II | Adverse effects | 1995 | *(358)* |
| | ASSIST | III | Adverse effects | 1997 | *(359)* |
| Aptiganel (Cerestat) | AAST | III | No effect | 1997 | – |
| CP-101,606 | – | II | – | 1999 | *(360)* |
| | – | III | Ongoing | – | – |
| NPS 1506 | NPS 1506 | Ib | Ongoing | – | – |
| Magnesium | – | III | No effect | 1995 | *(361)* |
| | Images | III | Ongoing | – | |
| Remacemide | – | Unknown | – | 1999 | *(362)* |
| ACEA 1021 (Licostinel) | – | I | No safety concerns | 1999 | *(363)* |
| GV150526 (Gavestinel®) | GAIN Americas | III | No effect | 2000 | – |
| | GAIN International | III | No effect | 2000 | *(364)* |
| Eliprodil | CVD 715 | II | No effect | – | – |
| | Eliprodil Phase III | III | Not available | – | – |
| | | | **AMPA/KA glutamate receptors** | | |
| YM872 (Zonampanel) | ARTIST + | III | Ongoing | – | – |
| | ARTIST MRI | II | Ongoing | – | – |
| ZK200775 (MPQX) | ZK-200775 | II | Not available | – | – |
| | | | **$Ca^{2+}$-channel blockers** | | |
| Nimodipine (Nimotop®) | 23 trials | III | None or adverse effects | 1992–2001 | *(101)* |
| Flunarizine (Sibelium®) | FIST | III | No effect | 1996 | *(102)* |
| | FIAS: pilot study | II | | 1990 | *(365)* |
| | | | **$Ca^{2+}$ chelator** | | |
| DP-b99 (DP-BAPTA) | 1 | II | Ongoing | – | – |
| | | | **Free radical scavengers/Antioxidants** | | |
| Ebselen (Harmokisane) | 1 | II | Improvement when started within 24 h<br>Ongoing | 1998 | *(121)* |
| | 1 | III | | – | |
| Tirilazad (Freedox®) | 5 | III | None to slight effects | 1998 | *(366) (367,368)* |
| | RANTASS | III | No effect | 1996 | |
| | RANTASS II | III | No effect | 1996 | |
| | | | **GABA agonists** | | |
| Clomethiazole (Zendra®) | CLASS | III | No effect | 1999 | *(369)* |
| | CLASS-IHT | III | No effect | 1999 | |
| | | | **Serotonin agonists** | | |
| Repinotan (Bay x 3702) | BRAINS | II | No adverse effects | 2000 | – |
| | RECT | III | Ongoing | – | – |
| | | | **Growth factors** | | |
| bFGF (or Trafermin, Fiblast®) | Fiblast Phase 2 | II | No serious adverse effects | 1998 | *(287)* |
| | Fiblast Phase 3 | III | No effect | 2000 | – |

potential importance in the mechanism of excitotoxicity, the roles of glutamate and $Ca^{2+}$ in ischemic lesions have received considerable attention.

### *Glutamate*

Glutamate is the predominant excitatory transmitter in the mammalian brain, and since the pioneering studies of Meldrum and colleagues *(34)* there has been compelling evidence of its deleterious effects in ischemia. Concomitant with perturbations of ionic homeostasis and the drop in ATP, extracellular glutamate levels rise during global ischemia by several fold, to reach up to 30 μ*M* within 15 min *(35).* Similar observations have been recorded in models of focal ischemia, in which extracellular glutamate levels rise to between 50 and 80 μ*M* for several hours in the core *(36,37),* and to 200 μ*M* in the striatum after 2 h of global ischemia *(38).* Current evidence ascribes the major source of glutamate to presynaptic terminals, possibly via reversal of the $Na^+$-glutamate transporter *(39),* rather than to postsynaptic elements or glia *(40).* In addition, overstimulation of glutamate receptors induces the intracellular accumulation of several ions, including $Ca^{2+}$ and $Na^+$ *(41).*

### NMDA Receptors

The toxic effect of glutamate on brain tissue was first demonstrated by Olney *(42),* giving rise to the excitotoxic theory of neuronal cell death. The appealing idea that excitotoxicity was the main cause of neuronal death in ischemia turned attention toward the ionotropic N-methyl-D-aspartate (NMDA) receptor, the major route of $Ca^{2+}$ entry in neurons. As a result, NMDA receptors have been at the center of neuroprotective strategies for decades. However, early studies reporting the protective effects of NMDA blockers *(34,43)* were rapidly countered by conflicting results showing that such compounds induce a profound reduction in brain temperature *(44,45).* Ischemic glutamate release is remarkably sensitive to hypothermia, which is in itself neuroprotective *(37).* Protection by MK-801, the major NMDA antagonist, is nevertheless observed in models of global ischemia in which temperature is carefully monitored. However, this effect requires high doses, suggesting that it is mediated by an action on $Na^+$ channels rather than by direct inhibition of glutamate receptors *(45).* In contrast, in focal ischemia—whether transient or permanent—MK-801 *(46,47),* NMDA glycine-site antagonists *(48),* competitive NMDA antagonists *(49,50),* and riluzole *(51)* all reduce lesion size.

As in the case of $Na^+$-channel blockers, glutamate blockers protect against focal ischemia when added several hours after the insult. Delayed neuroprotection is consistent with the fact that the excess of glutamate is maintained after the reperfusion step in global ischemia and in the penumbra in focal ischemia *(52,53).* In addition, although extracellular levels are much less important during the delayed degenerative period, glutamate effects are heightened by compromised energy production and by the rise in oxygen levels following reperfusion, rendering cells highly sensitive to even mild ischemic events.

The exciting possibility that inhibition of NMDA glutamate receptors could be neuroprotective in focal ischemia has failed transposition to the clinic. This may have been predicted based on the results of a number of studies. First, MK-801 has no effect in a thrombotic stroke model *(54)* or in spontaneously hypertensive rats. Negative results have also been reported in more standard paradigms *(55).* Secondly, various competitive and noncompetitive NMDA blockers induce major increases in blood flow in the core and the penumbra of focal infarcts *(56,57),* although this finding has been challenged by other studies that reported decreased *(58)* or unchanged *(59)* blood-flow levels. The damaging effects of NMDA antagonists have also been demonstrated *(60,61).* Finally, NMDA receptors are rapidly inactivated during ischemia *(62),* probably because of dephosphorylation resulting from ATP failure. In agreement with these findings, $Ca^{2+}$ entry after 10 min of ischemia is independent of NMDA receptors *(63).* Because of these dis-

couraging results and concerns about their potential neurotoxicity, the use of NMDA-receptor antagonists was discontinued in Phase I and Phase II clinical trials because of unacceptable adverse effects, including psychomimetic effects (agitation, hallucinations, paranoia, and delirium), sedation, and catatonia. Studies with CGS19755 (Selfotel®), aptiganel (Cerestat®), NPS 1506 and magnesium, and eliprodil and GV150526—which are respectively a competitive antagonist at the NMDA-binding site of the receptor, two non-competitive NMDA receptor antagonists that act as channel blockers, and ligands of the polyamine site and of the glycine site—have been prematurely terminated because of an unfavorable risk-benefit ratio (CGS 19755 and eliprodil *[64]*) and the lack of a significant effect from placebo (eliprodil). The weak NMDA antagonist memantine, an antiviral molecule derived from amantadine, acts on the magnesium site of the NMDA receptor $Ca^{2+}$ channel, and has neuroprotective activity in both focal and global ischemia *(65).* Memantine is currently used for the treatment of convulsion and spasticity, and clinical trials are still in progress for vascular dementia and Alzheimer's disease (AD), but not yet for stroke.

### AMPA/KA Receptors

More glutamate receptors may be involved in glutamate-induced calcium overload in ischemic cells, including alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and kainate (KA) receptors. AMPA receptors have a distribution that is parallel to that of NMDA receptors. Blockade of the AMPA receptor reduces intra-ischemic depolarization *(66)* and $Na^+$ entry *(63),* thus reducing toxic intracellular $Ca^{2+}$ accumulation. Another potential—although controversial *(67)*—mechanism for the participation of AMPA receptors in ischemic cell death is the so-called "GluR2 hypothesis" *(68).* Channels formed by GluR1, GluR3, or GluR4 subunits show high $Ca^{2+}$ permeability *(69),* and channels that heterooligomerize with the GluR2 subunit have low $Ca^{2+}$ permeability. The $Ca^{2+}$-repulsive property of GluR2 subunits is attributed to post-transcriptional editing—e.g., the substitution of a neutral glutamine residue by a positively charged arginine in the second transmembrane loop (M2 domain). The level of editing of the GluR2 subunits regulates the $Ca^{2+}$ permeability of AMPA receptors. Global ischemia induces a reduction of GluR2 synthesis in the CA1 pyramidal layer of the hippocampus in various species *(70,71),* which may account for the significant participation of AMPA receptors in calcium entry into damaged cells. Similar to GluR2, editing of the GluR6 subunit of KA receptors at three discrete sites *(72)* influences $Ca^{2+}$ permeability, although the rapid desensitization of KA receptors does not fit the profile of major contributors to glutamate-induced $Ca^{2+}$ influx in ischemia.

Protection by AMPA/KA-receptor antagonists, which include NBQX *(66),* LY-293558 *(73),* YM90K *(74),* YM872 *(75),* and ZK200775 *(76),* has been reported in permanent focal ischemia. Such studies still must be extended to global and transient focal ischemia. However, clinical trials performed with five AMPA antagonists—GYK 52466, NBQX, YM90K, ZK-200775 (MPQX)—have not been successful.

### Metabotropic Glutamate Receptors

The variety of roles played by glutamate receptors in ischemic brain damage was recently expanded by studies showing that metabotropic (mGlu) receptors may also participate significantly in increasing intracellular $Ca2^+$ concentrations. In addition to their role in modulating ionotropic receptor functions, mGlu receptors influence the release of $Ca^{2+}$ from storage sites in the ER via G protein-coupling and activation of phosphoinosityl triphosphate (IP3)- and ryanodine receptors *(77).* $Ca^{2+}$ released by the ER may enter adjacent mitochondria, where it causes damage by increasing oxidative metabolism *(78,79).* However, cyclopropyl- and phenylglycine derivatives, which are relatively selective agonists for group II mGlu receptors, can protect against neuronal death induced by combined hypoxia

and glucose deprivation in vitro *(80).* The combined group I/II agonist t-ACPD and the II/III agonist (S)-4-carboxy-3-hydroxyphenylglycine are also protective in focal and global ischemia, respectively *(81,82),* and inhibition of group I mGlu receptors can enhance the neuronal damage induced by mild hypoxia combined with glucose deprivation *(80),* or protect against excitotoxic degeneration *(83).* To our knowledge, no antagonist of mGlu receptors has undergone clinical development to date.

### *Calcium*

The rapid influx of $Ca^{2+}$ following glutamate-receptor activation quickly raises intracellular concentrations above the buffering capacities of neurons. $Ca^{2+}$ overloading has been associated with necrotic cell death during ischemia *(84).* In addition to NMDA receptors, several routes may be involved in cytosolic $Ca^{2+}$ accumulation—namely, L-channels, impaired $2Na^+/Ca^{2+}$ exchange by the plasmalemma caused by lowered $Ca^{2+}$-ATPase, and extrusion from intracellular calcium stores, like the ER *(85)* and the mitochondria *(86).* In accordance with this, the increase of cytosolic $Ca^{2+}$ in ischemized slices is greatly attenuated by compounds that inhibit these pathways, such as the inhibitor of the mitochondrial $Na^+/Ca^{2+}$ exchanger, CGP-37157 *(63),* the $Na^+$-channel blocker, lidocaine, the blocker of $Ca^{2+}$ release from the ER, dantrolene, and the AMPA-receptor blocker, CNQX.

Intracellular $Ca^{2+}$ has been found to rise by 25% in global ischemia *(87)* and in the focal ischemic core *(88).* Increases are much lower in the penumbra, where they are related to the period of intra-ischemic depolarization *(89).* Although these values may be overestimated, the ischemia-induced $Ca^{2+}$ increase remains sufficient to activate $Ca^{2+}$-dependent processes that lead to cell death. These include persistent protein kinase C (PKC) activation, the PLA2-induced formation of arachidonic acid, and the subsequent generation of free radicals with damage to mitochondria, breakdown of structural proteins, sustained protein phosphorylation, and the activation of nitric oxide (NO) synthase and of a number of proteases such as calpains and endonucleases. In addition to initial rises, cytosolic $Ca^{2+}$ increases are sustained in the post-ischemic phase in areas damaged by global ischemia *(90,91),* and in the ischemic penumbra *(89,92),* with obvious effects on the progression of the damage.

To limit $Ca^{2+}$-induced cellular damage, a variety of calcium entry-blocking drugs have been developed, and on the basis of experimental data, two have been approved for human use. Voltage-dependent $Ca^{2+}$ L- and T-channels are blocked by nimodipine and flunarizine, respectively. Studies with nimodipine—actually the first neuroprotective agent used for stroke—have suggested that it may be directly cerebroprotective *(93).* Nimodipine and flunarizine have long-lasting protective effects against ischemic damage in the gerbil *(94–96),* and in focal ischemia in the rat *(97).* Similar protection is observed with the N-channel blocker SNX-11 when administered after global ischemia in the rat *(98,99),* and after permanent or transient vascular occlusion *(100).* As with glutamate-receptor inhibitors, none of these compounds has proven to be effective in clinical settings to date. On the contrary, significant side effects have been reported: nimodipine (Nimotop®) induces detrimental hemodynamic effects *(101),* and the use of flunarizine (Sibelium®) has been suspended after negative results *(102).* However, it is important to mention that a meta-analysis revealed significant improvement in functional outcome in patients who had received nimodipine within 12 h post-stroke *(103).* Although the relationship between excessive glutamate release and $Ca^{2+}$-induced cellular damage is unequivocal, studies have not yet determined the extent to which glutamate-induced damage in ischemia is solely the result of excitotoxicity. In global ischemia, increases of extracellular glutamate are transient, and insufficient to induce irreversible cell damage without energy failure. The conditions are more appropriate in the focal ischemic penumbra, where energy metabolism disruption is mild and extracellular glutamate reaches levels that are actually excitotoxic in culture.

Although some similarities undoubtedly exist between excitotoxic and ischemic cell death, this relationship must be considered with caution. Ischemia is a more complex situation than glutamate excitotoxicity, and glutamate is but one actor in a multimodal play, which may partly explain the failures to improve outcome in stroke patients by using specific inhibitors of either glutamate or intracellular $Ca^{2+}$ effects.

## *The Mitochondria: Still Center Stage*

Mitochondria occupy up to 25% of cell volume in neurons and produce most of the cell's energy by oxidative phosphorylation *(104).* They are essential for cell viability, and are targeted by injuries that lead to both necrotic and apoptotic cell death *(21).* Mitochondria can trigger neuronal death in a number of ways, by disrupting electron transfer and energy metabolism, by accumulation of cytosolic $Ca^{2+}$, by releasing or activating proteins that mediate apoptosis, and by altering the cellular redox potential *(105).* Inhibition of oxidative phosphorylation induces the collapse of the mitochondrial inner-membrane potential, ATP depletion, colloidosmotic swelling of the matrix, and opening of a high conductance permeability transition (PT) pore [PTP, ~2.9 nm in diameter *(106)*], which allows solutes of high molecular mass (less than 1500 Da) to diffuse from the matrix to the cytosol. Although transient opening of the PTP may remove toxic concentrations of ROS (reactive oxygen species) *(107),* permanent opening is associated with the release of $Ca^{2+}$ and mitochondrial proteins critical to the apoptotic program (reviewed in ref. *108*), and with uncoupling of oxidative phosphorylation and mitochondrial swelling *(109).* Drugs that are able to modulate the PTP would thus have major clinical relevance, and cyclosporin A has been extensively studied in this regard. However, adverse effects caused by the lack of specificity of this immunosuppressive agent, and good evidence that cyclosporin A *per se* can cause oxidative stress *(110)* have halted its use for the treatment of stroke.

Severe inhibition of mitochondrial ATP formation, and accelerated ATP hydrolysis by the mitochondrial ATPase, leads to necrotic cell death. In contrast, during apoptosis, onset of the PTP and large-amplitude swelling *(109)* are concomitant with the release of soluble factors that amplify the cell-death program. Released pro-apoptotic molecules include cytochrome c *(111)* and apoptosis-inducing factor (AIF) from the matrix *(112).* AIF is a 50-kDa protein usually found in the intermembraneous space, which acts at the nuclear level to induce the cleavage of large fragments of DNA *(113).* Therefore, mitochondrial dysfunction plays an active role in apoptosis, although it is considered more "passive" in necrosis. Unlike necrosis, which involves mitochondrial swelling and rupture, the structural integrity of mitochondria is now considered a hallmark of apoptosis. Modifications in mitochondrial structure during apoptosis include condensation, with hyperdensity of the matrix and perinuclear clustering *(114).* This is reflected by morphological observations, which point to the well-preserved structure of mitochondria until the final disintegration of the cell into apoptotic bodies.

Because of the importance of mitochondria in ischemic neurodegeneration, a number of compounds have been developed to interfere with several of the major steps known to be involved in the process (reviewed in ref. *115*). These strategies have mainly been directed toward cardiac ischemia, and with the exception of antioxidant molecules, have not been applied to cerebral ischemia.

### *Oxidative Damage*

Free oxygen radicals (ROS) are produced in mitochondrial complexes I and II of the respiratory chain, at a level of 2–4% of the oxygen consumed. ROS are eliminated by several antioxidant systems, glutathione metabolism, superoxide dismutases, glyceraldehyde-3-phosphate dehydrogenase, and the antioxidant vitamins E and C. When production of ROS increases, these mechanisms may become insufficient, and oxidative damage ensues.

ROS contribute greatly to ischemic injury. They participate in the opening of the PTP *(109)*, are involved in cytochrome c release, and perform lipid oxidation. Their production is particularly significant during the reperfusion step *(116,117)*, when they dramatically exacerbate mitochondrial damage by depleting pyridine nucleotides and glutathione, the two reducing compounds that protect mitochondria from oxidative stress.

Several types of free radical scavengers such as lazaroids or chromane derivatives—including Tirilazad mesylate, the nitrone NXY-059 *(118)* and extracts from Gingko biloba—protect neurons in culture from the damage induced by increased formation of oxygen radicals. Bilobalide (EGB 761) has neuroprotective effects in transient ischemia *(119)* and has been used in perivascular and neurological diseases, but not in stroke—probably because of concerns regarding high dosage-induced intracerebral hemorrhage *(120)*. The seleno-organic compound ebselen (2-phenyl-1,2,-benzisoselenazol-3(2H)-one, Harmokisane) also reduces the cytosolic release of cytochrome c and DNA fragmentation in transient ischemia. Ebselen has been shown to protect the brain from stroke in humans *(121)*, but opposing pharmacological effects have been shown in animal studies. The mechanism of action for Lubeluzole, is still unknown, but may include inhibition of the nitric oxide synthase (NOS), was also ineffective in stroke *(33)*. A series of superoxide dismutase mimetics that selectively catalyze the dismutation of superoxide anions has also been developed for experimental research, with successful limitation of lesion volume in a model of myocardial ischemia/reperfusion injury *(122)*. To date, the most interesting neuroprotective effect by an inhibitor of ROS in an animal model comes from BN 80933, a compound with dual actions on neuronal NOS and lipid peroxidation *(123)*. Another compound with combined superoxide dismutase- and catalase-like effects, EUK-134, has also revealed potent neuroprotective effects in a rat model of focal ischemia when administered 3 h after arterial occlusion *(124)*. These two examples show that acting synergistically on different mechanisms of ischemic damage may result in additive beneficial effects.

### *Cytochrome c*

The leakage of cytochrome c into the cytoplasm has two dramatic effects on the apoptotic death sequence. Holocytochrome c is localized in the intermembraneous space in mitochondria, where it functions as a peripheral protein in the respiratory chain involved in the shuttling of electrons from Complex III. Loss of a component of the mitochondrial electron transport chain triggers an increased production of ROS *(125)*, which can in turn promote cytochrome c release from the mitochondria *(126)*, thus amplifying the damaging cascade. Once in the cytoplasm, cytochrome c binds to the apoptosis-activating factor-1 (Apaf-1, *(127)*) in a complex known as the apoptosome, which triggers the so-called "mitochondrial pathway" of caspase activation (*see* Fig. 2). This topic is described in more detail in the following section. Cytochrome c release is also distal to caspase activation, pointing to one of the amplification mechanisms that characterize caspase activity. First, the electron-transfer capacity of cytochrome c is lost after activation of caspase-8 or caspase-1 *(128)*. Second, activation of caspase-8, the prototypic initiator caspase of the death-receptor pathways, induces the cytosolic leakage of cytochrome c *(129,130)* before the formation of the PTP. Microinjection of cytochrome c into different cell types induces apoptosis with no apparent opening of the PTP *(131)*. These data suggest that PTP formation is sufficient, but not necessary, for cytochrome release. Several other factors can induce permeabilization of the outer mitochondrial membrane, including calcium overload, ROS, and pro-apoptotic members of the Bcl-2 family, which concur to the release of pro-apoptotic factors stored in the mitochondria.

### *Bcl-2-Related Proteins*

A major class of intracellular regulators of the apoptotic machinery acting at the mitochondrial level is represented by the 15 identi-

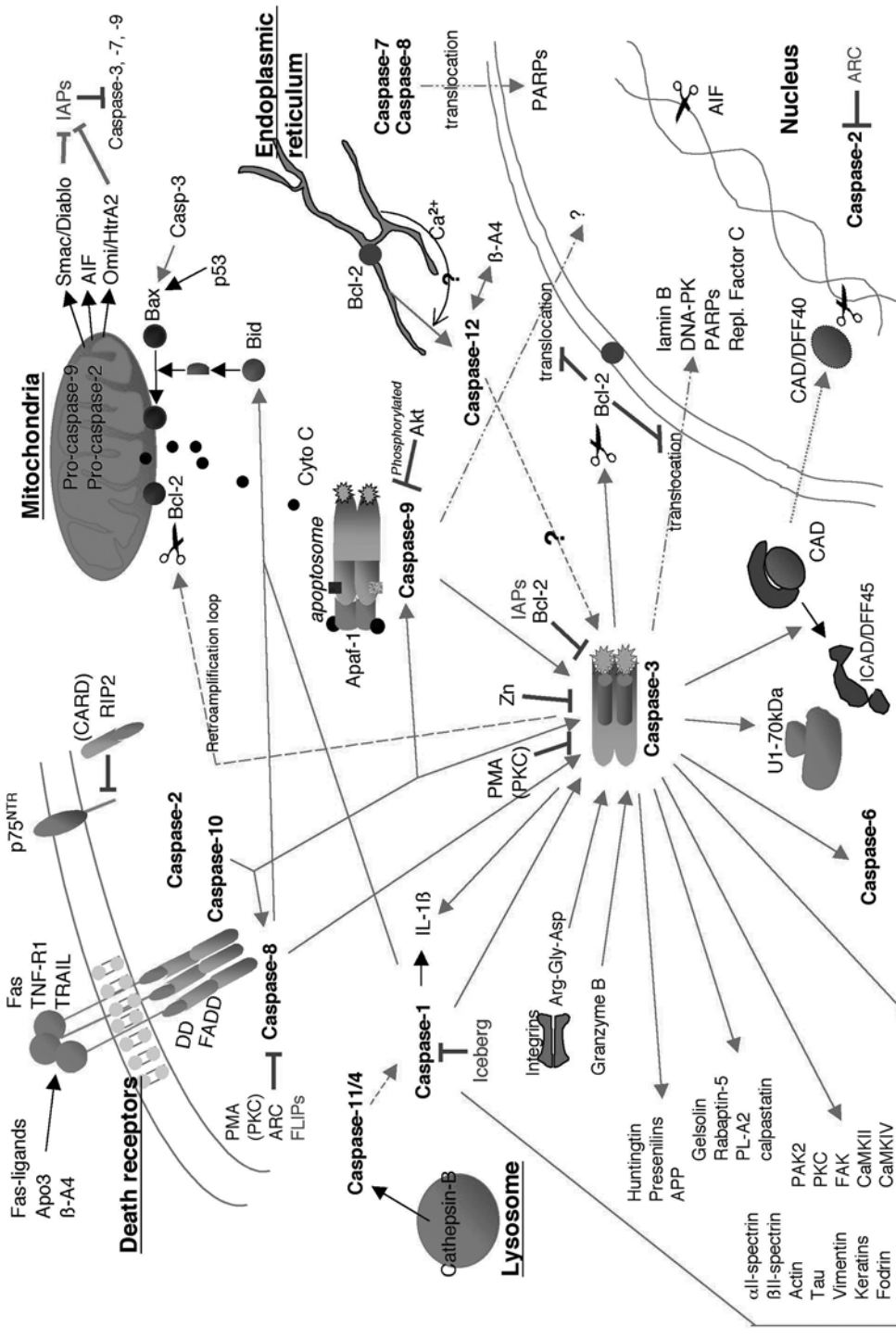


Fig. 2. Caspase activation cascades and their interactions. To the two extensively described pathways, initiated by mitochondrial dysfunction or the activation of death receptors, two still ill-defined pathways initiated by lysosomal (activation of caspase-11) and ER stress (caspase-12) are added. Amplification of the apoptotic signals results from interactions between the cascades, by either reciprocal activation of upstream and downstream caspases, or by the existence of links between pathways. Endogenous caspase inhibitors (IAPs and Flips) are further controlled by inhibitors.

fied members of the Bcl-2 family of proteins *(132).* Bcl-2, a mammalian homolog of the anti-death protein Ced-9 from Caenorhabditis elegans, is localized on the ER, and on mitochondrial and nuclear outer membranes, indicating that it may play a role in the transmembraneous transport of molecules. Members of this family act on mitochondria at different levels to integrate death signals *(133).* The pro-apoptotic members—including Bak, Bax, and BH3(Bcl-2 homology domain 3)-only members such as Bid and Bad—promote the formation of the PTP and the mitochondrial release of apoptotic factors *(134).* Anti-apoptotic members, such as Bcl-2 and Bcl-xL, protect against mitochondrial swelling and outer membrane breakdown *(135).* Bcl-2 prevents cytochrome c release *(136),* inhibits the mitochondrial generation of ROS *(137,138),* and prevents caspase activation *(136).* Bcl-2 family members interact with caspase cascades in multiple ways. For example, Bcl-2 is inactivated by caspase-3, giving rise to a truncated pro-apoptotic protein *(139).* Bcl-xL interacts with long prodomain caspases *(140),* presumably in association with Apaf-1, thereby inhibiting the function of the apoptosome *(141,142).*

Overexpression of the anti-apoptotic factors appears to be benefical in most paradigms *(143–145),* whereas their disruption exacerbates ischemia-induced damage *(146,147).* Bcl-2 overexpression protects against permanent middle cerebral artery (MCAO) *(148),* although the effect is only temporary in the infarcted area.

Bax has recently received great attention for its pore-forming capacity and involvement in the permeabilization of the mitochondrial membrane. Both mRNA and protein levels of Bax are increased in cells dying from ischemia *(149–151).* Mainly cytosolic, Bax is translocated by apoptotic signaling to the mitochondria *(152),* where it may play a role in cytochrome c release through the formation of anionic channels. Two models are currently being proposed to explain the participation of Bax in the mitochondrial cascade of apoptotic events (see ref. *153*). In the first model, Bax promotes the opening of the PTP complex by physical interaction. In transient focal ischemia, Bax is relocated at the mitochondrial level, where it interacts with two components of the PTP, VDAC (voltage-dependent anion channel) and ANT (adenine nucleotide translocator, the ADP/ATP antiporter) *(154).* In the second model, the release of apoptotic factors is mediated by tetrameric channels formed by Bax in the outer mitochondrial membrane, with no functional alteration of the mitochondria. Bid actively participates in neuronal cell death under ischemic conditions *(155),* probably by regulating Bax translocation.

### *Caspases, the New Molecular Cues of Apoptosis*

Caspases are cysteine ("c") proteases ("ase"), which cleave their substrates after an aspartate residue ("asp", *[156]*). Caspases exist in the cytoplasm of all nucleated cells as a single polypeptide chain that is a catalytically dormant zymogen. Caspases are activated by proteolytic cleavage through proximity-induced autoactivation or by the activity of other proteases, including themselves *(133,157,158).* This capacity for self- and reciprocal cleavage has led to the categorization of the 14 mammalian molecules *(159)* into initiator and effector caspases based on their position in the proteolytic cascades *(160–162).* Initiator caspases are directly activated by apoptotic signaling. They trigger and amplify the apoptotic process by activating effector caspases and other pro-apoptotic factors. Effector caspases (such as caspases 2, 3, 6, and 7) act at cytoplasmic sites, and are also translocated into various organelles—including the ER, mitochondria, and nucleus—to execute the proteolytic program that finalizes cell destruction *(163–165).* This classification has the advantage of being straightforward but suffers from a number of exceptions, and recent data indicate that most caspases have both initiator and effector roles *(159).* In addition to themselves, active caspases cleave a theoretical total of 200 polypeptides *(166),* each marking a milestone

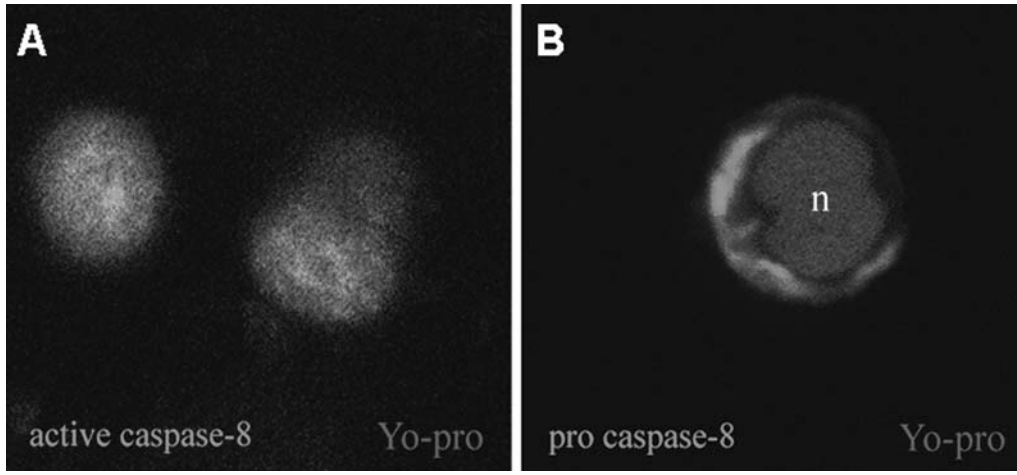


Fig. 3. Activation of caspase-8 in the nucleus of apoptotic cells. Fluorescence (A) and confocal (B) analyses performed on Jurkat T lymphocytes. **(A)** Active caspase-8 is localized in isolated nuclei of apoptotic Jurkat cells (yellow; the nucleus is labeled by Yo-Pro, red). The inactive pro-form of caspase-8 (green) is only observed in the cytoplasm (n, nucleus; labeled by Yo-Pro, red). Bars = **(A):** 8 ?m; **(B):** 15 ?m.

of apoptotic cell death *(164).* Substrates cleaved by caspases undergo functional changes that can be classified into one of three categories: activation, inactivation, or structural modification with organelle translocation (see refs. *167,168*). In addition to cleaving the effectors, initiator caspases also have direct targets. For instance, caspase-8 targets the cytoplasmic Bid. It is also translocated in its active form into the nucleus (Fig. 3) to cleave and inactivate poly(ADP-ribosyl) polymerases (Benchoua), which are also targets of caspase-3.

Strong evidence for the active participation of caspases in ischemia-induced cell death has been provided by inhibitor studies and by studies performed on animals with genetic modifications. Administration of the irreversible pan-caspase inhibitor, z-VAD-FMK, reduces infarct volume in both global *(169)* and focal *(170–172)* models of stroke, with or without reperfusion, but with variable efficacy depending on the severity of the injury and on the time and dose of injection *(169,170,172).* Protection against ischemic insults is also observed in animals that carry a dominant-negative form of caspase-1 *(173).*

The pathways leading to caspase activation following ischemic injuries are beginning to be characterized. They depend on the model studied, the severity of the insult, and the presence or absence of a reperfusion step. Two main pathways—the "mitochondrial pathway" and the "death-receptor pathway"—have been identified, and appear to be interrelated by feedforward loops of activation. Other pathways exist, more recently identified, and originating in cellular compartments such as the lysosomes and the ER *(174).* All pathways converge on the final effectors—caspases-3, -6, and -7—which execute the ultimate steps of the cell destruction program.

### *The Mitochondrial Pathway*

The mitochondrial pathway is initiated by the release of pro-apoptotic intramitochondrial factors, such as cytochrome c, AIF, and caspase-2 and -9 *(175).* All these processes are inhibited by Bcl-2. Once in the cytosol, cytochrome c is complexed to Apaf-1 *(127,176),* the mammalian homolog of Ced-4, which is upregulated by the key regulator of apoptosis, *p53 (177).* In the presence of ATP,

Apaf-1 couples cytochrome c to a multiproteic complex known as the apoptosome. The N-terminus of Apaf-1 contains a caspase recruitment domain (CARD), which allows binding to caspases, and is shared by caspases 1, 2, 3, 4, and 9 *(178).* Interactions between cytochrome c and Apaf-1 in the apoptosome promote the recruitment and auto-conversion of pro-caspase-9 into active caspase-9 *(127,176).* Thus caspase-9 cleaves effector caspases *(127),* leading the affected cell to the point of no return of the death process. The process is further controlled by the existence of two splice variants of pro-caspase-9, a long (L) and a short (S) form. The S form is not auto-processed and has no caspase-9 activity *(179).* It therefore has a dominant-negative effect. However, this unidirectional schema is only partially true, since caspases can cause mitochondrial dysfunction in some in vitro models *(180,181).* Caspase activation and mitochondrial dysfunction are probably linked by feedback loops *(182)* (*see* Fig. 2).

In global ischemia, cytochrome c release from the mitochondria is reported in CA1 and CA2 neurons, in coordination with cytosolic translocation of pro-caspase-9 from the mitochondria and its ensuing activation *(183).* In transient focal ischemia, cytochrome c release and caspase-9 activation are associated with the reperfusion period *(184).* Cytochrome c relocation and caspase-9 participation in ischemia-induced cell death have also been reported in permanent MCAO models, and are temporally and spatially correlated with the delayed degeneration of the penumbra *(185,186).* This finding is consistent with the fact that the formation of the apoptosome is energy-dependent *(187),* and that the involvement of the mitochondrial pathway requires relatively conserved ATP stores. Interestingly, the two forms of pro-caspase-9 behave differently after ischemia. Although the active L form is upregulated by transient MCAO, the non-catalytic S form is downregulated, possibly increasing the effectiveness of the pathway by downregulation of a potential inhibitor (*see* ref. *188*).

### *The Death-Domain-Receptor Pathway*

Death signals provided by the environment are integrated by receptors of the tumor necrosis factor (TNF)-receptor superfamily *(189),* the so-called "death receptors," which are characterized by the presence of an intracytoplasmic death domain (DD). They include the TNF-R1 (also known as *p55* or CD120, *190*]), Fas (Apo-1, CD95, [*140*]), DR3, DR4 (TRAIL-R1), and DR5 (TRAIL-R2, [*191*]). Binding of their ligands induces the trimerization of the receptor, and interaction between the DD and adaptator proteins containing a death effector domain (DED). The multiproteic complex thus formed, known as the death-inducing signal complex *(192),* promotes the recruitment and activation of initiator pro-caspases that contain a domain analogous to the DED in their prodomain—e.g., caspases -8 and -10 *(190,193–195).* Activation of caspase-8 and -10 occurs through the activation of Fas, TNF-R1, or DR3 *(196–199).* Active initiator caspases also propagate the apoptotic signal by directly activating downstream effector caspases, such as caspase-3, -6, and -7 *(200).*

Involvement of the death-receptor pathway in ischemic cell death is supported by several observations. Members of the TNF-R superfamily *(201,202)* and their ligands *(203,204)* are upregulated following both focal and global ischemia. Accordingly, immunosuppression of either Fas-L or TNF, or invalidation of Fas, protects against acute stroke *(205).* The factors involved in the upregulation of components of the Fas/TNF pathway are still unknown. Upregulation of both the receptors and their endogenous ligands can result from transcriptional activation by immediate early genes such as c-Jun. Activation of Jun by phosphorylation occurs in ischemic areas *(206),* and inhibition of Jun phosphorylation induces both decreased expression of the ligands and prevention of post-ischemic neuronal degeneration in a model of transient MCAO *(204).* In addition to direct activation by transcription factors, the increased availability of death-receptor ligands may result from local necrosis of non-neuronal cell types. We have demonstrated the exquisite sensitivity of protoplas-

mic astrocytes to severe ischemia in the cortex, through a process that clearly displays the morphological characteristics of necrosis—e.g., cell and organelle swelling, and membrane disruption *(207).* Death of protoplasmic astrocytes, the preponderant type of astrocytes in the cortical grey matter, is observed within the first hour following MCAO in mice. Massive necrosis induces a local inflammatory reaction and the production of several death ligands by microglia *(208),* which then act in a paracrine manner on surrounding neurons.

Caspase-8 processing and activity in permanent focal ischemia are associated with both primary degeneration in the core and secondary neuronal death in the penumbra *(186,209).* In contrast, minor variations in active caspase-8 levels are detected in CA1 neurons after global ischemia, yet caspase-10 activity increases significantly in this paradigm. This suggests that caspase-10 is the initiator caspase of the death-receptor pathway in global ischemia *(202),* and caspase-8 would be the major initiator caspase in focal ischemia. Caspase-8 propagates the apoptotic signal by activating downstream caspases and by inducing mitochondrial damage, which in turn promotes the caspase-9-dependent proteolytic cascade *(210,211).* Bid, a member of the Bcl-2 family endowed with pro-apoptotic properties, represents the critical link between the activation of caspase-8 and downstream mitochondria-induced neuronal damage. Full-length Bid is cleaved by caspase-8 to generate a truncated form that has the ability to promote permeabilization of the mitochondrial membrane and cytochrome c release *(212).* Bid does not induce cytochrome c release by direct interaction with the mitochondrial membrane *(213),* but more likely, by increasing Bax activation (*see* ref. *153*). Truncated Bid is detected in ischemized cortices 4 h after transient MCAO, and Bid knockout mice are partially protected against this type of injury *(155).*

### *Other Pathways*

The role of caspases in cell death was discovered through the identification of Ced-3 and its mammalian homolog, the interleukin-1β-converting enzyme (ICE or caspase-1 *(214,215)*). In an apoptotic context, ICE can act as an initiator caspase by cleaving and activating the effector caspase-3 *(216,217).* ICE is activated in several ischemic paradigms, in association with either the ischemic insult or the reperfusion step *(186,218,219).* The expression of ICE is mainly upregulated in microglia and endothelial cells, consistent with its potential role in the inflammatory process *(218,220),* but it is also detected in neuronal populations, where it may directly participate in apoptotic cascades of caspase activation. Selective inactivation of ICE is beneficial in most ischemic models. Pharmacological inhibition of ICE by the synthetic peptides YVAD-CMK or WEHD-CHO results in a significant reduction of infarct size in models of acute or global ischemic injuries *(219,220).* A decrease in infarct volume has also been shown, both in mice strains in which ICE has been inactivated and mutant mice expressing a dominant-negative protein *(221–223).* ICE inactivation appears to have a beneficial effect by interfering with inflammatory processes through a reduction in IL-1β maturation, but also by counteracting apoptotic mechanisms by defective caspase-3 activation. Furthermore, in transient models, the reduction in infarct size is associated with a decreased incidence of the edema induced by reperfusion.

The glial expression of ICE is related to its implication in the inflammatory response. However, the factors leading to neuronal ICE activation have not yet been elucidated. One attractive hypothesis is provided by the identification of caspase-11 as an activator of ICE. Caspase-11 cleaves and activates caspase-1 both in vitro and in vivo under pathological conditions, including cerebral ischemia. Ex vivo, caspase-11 is processed by cathepsin B *(224),* a lysosomal protease also involved in ischemia-induced neuronal damage *(225–227).* Theoretically, activation of caspase-1 under ischemic conditions could result from the activation of caspase-11, as a result of cathepsin B release from ruptured lysosomes. In support of this hypothesis, inhibitors of cathepsins have

neuroprotective properties in models of focal *(227,228)* and global *(229)* ischemia. This implies a new pathway of caspase activation related to lysosomal stress, which sequentially includes the release of cathepsin B, activation of caspase-11 and activation of caspase-1.

Another intrinsic pathway leading to caspase activation has emerged with the identification of caspase-12. Caspase-12 is a protease resident in the ER, which is activated in response to ER stress *(174,230,231)* or directly by m-calpain *(232).* These two events are both linked to intracellular calcium homeostasis dysregulation, and are both potentially involved in ischemia-induced neuronal damage.

### *The Final Effectors*

The final molecular events before the "death commitment point"—defined as the point of irreversibility of the apoptotic process *(233)*—temporally correlate with the activation of effector caspases, the prototype of which is caspase-3. Two lines of evidence indicate that caspase-3 plays a crucial role in ischemic cell death. First, caspase-3 is activated in apoptotic cells following global ischemia *(234),* and transient *(235)* or permanent *(185,209)* focal ischemia. Second, inhibitors of caspase-3 have neuroprotective effects in global ischemia and hypoxia *(171,235–238).* Neuroprotection is still achieved when administration of the inhibitors is delayed for 6–9 h after mild transient ischemia *(237,239)* or hypoxia *(240).*

In global ischemia models, caspase-3 is activated in vulnerable hippocampal CA1 neurons several hours or days after the insult—e.g., with a time-course parallel to neuronal death. Increased activity is accompanied by the upregulation of pro-caspase-3 mRNA and protein levels *(236,241–244).* Activation of caspase-3 occurs earlier in focal models—for example, when the injury is more severe—and is also accompanied by the upregulation of the pro-caspase mRNA, and caspase-3 protein. In accordance with this, administration of a caspase-3-like inhibitor, the zDEVD-FMK, reduces infarct volumes in most, if not all, ischemic paradigms *(171,236,237,239,243,245,246).* Caspase-7, the other effector caspase with DEVDase activity, is processed in a model of transient focal ischemia in rodents *(247).* We have also detected caspase-6 activity (VEIDase) 1 h after permanent MCAO in mice.

The striking efficacy of the caspases is ensured both by the number and nature of their substrates, and by the existence of positive feedforward mechanisms that protect against the failure of apoptosis. The interplay between pro- and anti-apoptotic members of the Bcl-2 family of proteins and caspases is highly revealing in this respect. The interrelationship between both families and their apparently redundant interactions actually reveals an astonishing fail-safe system.

## *Trophic Factors: Still Waiting in the Wings*

Our interest in factors with neurotrophic properties arises from the fact that they can act on the two main causes of ischemia-related functional impairment, and are therefore considered excellent candidates to limit both structural and functional damage. First, neurotrophic factors can directly limit cell death through inhibition of the pathological cascades. A number of experiments have shown the anti-apoptotic properties of various neurotrophic factors (see ref. *248*). Second, neurotrophic factors have major implications in brain plasticity, which is probably the main effector of the striking improvement of neurological functions observed post-stroke.

Various growth factors, especially neurotrophic factors, have been shown to be highly neuroprotective in animal models of stroke *(249–251).* Members of the neurotrophin family, as well as members of the ciliary neurotrophic factor (CNTF), the transforming growth factor (TGF), the glial-derived neurotrophic factor (GDNF), the insulin-like growth factors (IGF), the tumor necrosis factor (TNF), and the fibroblast growth factor (FGF) families, have all revealed neuroprotective capacities in models of global and focal ischemia.

NGF promotes neuronal survival by acting through the TrkA receptor tyrosine kinase. During development, TrkA exerts its survival effect by silencing the apoptotic signal mediated by the low-affinity neurotrophin receptor, $p75^{NTR}$ *(252).* In the adult, the TrkA-signaling pathway includes the activation of the phosphoinositide-3-kinase (PI3K) pathway, which leads to the production of phosphatidylinositol-3,4-biphosphate and subsequent activation of the serine-threonine protein kinase Akt (also called p21-Ras/protein kinase B) (*see* ref. *253*). Akt interferes with the molecular cascades of apoptosis observed in ischemia by at least two means. First, Akt keeps Bad, the non-membrane-bound Bcl-2 relative, in a phosphorylated state in the cytoplasm *(254,255),* where it is sequestered by the phosphoserine-binding protein 14-3-3, thus inhibiting its pro-apoptotic interaction with Bcl-X *(256).* Second, Akt phosphorylates caspase-9, thus inhibiting its activity *(257).* Akt also phosphorylates the Forkhead transcription factors *(258),* but the importance of this phosphorylation event in ischemic damage has not been established. Akt is degraded by death-receptor signaling *(259).*

Concerns about the use of NGF as a neuroprotective agent in stroke have been raised by evidence of pro-apoptotic properties through $p75^{NTR}$ signaling. $P75^{NTR}$, a member of the Fas/TNFR family of death receptors *(260),* interacts with all neurotrophins *(261,262),* and stimulates the expression or activation of apoptotic factors such as ceramide, NFκB, and Jun kinase. However, NFκB also has neuroprotective properties, and recent data show that $p75^{NTR}$ is necessary to the survival effect of TrkA *(263).* Therefore, the actual involvement of $p75^{NTR}$ in ischemic lesions has not yet been determined.

## Therapeutic Approaches to Be Redefined

Despite the significant number of neuroprotective drugs that have been developed to limit ischemic brain damage and improve the outcome for stroke patients, ischemic stroke is still a leading cause of death and long-term disability. A dramatic discrepancy therefore exists between the encouraging experimental data and the ineffectiveness of the same compounds when applied to humans. Although it is not within the scope of this article to analyze the reasons for these failures, consideration should be given to the fact that the initial targets of therapeutic research may not have been properly defined. Since the early 1980s, the theory that ischemic cell death is necrotic has focused on attempts to find a pharmacological treatment for acute stroke by the modulation of excitotoxicity. Our increasing knowledge of the pathophysiology of stroke during the past decade, and the notion that apoptotic mechanisms are involved in ischemic damage, have fostered a new generation of strategies based on more distal mechanisms. In addition to "classical" strategies aimed at limiting glutamate and $Ca^{2+}$ toxicity, still the object of intensive research, new strategies are being developed, which are designed to limit cell death, and to improve functional recovery or replace lost cells.

### *Limiting Cell Death*

#### *Anti-Apoptotic Agents*

The theory that apoptosis plays a major role in ischemic brain lesions is now widely accepted, and has a great impact on the development of new therapeutic strategies for stroke. In order to identify the most relevant neuroprotective agents, many studies have addressed the role of endogenous regulators of apoptosis in ischemia-induced cell death. The importance of the mitochondrial PTP in ischemic cell death is demonstrated by the neuroprotective potential of drugs that inhibit its opening, such as cyclosporin A *(264).* The immunosuppressive properties of cyclosporin A are currently used in transplantation, but its renal toxicity does not allow for long-term treatment. An important target for the pharmaceutical industry is the development of block-

ers of the PTP that lack the adverse effects and immunosuppressive actions of cyclosporin A.

Protease inhibition also holds tremendous neuroprotective potential. The possibility that caspase inhibitors could be used therapeutically in a variety of neurological and non-neurological diseases associated with abnormal apoptosis has raised high hopes, and small-molecule inhibitors are actively being developed by the industry (Idun Pharm. Inc., Vertex Pharm./Serono, Merck Frosst). However, peptide-based inhibitors may preserve cellular functionality, but only in the short period following ischemic shock, and not in all cases *(243).* Other obstacles that have not been overcome include concerns regarding toxicity, blood-brain barrier penetration, and caspase selectivity, potency, and pharmacokinetic properties. For example, broad-range inhibitors may interact with vital cysteine proteases, resulting in the deregulation of apoptosis, but may also affect critical functions, overloading the cell-death pathways and switching the outcome from apoptosis to necrosis *(265–267).*

Caspase activation is naturally controlled by a set of endogenous molecules residing in the cytosol and in the mitochondria. The inhibitor of apoptosis (IAP) family of proteins, which includes the neuronal apoptosis inhibitory protein (NAIP), the X-chromosome-linked IAP (XIAP), and human IAP-1 and -2, constitute cellular regulators of cell death that are very well-conserved across species *(268).* The IAP proteins are defined by the presence of three domains of an ~80 amino acid motif termed the baculoviral inhibitor of apoptosis repeat (BIR). BIR domains are indispensable for the anti-apoptotic activities of IAP members by virtue of their ability to bind and inhibit distinct caspases *(268–270).* XIAP is the most potent inhibitor in the family, and protects against apoptosis by binding to caspase-9, -7, and -3 *(268).* Intrahippocampal injection of adenoviral constructs containing NAIP and XIAP prevents both the production of active caspase-3 and the degeneration of CA1 neurons after transient forebrain ischemia in the rat *(234,271).* Interestingly, in contrast to synthetic inhibitors, XIAP also maintains the homeostasis of hippocampal neurons and reduces spatial memory loss following ischemic injury *(234).* In addition to IAP family members, common anti-caspase properties may be presented by other endogenous inhibitors of apoptosis, such as the viral proteins CrmA (Cowpox viral cytokine-response modifier A serpin), a selective inhibitor of caspase-1 and caspase-8, and *p35 (272),* which can potently inhibit most known caspases *(273).* Although the precise mechanism of action of these inhibitors is not fully understood, it has been established that the selective expression of *p35* in the oligodendrocytes of transgenic mice confers significant protection against focal ischemia-induced cell death *(274).*

The mammalian homolog of the equine herpes virus protein E8 is c-FLIP (also called CASPER, I-FLICE, Flame, CASH, CLARP, MRIT, or usurpin). The full-length protein variant, c-FLIPL, is the most potent known inhibitor of apoptotic "death-receptor pathways" *(275).* However, levels of transiently produced FLIPs result in cell death in vitro *(276),* and relatively little is known about the potential role of FLIP in the nervous system. FLIP protein is weakly expressed in cortical cells during development *(277),* and high levels of Flip expression are present in spinal motoneurons that are resistant to Fas-induced death during the phase of developmental programmed cell death *(278).* Although no data are currently available regarding models of cerebral ischemia, observations in cardiac tissue, where the highest levels of FLIP are found, have revealed a lack of FLIP in cells undergoing apoptosis following ischemia/reperfusion injury. In the surrounding healthy tissue, FLIP levels remain high, suggesting an important role for FLIP in regulating the susceptibility of cardiac myocytes to apoptotic stimuli *(279).* Interestingly, FLIP and XIAP act on distinct caspase activation cascades. XIAP potently blocks the enzymatic activity of group II caspases, including caspase-3 and -7 as well as caspase-9, thus targeting the "mitochondrial apoptotic pathway." FLIP acts as a dominant-

negative protein for caspase-8, the activation of which is linked to the "death-receptors pathway." The association of both types of inhibitors may therefore increase the neuroprotective potential of these compounds.

Although the use of endogenous inhibitors may prove effective in protecting against stroke-induced brain damage, a number of questions must be answered before caspase inhibition enters the clinic, including i) the problem of intracerebral delivery of the inhibitors, and ii) the actual fate of rescued neurons. Inhibition of caspases does prevent acquisition of the morphological features of apoptosis, but does not always prevent irreversible loss of cellular function. As described for pro-caspase-9 splice variants during ischemia, inhibition of caspase activity may lead to the accumulation of the long proforms, with potential delayed activation. This delayed activation may, as observed in Bcl-2-overexpressing mice, lead to the failure to mediate long-term protection. In another example, sympathetic neurons cultured from Bax-knockout mice survive without trophic support, but they remain atrophic unless NGF is supplied *(280).* A comprehensive analysis of the effects of apoptosis inhibitors in stroke models, including behavioral and imaging studies to evaluate restoration of function, is now needed.

### *Neurotrophic Factors*

The neuroprotective effect of NGF has been demonstrated in global cerebral ischemia after intracerebroventricular infusion of NGF *(281),* delivery by viral vectors *(282),* or implantation of genetically modified fibroblasts *(283).* Such invasive neurosurgical procedures are not conceivable in stroke patients, and, more than for any other class of molecule, the clinical use of trophic factors is subject to the development of reliable delivery systems. Neurotrophic factors are large molecules, and systemically administering them in sufficiently high doses to obtain even minimal crossing of the blood-brain barrier has inevitably resulted in toxic side effects in previous trials. Among the strategies developed to deliver neurotrophic factors to the CNS—e.g., direct intracerebral delivery, either acute or by means of osmotic minipumps, the use of transplanted cells genetically engineered to secrete neurotrophic factors, and direct gene transfer using viral vectors—none has proven satisfactory. All these strategies have disadvantages, such as limited diffusion into the brain parenchyma, non-restricted delivery throughout the CNS, tissue damage at the injection site, severe side effects, short-lived production, and immune responses *(284,285).* Clinical use of NGF in a pilot study in Alzheimer's disease was stopped prematurely *(286).* Only one trial has been performed in stroke, using basic fibroblast growth factor (bFGF) (Trafermin, the Fiblast® Phase III trial), with a negative outcome *(287).*

The possibility of increasing the levels of endogenous growth factors by upregulation of their synthesis has also been approached. Clembuterol, a lipophilic β2-mimetic drug, and selegiline, respectively increase the synthesis of NGF and CNTF in vitro *(288,289),* and have neuroprotective effects against ischemic brain damage *(290).* However, no clinical development has been carried out to date, probably because of previous trial failures. Despite evidence of their efficacy, work on trophic factors has also suffered from the apparent controversy concerning the roles of the two NGF receptors. Aside from "classical" neurotrophic factors, new factors with neurotrophic activities have been identified. For instance, the neuropeptide pituitary adenylate cyclase-activating peptide (PACAP) for example, is involved in the proliferation of neuronal precursors *(291,292),* as well as the differentiation and/or survival of a wide variety of neurons (for review, *see* ref. *293*). PACAP crosses the blood-brain barrier *(294),* and has a neuroprotective effect in animal models of ischemia *(295),* even when administered after a delay *(296),* making it an ideal candidate for further development. Molecules such as these, and their effects in cerebral ischemia, must be investigated further.

## Improving Plasticity

Stroke disrupts general behavioral functions that depend upon the integrative activity of the entire brain. Recovery after stroke is largely unpredictable, but can be spectacular. Complete recovery of cognitive, sensorimotor, and functional abilities is observed in approx 10% of patients, so that they have no need for rehabilitation. This is correlated with functional imaging data, which show the activation of new brain areas after stroke and by experimental evidence of axonal elongation and synaptogenesis after ischemia *(297).* The activation of remote brain areas suggests a reorganization of cortical maps, which may be optimized by local or remote modifications of the synaptic network. In this regard, the state of the ischemic penumbra is crucial, since is determines the final extent of the lesion, in terms of neuronal death as well as in terms of the loss of other cell types. For instance, the survival of some oligodendrocytes in the lesioned area may improve axonal regeneration.

Neurotrophic factors play an important role in modulating this conspicuous post-lesion self-repair capacity of the brain, as demonstrated by both clinical and experimental results. Thanks to both their survival properties and involvement in brain plasticity, neurotrophic factors are by far the best candidates to limit stroke-induced neurological impairments. However, as previously mentioned, much work must be done before theory can be translated into practice.

## Replacing Lost Cells

In addition to strategies aimed at promoting neuroprotection and limiting cell death, new therapeutic hopes have recently emerged from the fields of adult neurogenesis and transplantation. The presence of potential sources of newly generated neurons in the adult brain has generated an exciting area of research, and the clinical potential of cell therapy has been demonstrated in neurodegenerative diseases such as Parkinson's disease (PD) and Huntington's disease *(298–301).* A new era of preclinical research is currently developing, which takes advantage of the knowledge generated by both the fields of adult neurogenesis and neuronal transplantation, to analyze the therapeutic potential of multipotent embryonic and adult stem cells in acute and progressive neurological diseases *(302–304).*

### *Endogenous Stem Cells: The Ideal Pool of Precursors for Brain Repair*

The discovery that the adult mammalian nervous system possesses precursor cells which can generate new neurons throughout life (reviewed in ref. *305*) has changed our view of the adaptive potential of the postnatal brain in a considerable way. Although reports of dividing cells in the adult brain date back to the sixties *(306),* neurogenesis in adult mammals was not widely accepted until the last decade, when it began to be recognized as a fundamental mechanism of neuronal renewal closely linked to the adaptive capacities, or plasticity, of the brain. However, in contrast to the high rate of neurogenesis observed throughout the brain in many non-mammalian vertebrates (for example, *see* ref. *307*), neurogenesis in the adult mammalian brain under normal conditions is restricted to two regions, the dentate gyrus of the hippocampal formation *(308–312),* and the subventricular zone (SVZ) *(309,313–315).* In the dentate gyrus, newly generated cells arise from progenitors located in the subgranular zone of the hilus and migrate to the adjacent granule-cell layer, where they differentiate into neurons *(310,316).* In contrast, progenitors in the SVZ form part of a complex structure consisting of at least four distinct cell types, and give rise to new neurons that migrate anteriorly through the rostral migratory stream and into the olfactory bulb, where they mature into local interneurons *(315,317,318).* In vitro, these latter progenitors have been demonstrated to have the capacity for self-renewal, and to give rise to all three major neural cell types—neurons, astrocytes, and oligodendrocytes, resulting in their classification as "stem cells" (for review, *see* ref. *319*).

The precursors of new neurons in both the SVZ and the hippocampus appear to express GFAP *(318,320)*, a marker for cells of the astrocytic lineage, suggesting that differences between neuronal and glial lineages may not be as clear-cut as previously believed.

Although less frequently seen under non-pathological conditions, the possibility exists that neural stem cells may also be disseminated throughout other regions of the CNS, including the cortex, striatum, olfactory bulb, and spinal cord *(321–324)*, giving rise to renewed hopes that they may be used therapeutically for the recovery and rearrangement of neuronal networks after injury or disease. First, adult-generated cells with neuronal characteristics have been found in the neocortex of several mammalian species, including primates (for review, *see* ref. *325*). Second, the recruitment of new neurons may be affected by environmental, epigenetic, and intrinsic, genetic variables. The proliferation of progenitors and survival of newly generated cells are controlled—for example, by activity or experience *(326,327)*, by stress *(328,329)*, by reproductive hormones *(330)*, and by exercise *(331)*. In addition, not all animals of a species exhibit similar levels of neurogenesis—for example, different mouse strains have been found to produce different numbers of new neurons *(332)*. Interestingly, stem cells or neuronal precursors may also be uniquely responsive to neurodegenerative environments. Experimental ablation of populations of neurons leads to increased neurogenesis and neuronal replacement in several paradigms (for example, *see* ref. *333*). Neuronal proliferation has been found to be enhanced in different models of seizure induction *(334–336)*, and various models of ischemia accelerate the proliferation of SVZ *(337)* and dentate gyrus progenitors *(338,339)*, the latter by a factor of 10, giving rise to a 60% proportion of neurons *(340,341)*. Focal cerebral ischemia also increases the number of new neurons derived from the SVZ that are ipsilateral to the lesioned side *(342)*. Although it is not yet known whether such proliferation can aid in the repair and recovery of the brain, initial studies indicate that some of these new neurons do indeed make synaptic connections, a prerequisite for functional replacement.

Despite these studies, our knowledge of the signaling pathways that allow the proliferation and migration of new neurons, and their integration into the functional circuitry of the mature brain is still in its infancy, although some cues are beginning to emerge (for example, *see* refs. *343,344*). Before harnessing the phenomenon of endogenous neurogenesis for brain repair, therefore, we must elaborate the mechanisms that control it—a goal that might be achieved indirectly by studies on exogenous stem cells.

### *Exogenous Stem Cells: An Unlimited Source of Neurons*

Intracerebral transplantation of fetal tissue is a well-established procedure for the treatment of various neurodegenerative disorders. Transplanted fetal neuroblasts have already provided positive clinical results in PD and Huntington's disease *(345,346)*. Although the reconstruction of lost circuitry is only partial, newly developing neurons have been demonstrated both to restore function and to promote the self-repair capacity of the adult brain *(302)*. Also in the case of ischemia, fetal rat hippocampal neurons transplanted into the hippocampal lesion survive and form clusters *(347)*, and grafts from CA1 hippocampal tissue promote recovery from cognitive deficits in marmosets *(348)*. However, transplantation of fetal human tissue raises practical and ethical concerns that make it unsuitable for widespread use. These concerns are allayed by stem cells, which can be expanded in vitro and therefore provide an unlimited supply of precursors for intracerebral transplantation. Before neural stem cells can be widely used in the treatment of neurological diseases or injury, including ischemia, several questions must be answered: i) will neural stem cells or their progeny integrate functionally into an adult brain? ii) what cells should be used? iii) when and where should they be transplanted?

iv) how can their survival, differentiation, and functional integration be enhanced?

If there is a lesson to be learned from transplantation studies in the adult brain, it is that brain structures are receptive to the migration, differentiation, and functional integration of immature neurons. The developing brain contains many structural and functional adaptations that facilitate these processes. Although in many cases these are missing or modified in the adult, enough loopholes exist for the continuation of neurogenesis along the SVZ and hippocampus of adults. New neurons born in the embryonic ventricular zone use specialized structures to travel to their appropriate destinations through the adult parenchyma. Molecules such as PSA-NCAM, which are expressed by immature neurons in the embryo and in the adult and which aid in their migration, are upregulated in the adult brain under conditions of injury or neurodegeneration, including ischemia *(349–351).* When transplanted into a lesioned CNS, developing cells can receive and send connections, release neurotransmitters, and alleviate some functional deficits induced in animal models of neurological diseases *(345).*

In agreement, studies that focus directly on various populations of stem or precursor cells have also begun to yield useful data. When transplanted into neonatal mice, stem cells colonize the host parenchyma. If the animal is subjected to brain ischemia a week later, these stem cells specifically occupy the site of the lesion to form clusters around the cavity *(342).* Even when transplanted into the contralateral hemisphere, the cells migrate through white-matter tracts to the infarcted region, where a small proportion differentiate into neurons, yet no newly generated neurons are found on the side opposite the lesion. However, migration and differentiation are not observed if transplantation is postponed for several weeks post-ischemia, demonstrating the role of the lesion in these processes. In other examples, hematopoietic myeloid progenitor cells from the adult bone marrow survive implantation into the penumbra of a striatal ischemic lesion, a condition that is improved by adding the supporting tissue or stroma to the cells. In addition, this paradigm increases endogenous neurogenesis, emphasizing the importance of the microenvironment created by transplanted immature cells and the existence of a dynamic interaction between transplanted cells and the host. Several studies have also been performed using cell lines such as the neuroepithelial stem-cell line MHP36 *(352),* which integrates into the ischemic striatum and cortex and reverses sensorimotor deficits in rats. Furthermore, in a pilot clinical trial inspired by animal studies on brain repair after stroke, cultured neuronal cells derived from a teratocarcinoma cell line were successfully transplanted into patients with basal ganglia stroke *(353),* and long-term evaluations of functional outcome are still anticipated.

One development of the last decade that has proven of immense value in the in vitro propagation and study of neural stem cells, is the culture of neurospheres *(314,354).* Neurospheres are clonal colonies derived from neurogenic areas of immature or mature brains using specific culture methods. Under optimal conditions, they are generated by a single multipotent progenitor or stem cell that renews itself and gives rise to more committed precursor populations or differentiated neural cells. Neurospheres give rise to the three major neural cell types *(355).* The last—but not the least—advantage of neurospheres is that they can be derived from human brain tissue as well as from animal tissue, making them ideal for development as a source of cells for transplantation in the clinical setting. In the case of ischemia and other conditions, neurospheres offer a distinct advantage—the ability to generate both neurons and glia. Apart from the notion that an ideal transplant would be one that would replace all the different cell types lost, co-transplantation of glia with neurons might actually serve to enhance the repair process. For example, transplanted glial cells may be useful in modifying the response to injury and assisting in structural repair *(356).* Our data also show

that transplanting glia may be clinically relevant in stroke by protecting threatened neurons from further degeneration *(207).*

When considering both the time and the place of transplantation, it should be kept in mind that both will influence and be influenced by the choice of cells to be transplanted. For example, more immature cells with higher proliferation and migration capacity may be used for transplants designed to replace dead neurons, or for transplants placed outside the ischemic core. However, more mature cells that already express several neurotrophic factors, but which may be less plastic or mobile, might do better in transplants aimed at rescuing endogenous cells.

## Conclusions

A comparison between earlier clinical studies on potentially neuroprotective agents and approaches currently being developed in the pharmaceutical industry clearly illustrates that new targets are being defined for drug research in acute stroke. Although it cannot be guaranteed that the new generations of agents will be more effective than their predecessors, it appears that the disappointing results of previous clinical trials may be the result of the premature application of experimental data as well as protocol problems and underestimation of the neuroprotective effects of drugs (*see* ref. *357*). With our increasing knowledge of the pathways involved in ischemic cell death, the prospect of successful therapy in the future looks promising. However, it is probable that the association of several molecules will be necessary to block cell death and improve functional outcome. Alternatively, compounds are being designed that act on multiple processes simultaneously, and which have already proven to have greater efficacy than their forerunners. In addition, the combination of such conventional approaches with strategies aimed at enhancing endogenous neuronal plasticity or replacing dead cells or damaged neurons may be more effective than drugs alone.

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## References

1. Wardlaw J. M., Dennis M. S., Lindley R. I., Sellar R. J., and Warlow C. P. (1996) The validity of a simple clinical classification of acute ischaemic stroke. *J. Neurol.* **243,** 274–279.
2. Allan W. C. (1990) The IVH complex of lesions: cerebrovascular injury in the preterm infant. *Neurol Clin.* **8,** 529–551.
3. Davenport R. and Dennis M. (2000) Neurological emergencies: acute stroke. *J. Neurol. Neurosurg. Psychiatry* **68,** 277–288.
4. Furlan A., Higashida R., Wechsler L., Gent M., Rowley H., Kase C., et al. (1999) Intra-arterial prourokinase for acute ischemic stroke. The PROACT II study: a randomized controlled trial. *Prolyse in Acute Cerebral Thromboembolism JAMA* **282,** 2003–2011.
5. Conference T. a. b. o. t. i. h. h. (1992), in *National Health and Welfare,* pp. 12–23, NINDS Publ., Victoria.
6. Garcia J. H., Liu K. F., and Ho K. L. (1995) Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. *Stroke* **26,** 636–642, discussion 643.
7. Lipton P. (1999) Ischemic cell death in brain neurons. *Physiol. Rev.* **79,** 1431–1568.
8. Linnik M. D., Zobrist R. H., and Hatfield M. D. (1993) Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. *Stroke* **24,** 2002–2008, discussion 2008–2009.
9. Charriaut-Marlangue C., Margaill I., Represa A., Popovici T., Plotkine M., and Ben-Ari Y. (1996) Apoptosis and necrosis after reversible focal ischemia: an in situ DNA fragmentation analysis. *J. Cereb Blood Flow Metab.* **16,** 186–194.
10. Guégan C., Ceballos-Picot I., Nicole A., Kato H., Onténiente B., and Sola B. (1998) Recruitment of several neuroprotective pathways

after permanent focal ischemia in mice. *Exp. Neurol.* **154,** 371–380.
11. Clarke C. J., Hales A., Hunt A., and Foxwell B. M. (1998) IL-10-mediated suppression of TNF-alpha production is independent of its ability to inhibit NF kappa B activity. *Eur. J. Immunol.* **28,** 1719–1726.
12. Zeng Y. S. and Xu Z. C. (2000) Co-existence of necrosis and apoptosis in rat hippocampus following transient forebrain ischemia. *Neurosci. Res.* **37,** 113–125.
13. Wyllie A. H., Kerr J. F., and Currie A. R. (1980) Cell death: the significance of apoptosis. *Int. Rev. Cytol.* **68,** 251–306.
14. Colbourne F., Sutherland G. R., and Auer R. N. (1999) Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia. *J. Neurosci.* **19,** 4200–4210.
15. Portera-Cailliau C., Price D. L., and Martin L. J. (1997) Non-NMDA and NMDA receptor-mediated excitotoxic neuronal deaths in adult brain are morphologically distinct: further evidence for an apoptosis-necrosis continuum. *J. Comp. Neurol.* **378,** 88–104.
16. Martin L. J., Al-Abdulla N. A., Brambrink A. M., Kirsch J. R., Sieber F. E., and Portera-Cailliau C. (1998) Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. *Brain Res. Bull.* **46,** 281–309.
17. Choi D. W. and Gage F. H. (1996) Disease, transplantation and regeneration. *Curr. Opin. Neurobiol.* **6,** 635–637.
18. Gwag B. J., Lobner D., Koh J. Y., Wie M. B., and Choi D. W. (1995) Blockade of glutamate receptors unmasks neuronal apoptosis after oxygen-glucose deprivation in vitro. *Neuroscience* **68,** 615–619.
19. Leist M., Single B., Castoldi A. F., Kuhnle S., and Nicotera P. (1997) Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J. Exp. Med.* **185,** 1481–1486.
20. Melino G., Catani M. V., Corazzari M., Guerrieri P., and Bernassola F. (2000) Nitric oxide can inhibit apoptosis or switch it into necrosis. *Cell Mol. Life Sci.* **57,** 612–622.
21. Lemasters J. J., Qian T., Bradham C. A., Brenner D. A., Cascio W. E., Trost L. C., et al. (1999) Mitochondrial dysfunction in the pathogenesis of necrotic and apoptotic cell death. *J. Bioenerg. Biomembr.* **31,** 305–319.
22. Hata R., Mies G., Wiessner C., Fritze K., Hesselbarth D., Brinker G., et al. (1998) A reproducible model of middle cerebral artery occlusion in mice: hemodynamic, biochemical, and magnetic resonance imaging. *J. Cereb. Blood Flow Metab* **18,** 367–375.
23. Johnson D. E. (2000) Programmed cell death regulation: basic mechanisms and therapeutic opportunities. *Leukemia* **14,** 1340–1344.
24. Martin R. L., Lloyd H. G., and Cowan A. I. (1994) The early events of oxygen and glucose deprivation: setting the scene for neuronal death? *Trends Neurosci.* **17,** 251–257.
25. Sun G. Y. and Foudin L. L. (1984) On the status of lysolecithin in rat cerebral cortex during ischemia. *J. Neurochem.* **43,** 1081–1086.
26. Graham S. H., Chen J., Lan J., Leach M. J., and Simon R. P. (1994) Neuroprotective effects of a use-dependent blocker of voltage-dependent sodium channels, BW619C89, in rat middle cerebral artery occlusion. *J. Pharmacol. Exp. Ther.* **269,** 854–859.
27. Smith S. E. and Meldrum B. S. (1995) Cerebroprotective effect of lamotrigine after focal ischemia in rats. *Stroke* **26,** 117–121; discussion 121–122.
28. Fujitani T., Adachi N., Miyazaki H., Liu K., Nakamura Y., Kataoka K., et al. (1994) Lidocaine protects hippocampal neurons against ischemic damage by preventing increase of extracellular excitatory amino acids: a microdialysis study in Mongolian gerbils. *Neurosci. Lett.* **179,** 91–94.
29. Lysko P. G., Webb C. L., Yue T. L., Gu J. L., and Feuerstein G. (1994) Neuroprotective effects of tetrodotoxin as a Na+ channel modulator and glutamate release inhibitor in cultured rat cerebellar neurons and in gerbil global brain ischemia. *Stroke* **25,** 2476–2482.
30. Crumrine R. C., Bergstrand K., Cooper A. T., Faison W. L., and Cooper B. R. (1997) Lamotrigine protects hippocampal CA1 neurons from ischemic damage after cardiac arrest. *Stroke* **28,** 2230–2236; discussion 2237.
31. Lekieffre D. and Meldrum B. S. (1993) The pyrimidine-derivative, BW1003C87, protects CA1 and striatal neurons following transient severe forebrain ischaemia in rats. A microdialysis and histological study. *Neuroscience* **56,** 93–99.
32. Rataud J., Debarnot F., Mary V., Pratt J., and Stutzmann J. M. (1994) Comparative study of voltage-sensitive sodium channel blockers in

focal ischaemia and electric convulsions in rodents. *Neurosci Lett.* **172,** 19–23.
33. Diener H. C., Cortens M., Ford G., Grotta J., Hacke W., Kaste M., et al. (2000) Lubeluzole in acute ischemic stroke treatment: a double-blind study with an 8-hour inclusion window comparing a 10-mg daily dose of lubeluzole with placebo. *Stroke* **31,** 2543–2451.
34. Simon R. P., Swan J. H., Griffiths T., and Meldrum B. S. (1984) Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science* **226,** 850–852.
35. Benveniste H., Drejer J., Schousboe A., and Diemer N. H. (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J. Neurochem.* **43,** 1369–1374.
36. Shimada N., Graf R., Rosner G., Wakayama A., George C. P., and Heiss W. D. (1989) Ischemic flow threshold for extracellular glutamate increase in cat cortex. *J. Cereb. Blood Flow Metab.* **9,** 603–606.
37. Baker C. J., Fiore A. J., Frazzini V. I., Choudhri T. F., Zubay G. P., and Solomon R. A. (1995) Intraischemic hypothermia decreases the release of glutmate in the cores of permanent focal cerebral infarcts. *Neurosurgery* **36,** 994–1001; discussion 1001–1002.
38. Obrenovitch T. P., Urenjak J., Richards D. A., Ueda Y., Curzon G., and Symon L. (1993) Extracellular neuroactive amino acids in the rat striatum during ischaemia: comparison between penumbral conditions and ischaemia with sustained anoxic depolarisation. *J. Neurochem.* **61,** 178–186.
39. Rossi D. J., Oshima T., and Attwell D. (2002) Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature* **403,** 316–321.
40. Mitani A., Andou Y., Matsuda S., Arai T., Sakanaka M., and Kataoka K. (1994) Origin of ischemia-induced glutamate efflux in the CA1 field of the gerbil hippocampus: an in vivo brain microdialysis study. *J. Neurochem.* **63,** 2152–2164.
41. Choi D. W. and Rothman S. M. (1990) The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu. Rev. Neurosci.* **13,** 171–182.
42. Olney J. W. (1969) Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* **164,** 719–721.
43. Novelli A., Reilly J. A. Lysko P. G., and Henneberry R. C. (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res.* **451,** 205–212.
44. Buchan A. and Pulsinelli W. A. (1990) Hypothermia but not the N-methyl-D-aspartate antagonist, MK-801, attenuates neuronal damage in gerbils subjected to transient global ischemia. *J. Neurosci.* **10,** 311–316.
45. Hoffman C. A. and Boast C. A. (1995) Neuroprotection by MK-801 in temperature maintained gerbils. *Brain Res. Bull.* **38,** 405–409.
46. Park C. K., Nehls D. G., Graham D. I., Teasdale G. M., and McCulloch J. (1988) The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. *Ann. Neurol.* **24,** 543–551.
47. Uematsu D., Araki N., Greenberg J. H., Sladky J., and Reivich M. (1991) Combined therapy with MK-801 and nimodipine for protection of ischemic brain damage. *Neurology* **41,** 88–94.
48. Gill R., Hargreaves R. J., and Kemp J. A. (1995) The neuroprotective effect of the glycine site antagonist 3R-(+)-cis-4-methyl-HA966 (L-687,414) in a rat model of focal ischaemia. *J. Cereb. Blood Flow Metab.* **15,** 197–204.
49. Bullock R., Graham D. I., Chen M. H., Lowe D., and McCulloch J. (1990) Focal cerebral ischemia in the cat: pretreatment with a competitive NMDA receptor antagonist, D-CPP-ene. *J. Cereb. Blood Flow Metab.* **10,** 668–674.
50. Sauer D., Weber E., Luond G., Da Silva F., and Allegrini P. R. (1995) The competitive NMDA antagonist CGP 40116 permanently reduces brain damage after middle cerebral artery occlusion in rats. *J. Cereb. Blood Flow Metab.* **15,** 602–610.
51. Lang-Lazdunski L., Heurteaux C., Vaillant N., Widmann C., and Lazdunski M. (1999) Riluzole prevents ischemic spinal cord injury caused by aortic crossclamping. *J. Thorac. Cardiovasc. Surg.* **117,** 881–889.
52. Andine P., Orwar O., Jacobson I., Sandberg M., and Hagberg H. (1991) Changes in extracellular amino acids and spontaneous neuronal activity during ischemia and extended reflow in the CA1 of the rat hippocampus. *J. Neurochem.* **57,** 222–229.
53. Matsumoto K., Lo E. H., Pierce A. R., Halpern E. F., and Newcomb R. (1996) Secondary elevation of extracellular neurotransmitter amino

acids in the reperfusion phase following focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* **16,** 114–124.
54. Yao H., Markgraf C. G., Dietrich W. D., Prado R., Watson B. D., and Ginsberg M. D. (1994) Glutamate antagonist MK-801 attenuates incomplete but not complete infarction in thrombotic distal middle cerebral artery occlusion in Wistar rats. *Brain Res.* **642,** 117–122.
55. Nakamura K., Hatakeyama T., Furuta S., and Sakaki S. (1993) The role of early Ca2+ influx in the pathogenesis of delayed neuronal death after brief forebrain ischemia in gerbils. *Brain Res.* **613,** 181–192.
56. Perkins W. J., Lanier W. L., Karlsson B. R., Milde J. H., and Michenfelder J. D. (1989) The effect of the excitatory amino acid receptor antagonist dizocilipine maleate (MK-801) on hemispheric cerebral blood flow and metabolism in dogs: modification by prior complete cerebral ischemia. *Brain Res.* **498,** 34–44.
57. Takizawa S., Hogan M., and Hakim A. M. (1991) The effects of a competitive NMDA receptor antagonist (CGS-19755) on cerebral blood flow and pH in focal ischemia. *J. Cereb. Blood Flow Metab.* **11,** 786–793.
58. Robertson S. C., Wetjen N. M., Beer B. J., and Loftus C. M. (1997) Pre- and postischemic effects of the NMDA receptor antagonist dizocilpine maleate (MK-801) on collateral cerebral blood flow. *J. Neurosurg.* **87,** 927–933.
59. Morikawa E., Mori H., Kiyama Y., Mishina M., Asano T., and Kirino T. (1998) Attenuation of focal ischemic brain injury in mice deficient in the epsilon 1 (NR2A) subunit of NMDA receptor. *J. Neurosci.* **18,** 9727–9732.
60. Buchan A. M., Li H., Cho S., and Pulsinelli W. A. (1991) Blockade of the AMPA receptor prevents CA1 hippocampal injury following severe but transient forebrain ischemia in adult rats. *Neurosci Lett.* **132,** 255–258.
61. Li H. and Buchan A. M. (1993) Treatment with an AMPA antagonist 12 hours following severe normothermic forebrain ischemia prevents CA1 neuronal injury. *J. Cereb. Blood Flow Metab.* **13,** 933–939.
62. Lobner D. and Lipton P. (1993) Intracellular calcium levels and calcium fluxes in the CA1 region of the rat hippocampal slice during in vitro ischemia: relationship to electrophysiological cell damage. *J. Neurosci.* **13,** 4861–4871.
63. Zhang Y. and Lipton P. (1999) Cytosolic Ca2+ changes during in vitro ischemia in rat hippocampal slices: major roles for glutamate and Na+-dependent Ca2+ release from mitochondria. *J. Neurosci.* **19,** 3307–3315.
64. Davis M. and Barer D. (1999) Neuroprotection in acute ischaemic stroke. II: Clinical potential. *Vasc. Med.* **4,** 149–163.
65. Seif el Nasr M., Peruche B., Rossberg C., Mennel H. D., and Krieglstein J. (1990) Neuroprotective effect of memantine demonstrated in vivo and in vitro. *Eur. J. Pharmacol.* **185,** 19–24.
66. Mies G., Kohno K. and Hossmann K. A. (1994) Prevention of periinfarct direct current shifts with glutamate antagonist NBQX following occlusion of the middle cerebral artery in the rat. *J. Cereb. Blood Flow Metab.* **14,** 802–807.
67. Ben-Ari Y. and Khrestchatisky M. (1998) The GluR2 (GluRB) hypothesis in ischemia: missing links. *Trends Neurosci.* **21,** 241–242.
68. Pellegrini-Giampietro D. E., Gorter J. A., Bennett M. V., and Zukin R. S. (1997) The GluR2 (GluR-B) hypothesis: Ca(2+)-permeable AMPA receptors in neurological disorders [see comments]. *Trends Neurosci.* **20,** 464–470.
69. Hollmann M., Hartley M., and Heinemann S. (1991) Ca2+ permeability of KA-AMPA—gated glutamate receptor channels depends on subunit composition. *Science* **252,** 851–853.
70. Pollard H., Heron A., Moreau J., Ben-Ari Y., and Khrestchatisky M. (1993) Alterations of the GluR-B AMPA receptor subunit flip/flop expression in kainate-induced epilepsy and ischemia. *Neuroscience* **57,** 545–554.
71. Heurteaux C., Lauritzen I., Widmann C., and Lazdunski M. (1995) Essential role of adenosine, adenosine A1 receptors, and ATP-sensitive K+ channels in cerebral ischemic preconditioning. *Proc. Natl. Acad. Sci. USA* **92,** 4666–4670.
72. Kohler M., Burnashev N., Sakmann B., and Seeburg P. H. (1993) Determinants of Ca2+ permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity by RNA editing. *Neuron* **10,** 491–500.
73. Bullock R., Graham D. I., Swanson S., and McCulloch J. (1994) Neuroprotective effect of the AMPA receptor antagonist LY-293558 in focal cerebral ischemia in the cat. *J. Cereb. Blood Flow Metab.* **14,** 466–471.
74. Yatsugi S., Takahashi M., Kawasaki-Yatsugi S., Koshiya K., Sakamoto S., Uematsu D., et al. (1996) Neuroprotective effect of YM90K, a novel AMPA/kainate receptor antagonist, in focal cerebral ischemia in cats. *J. Cereb. Blood Flow Metab.* **16,** 959–966.

75. Shimizu-Sasamata M., Kano T., Rogowska J., Wolf G. L., Moskowitz M. A., and Lo E. H. (1998) YM872, a highly water-soluble AMPA receptor antagonist, preserves the hemodynamic penumbra and reduces brain injury after permanent focal ischemia in rats. *Stroke* **29,** 2141–2148.
76. Turski L., Huth A., Sheardown M., McDonald F., Neuhaus R., Schneider H. H., et al. (1998) ZK200775: a phosphonate quinoxalinedione AMPA antagonist for neuroprotection in stroke and trauma. *Proc. Natl. Acad. Sci. USA* **95,** 10,960–10,965.
77. Fagni L., Chavis P., Ango F., and Bockaert J. (2000) Complex interactions between mGluRs, intracellular Ca2+ stores and ion channels in neurons. *Trends Neurosci.* **23,** 80–88.
78. Ankarcrona M., Dypbukt J. M., Orrenius S., and Nicotera P. (1996) Calcineurin and mitochondrial function in glutamate-induced neuronal cell death. *FEBS Lett.* **394,** 321–324.
79. Budd S. L. and Nicholls D. G. (1996) Mitochondria, calcium regulation, and acute glutamate excitotoxicity in cultured cerebellar granule cells. *J. Neurochem.* **67,** 2282–2291.
80. Buisson A. and Choi D. W. (1995) The inhibitory mGluR agonist, S-4-carboxy-3-hydroxy-phenylglycine selectively attenuates NMDA neurotoxicity and oxygen-glucose deprivation-induced neuronal death. *Neuropharmacology* **34,** 1081–1087.
81. Chiamulera C., Costa S., and Reggiani A. (1990) Effect of NMDA- and strychnine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning. *Psychopharmacology* **102,** 551–552.
82. Henrich-Noack P., Hatton C. D., and Reymann K. G. (1998) The mGlu receptor ligand (S)-4C3HPG protects neurons after global ischaemia in gerbils. *Neuroreport* **9,** 985–988.
83. Pizzi M., Fallacara C., Arrighi V., Memo M., and Spano P. F. (1993) Attenuation of excitatory amino acid toxicity by metabotropic glutamate receptor agonists and aniracetam in primary cultures of cerebellar granule cells. *J. Neurochem.* **61,** 683–689.
84. Kristián T. and Siesjö B. K. (1996) Calcium-related damage in ischemia. *Life Sci.* **59,** 357–367.
85. Blaustein M. P. and Golovina V. A. (2001) Structural complexity and functional diversity of endoplasmic reticulum Ca(2+) stores. *Trends Neurosci.* **24,** 602–608.
86. Scarpa A. and Azzone G. F. (1970) The mechanism of ion translocation in mitochondria. 4. Coupling of K+ efflux with Ca2+ uptake. *Eur. J. Biochem.* **12,** 328–335.
87. Kass I. S. and Lipton P. (1986) Calcium and long-term transmission damage following anoxia in dentate gyrus and CA1 regions of the rat hippocampal slice. *J. Physiol.* **378,** 313–334.
88. Harris R. J., Symon L., Branston N. M., and Bayhan M. (1981) Changes in extracellular calcium activity in cerebral ischaemia. *J. Cereb. Blood Flow Metab.* **1,** 203–209.
89. Kristián T., Gido G., Kuroda S., Schutz A., and Siesjö B. K. (1998) Calcium metabolism of focal and penumbral tissues in rats subjected to transient middle cerebral artery occlusion. *Exp. Brain Res.* **120,** 503–509.
90. Andine P., Jacobson I., and Hagberg H. (1992) Enhanced calcium uptake by CA1 pyramidal cell dendrites in the postischemic phase despite subnormal evoked field potentials: excitatory amino acid receptor dependency and relationship to neuronal damage. *J. Cereb. Blood Flow Metab.* **12,** 773–783.
91. Ohta S., Furuta S., Matsubara I., Kohno K., Kumon Y., and Sakaki S. (1996) Calcium movement in ischemia-tolerant hippocampal CA1 neurons after transient forebrain ischemia in gerbils. *J. Cereb. Blood Flow Metab.* **16,** 915–922.
92. DeGraba T. J., Ostrow P. T., and Grotta J. C. (1993) Threshold of calcium disturbances after focal cerebral ischemia in rats. Implications of the window of therapeutic opportunity. *Stroke* **24,** 1212–1216; discussion 1216–1217.
93. Meyer F. B. (1990) Calcium antagonists and vasospasm. *Neurosurg. Clin. N. Am.* **1,** 367–376.
94. Deshpande J. K. and Wieloch T. (1986) Flunarizine, a calcium entry blocker, ameliorates ischemic brain damage in the rat. *Anesthesiology* **64,** 215–224.
95. Mossakowski M. J. and Gadamski R. (1990) Nimodipine prevents delayed neuronal death of sector CA1 pyramidal cells in short-term forebrain ischemia in Mongolian gerbils. *Stroke* **21,** IV120–122.
96. Nuglisch J., Karkoutly C., Mennel H. D. Rossberg C., and Krieglstein J. (1990) Protective effect of nimodipine against ischemic neuronal damage in rat hippocampus without changing postischemic cerebral blood flow. *J. Cereb. Blood Flow Metab.* **10,** 654–659.

97. Uematsu D., Greenberg J. H., Hickey W. F., and Reivich M. (1989) Nimodipine attenuates both increase in cytosolic free calcium and histologic damage following focal cerebral ischemia and reperfusion in cats. *Stroke* **20,** 1531–1537.
98. Valentino K., Newcomb R., Gadbois T., Singh T., Bowersox S., Bitner S., et al. (1993) A selective N-type calcium channel antagonist protects against neuronal loss after global cerebral ischemia. *Proc. Natl. Acad. Sci. USA* **90,** 7894–7897.
99. Buchan A. M., Gertler S. Z., Li H., Xue D., Huang Z. G., Chaundy K. E., et al. (1994) A selective N-type Ca(2+)-channel blocker prevents CA1 injury 24 h following severe forebrain ischemia and reduces infarction following focal ischemia. *J. Cereb. Blood Flow Metab.* **14,** 903–910.
100. Takizawa S., Matsushima K., Fujita H., Nanri K., Ogawa S., and Shinohara Y. (1995) A selective N-type calcium channel antagonist reduces extracellular glutamate release and infarct volume in focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* **15,** 611–618.
101. Horn J., de Haan R. J., Vermeulen M., and Limburg M. (2001) Very Early Nimodipine Use in Stroke (VENUS): a randomized, double-blind, placebo-controlled trial. *Stroke* **32,** 461–465.
102. Franke C. L., Palm R., Dalby M., Schoonderwaldt H. C., Hantson L., Eriksson B., et al. (1996) Flunarizine in stroke treatment (FIST): a double-blind, placebo-controlled trial in Scandinavia and the Netherlands. *Acta Neurol. Scand.* **93,** 56–60.
103. Mohr J., Orgogozo J., Harrison M., Hennerici M., Wahlgren N., Gelmers J., et al. (1994) Meta-analysis of oral nimodipine trials in acute ischemic stroke. *Cerebrovasc. Dis.* **4,** 177–210.
104. Mitchell P. (1961) *Nature* **191,** 144–148.
105. Morin D., Hauet T., Spedding M., and Tillement J. (2001) Mitochondria as target for anti-ischemic drugs. *Adv. Drug Deliv. Rev.* **49,** 151–174.
106. Haworth R. A. and Hunter D. R. (1979) The Ca2+-induced membrane transition in mitochondria. II. Nature of the Ca2+ trigger site. *Arch. Biochem. Biophys.* **195,** 460–467.
107. Skulachev V. P. (1996) Why are mitochondria involved in apoptosis? Permeability transition pores and apoptosis as selective mechanisms to eliminate superoxide-producing mitochondria and cell. *FEBS Lett.* **397,** 7–10.
108. Petit P. X., Susin S. A., Zamzami N., Mignotte B., and Kroemer G. (1996) Mitochondria and programmed cell death: back to the future. *FEBS Lett.* **396,** 7–13.
109. Zoratti M. and Szabo I. (1995) The mitochondrial permeability transition. *Biochim. Biophys. Acta* **1241,** 139–176.
110. Wolf A., Trendelenburg C. F., Diez-Fernandez C., Prieto P., Houy S., Trommer W. E., et al. (1997) Cyclosporine A-induced oxidative stress in rat hepatocytes. *J. Pharmacol. Exp. Ther.* **280,** 1328–1334.
111. Liu X., Kim C. N., Yang J., Jemmerson R., and Wang X. (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* **86,** 147–157.
112. Susin S. A., Zamzami N., Castedo M., Hirsch T., Marchetti P., Macho A., et al. (1996) Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. *J. Exp. Med.* **184,** 1331–1341.
113. Lorenzo H. K., Susin S. A., Penninger J., and Kroemer G. (1999) Apoptosis inducing factor (AIF): a phylogenetically old, caspase- independent effector of cell death. *Cell Death Differ.* **6,** 516–524.
114. Desagher S. and Martinou J. C. (2000) Mitochondria as the central control point of apoptosis. *Trends Cell Biol.* **10,** 369–377.
115. Morin D., Papadopoulos V., and Tillement J.-P. (2002) Prevention of cell damage in ischaemia: novel molecular targets in mitochondria. *Expt. Opin. Ther. Targets* **6,** 315–334.
116. Love S. (1999) Oxidative stress in brain ischemia. *Brain Pathol.* **9,** 119–131.
117. Siesjö B. K., Elmer E., Janelidze S., Keep M., Kristian T., Ouyang Y. B., et al. (1999) Role and mechanisms of secondary mitochondrial failure. *Acta Neurochir. Suppl.* **73,** 7–13.
118. Marshall J. W., Duffin K. J., Green A. R., and Ridley R. M. (2001) NXY-059, a free radical—trapping agent, substantially lessens the functional disability resulting from cerebral ischemia in a primate species. *Stroke* **32,** 190–198.
119. Spinnewyn B., Blavet N., and Clostre F. (1986) [Effects of Ginkgo biloba extract on a cerebral ischemia model in gerbils]. *Presse Med.* **15,** 1511–1515.
120. Clark W. M., Rinker L. G., Lessov N. S., Lowery S. L., and Cipolla M. J. (2001) Efficacy of antioxidant therapies in transient focal ischemia in mice. *Stroke* **32,** 1000–1004.
121. Yamaguchi T., Sano K., Takakura K., Saito I., Shinohara Y., Asano T., et al. (1998) Ebselen in

acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. *Stroke* **29,** 12–17.
122. Cuzzocrea S., Riley D. P., Caputi A. P., and Salvemini D. (2001) Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol. Rev.* **53,** 135–139.
123. Chabrier P. E., Auguet M., Spinnewyn B., Auvin S., Cornet S., Dermerle-Pallardy C., et al. (1999) BN 80933, a dual inhibitor of neuronal nitric oxide synthase and lipid peroxidation: a promising neuroprotective strategy. *Proc. Natl. Acad. Sci. USA* **96,** 10,824–10,829.
124. Baker K., Marcus C. B., Huffman K., Kruk H., Malfroy B., and Doctrow S. R. (1998) Synthetic combined superoxide dismutase/catalase mimetics are protective as a delayed treatment in a rat stroke model: a key role for reactive oxygen species in ischemic brain injury. *J. Pharmacol. Exp. Ther.* **284,** 215–221.
125. Cai J. and Jones D. P. (1998) Superoxide in apoptosis. Mitochondrial generation triggered by cytochrome c loss. *J. Biol. Chem.* **273,** 11,401–11,404.
126. Madesh M. and Hajnoczky G. (2001) VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. *J. Cell. Biol.* **155,** 1003–1015.
127. Li P., Nijhawan D., Budihardjo I., Srinivasula S. M., Ahmad M., Alnemri E. S., et al. (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **91,** 479–489.
128. Zhivotovsky B., Burgess D. H., Schlegel J., Porn M. I., Vanags D., and Orrenius S. (1997) Proteases in Fas-mediated apoptosis. *J. Cell Biochem.* **64,** 43–49.
129. Krippner A., Matsuno-Yagi A., Gottlieb R. A., and Babior B. M. (1996) Loss of function of cytochrome c in Jurkat cells undergoing fas-mediated apoptosis. *J. Biol. Chem.* **271,** 21,629–21,636.
130. Stefanelli C., Bonavita F., Stanic I., Farruggia G., Falcieri E., Robuffo I., et al. (1997) ATP depletion inhibits glucocorticoid-induced thymocyte apoptosis. *Biochem. J.* **322,** 909–917.
131. Li F., Srinivasan A., Wang Y., Armstrong R. C., Tomaselli K. J., and Fritz L. C. (1997) Cell-specific induction of apoptosis by microinjection of cytochrome c. Bcl-xL has activity independent of cytochrome c release. *J. Biol. Chem.* **272,** 30,299–30,305.
132. Gross A., McDonnell J. M., and Korsmeyer S. J. (1999) BCL-2 family members and the mitochondria in apoptosis. *Genes Dev.* **13,** 1899–1911.
133. Green D. R., and Reed J. C. (1998) Mitochondria and apoptosis. *Science* **281,** 1309–1312.
134. Jurgensmeier J. M., Xie Z., Deveraux Q., Ellerby L., Bredesen D., and Reed J. C. (1998) Bax directly induces release of cytochrome c from isolated mitochondria. *Proc. Natl. Acad. Sci. USA* **95,** 4997–5002.
135. Vander Heiden M. G., Chandel N. S., Williamson E. K., Schumacker P. T., and Thompson C. B. (1997) Bcl-xL regulates the membrane potential and volume homeostasis of mitochondria [see comments]. *Cell* **91,** 627–637.
136. Kluck R. M., Bossy-Wetzel E., Green D. R., and Newmeyer D. D. (1997) The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* **275,** 1132–1136.
137. Hockenbery D. M., Oltvai Z. N., Yin X. M., Milliman C. L., and Korsmeyer S. J. (1993) Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* **75,** 241–251.
138. Kane D. J., Sarafian T. A., Anton R., Hahn H., Gralla E. B., Valentine J. S., et al. (1993) Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* **262,** 1274–1277.
139. Chen D. F., Schneider G. E., Martinou J. C., and Tonegawa S. (1997) Bcl-2 promotes regeneration of severed axons in mammalian CNS. *Nature* **385,** 434–439.
140. Chinnaiyan A. M., O'Rourke K., Tewari M., and Dixit V. M. (1995) FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* **81,** 505–512.
141. Seshagiri S. and Miller L. K. (1997) Caenorhabditis elegans CED-4 stimulates CED-3 processing and CED-3-induced apoptosis. *Curr. Biol.* **7,** 455–460.
142. Pettmann B. and Henderson C. E. (1998) Neuronal cell death. *Neuron* **20,** 633–647.
143. Antonawich F. J., Federoff H. J., and Davis J. N. (1999) BCL-2 transduction, using a herpes simplex virus amplicon, protects hippocampal neurons from transient global ischemia. *Exp. Neurol.* **156,** 130–137.

144. Wiessner C., Allegrini P. R., Rupalla K., Sauer D., Oltersdorf T., McGregor A. L., et al. (1999) Neuron-specific transgene expression of Bcl-XL but not Bcl-2 genes reduced lesion size after permanent middle cerebral artery occlusion in mice. *Neurosci. Lett.* **268,** 119–122.
145. Yan C., Chen J., Chen D., Minami M., Pei W., Yin X. M., et al. (2000) Overexpression of the cell death suppressor Bcl-w in ischemic brain: implications for a neuroprotective role via the mitochondrial pathway. *J. Cereb. Blood Flow Metab.* **20,** 620–630.
146. Hata R., Gillardon F., Michaelidis T. M., and Hossmann K. A. (1999) Targeted disruption of the bcl-2 gene in mice exacerbates focal ischemic brain injury. *Metab. Brain Dis.* **14,** 117–124.
147. Chen J., Simon R. P., Nagayama T., Zhu R., Loeffert J. E., Watkins S. C., et al. (2000) Suppression of endogenous bcl-2 expression by antisense treatment exacerbates ischemic neuronal death. *J. Cereb. Blood Flow Metab.* **20,** 1033–1039.
148. Martinou J. C., Dubois-Dauphin M., Staple J. K., Rodriguez I., Frankowski H., Missotten M., et al. (1994) Overexpression of BCL-2 in transgenic mice protects neurons from naturally occurring cell death and experimental ischemia. *Neuron* **13,** 1017–1030.
149. Krajewski S., Mai J. K., Krajewska M., Sikorska M., Mossakowski M. J., and Reed J. C. (1995) Upregulation of bax protein levels in neurons following cerebral ischemia. *J. Neurosci.* **15,** 6364–6376.
150. Gillardon F., Lenz C., Waschke K. F., Krajewski S., Reed J. C., Zimmermann M., et al. (1996) Altered expression of Bcl-2, Bcl-X, Bax, and c-Fos colocalizes with DNA fragmentation and ischemic cell damage following middle cerebral artery occlusion in rats. *Brain Res. Mol. Brain Res.* **40,** 254–260.
151. Isenmann S., Stoll G., Schroeter M., Krajewski S., Reed J. C., and Bahr M. (1998) Differential regulation of Bax, Bcl-2, and Bcl-X proteins in focal cortical ischemia in the rat. *Brain Pathol.* **8,** 49–62; discussion 62–63.
152. Wolter K. G., Hsu Y. T., Smith C. L., Nechushtan A., Xi X. G., and Youle R. J. (1997) Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell Biol.* **139,** 1281–1292.
153. Degterev A., Boyce M., and Yuan J. (2001) The channel of death. *J. Cell Biol.* **155,** 695–696.
154. Cao G., Minami M., Pei W., Yan C., Chen D., O'Horo C., et al. (2001) Intracellular Bax translocation after transient cerebral ischemia: implications for a role of the mitochondrial apoptotic signaling pathway in ischemic neuronal death. *J. Cereb. Blood Flow Metab.* **21,** 321–333.
155. Plesnila N., Zinkel S., Le D. A., Amin-Hanjani S., Wu Y., Qiu J., et al. (2001) BID mediates neuronal cell death after oxygen/ glucose deprivation and focal cerebral ischemia. *Proc. Natl. Acad. Sci. USA* **98,** 15,318–15,323.
156. Alnemri E. S., Livingston D. J., Nicholson D. W., Salvesen G., Thornberry N. A., Wong W. W., et al. (1996) Human ICE/CED-3 protease nomenclature [letter]. *Cell* **87,** 171.
157. Nicholson D. W. and Thornberry N. A. (1997) Caspases: killer proteases. *Trends Biochem. Sci.* **22,** 299–306.
158. Wolf C. M. and Eastman A. (1999) The temporal relationship between protein phosphatase, mitochondrial cytochrome c release, and caspase activation in apoptosis. *Exp. Cell Res.* **247,** 505–513.
159. Nicholson D. W. (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ.* **6,** 1028–1042.
160. Nunez G., Benedict M. A., Hu Y., and Inohara N. (1998) Caspases: the proteases of the apoptotic pathway. *Oncogene* **17,** 3237–3245.
161. Earnshaw W. C., Martins L. M., and Kaufmann S. H. (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu. Rev. Biochem.* **68,** 383–424.
162. Zhivotovsky B., Samali A., Gahm A., and Orrenius S. (1999) Caspases: their intracellular localization and translocation during apoptosis. *Cell Death Differ.* **6,** 644–651.
163. Cryns V. and Yuan J. (1998) Proteases to die for. *Genes Dev.* **12,** 1551–1570.
164. Thornberry N. A. and Lazebnik Y. (1998) Caspases: enemies within. *Science* **281,** 1312–1316.
165. Slee E. A., Adrain C., and Martin S. J. (1999) Serial killers: ordering caspase activation events in apoptosis. *Cell Death Differ.* **6,** 1067–1074.
166. Brockstedt E., Rickers A., Kostka S., Laubersheimer A., Dorken B., Wittmann-Liebold B., et al. (1998) Identification of apoptosis-associated proteins in a human Burkitt lymphoma cell line. Cleavage of heterogeneous nuclear ribonucleoprotein A1 by caspase 3. *J. Biol. Chem.* **273,** 28,057–28,064.

167. Schwartz L. M. and Milligan C. E. (1996) Cold thoughts of death: the role of ICE proteases in neuronal cell death. *Trends Neurosci.* **19,** 555–562.
168. Villa P., Kaufmann S. H., and Earnshaw W. C. (1997) Caspases and caspase inhibitors. *Trends Biochem Sci.* **22,** 388–393.
169. Rami A., Agarwal R., Botez G., and Winckler J. (2000) mu-Calpain activation, DNA fragmentation, and synergistic effects of caspase and calpain inhibitors in protecting hippocampal neurons from ischemic damage. *Brain Res.* **866,** 299–312.
170. Loddick S. A., MacKenzie A., and Rothwell N. J. (1996) An ICE inhibitor, z-VAD-DCB attenuates ischaemic brain damage in the rat. *Neuroreport* **7,** 1465–1468.
171. Hara H., Friedlander R. M., Gagliardini V., Ayata C., Fink K., Huang Z., et al. (1997) Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. *Proc. Natl. Acad. Sci. USA* **94,** 2007–2012.
172. Wiessner C., Sauer D., Alaimo D., and Allegrini P. R. (2000) Protective effect of a caspase inhibitor in models for cerebral ischemia in vitro and in vivo. *Cell Mol. Biol.* (Noisy-legrand). **46,** 53–62.
173. Friedlander R. M., Gagliardini V., Hara H., Fink K. B., Li W., MacDonald G., et al. (1997) Expression of a dominant negative mutant of interleukin-1 beta converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischemic brain injury. *J. Exp. Med.* **185,** 933–940.
174. Nakagawa T., Zhu H., Morishima N., Li E., Xu J., Yankner B. A., et al. (2000) Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* **403,** 98–103.
175. Susin S. A., Lorenzo H. K., Zamzami N., Marzo I., Brenner C., Larochette N., et al. (1999) Mitochondrial release of caspase-2 and -9 during the apoptotic process. *J. Exp. Med.* **189,** 381–394.
176. Zou H., Henzel W. J., Liu X., Lutschg A., and Wang X. (1997) Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3 [see comments]. *Cell* **90,** 405–413.
177. Fortin A., Cregan S. P., MacLaurin J. G., Kushwaha N., Hickman E. S., Thompson C. S., et al. (2001) APAF1 is a key transcriptional target for p53 in the regulation of neuronal cell death. *J. Cell Biol.* **155,** 207–216.
178. Hofmann K., Bucher P., and Tschopp J. (1997) The CARD domain: a new apoptotic signalling motif. *Trends Biochem. Sci.* **22,** 155–156.
179. Fujita E., Urase K., Egashira J., Miho Y., Isahara K., Uchiyama Y., et al. (2000) Detection of caspase-9 activation in the cell death of the Bcl-x-deficient mouse embryo nervous system by cleavage sites-directed antisera. *Brain Res. Dev. Brain Res.* **122,** 135–147.
180. Susin S. A., Zamzami N., Castedo M., Daugas E., Wang H. G., Geley S., et al. (1997) The central executioner of apoptosis: multiple connections between protease activation and mitochondria in Fas/APO-1/CD95- and ceramide-induced apoptosis. *J. Exp. Med.* **186,** 25–37.
181. Marzo I., Susin S. A., Petit P. X., Ravagnan L., Brenner C., Larochette N., et al. (1998) Caspases disrupt mitochondrial membrane barrier function. *FEBS Lett.* **427,** 198–202.
182. Green D. and Kroemer G. (1998) The central executioners of apoptosis: caspases or mitochondria? *Trends Cell Biol.* **8,** 267–271.
183. Krajewski S., Krajewska M., Ellerby L. M., Welsh K., Xie Z., Deveraux Q. L., et al. (1999) Release of caspase-9 from mitochondria during neuronal apoptosis and cerebral ischemia. *Proc. Natl. Acad. Sci. USA* **96,** 5752–5757.
184. Noshita N., Sugawara T., Fujimura M., Morita-Fujimura Y., and Chan P. H. (2001) Manganese superoxide dismutase affects cytochrome c release and caspase-9 activation after transient focal cerebral ischemia in mice. *J. Cereb. Blood Flow Metab.* **21,** 557–567.
185. Guégan C. and Sola B. (2000) Early and sequential recruitment of apoptotic effectors after focal permanent ischemia in mice. *Brain Res.* **856,** 93–100.
186. Benchoua A., Guegan C., Couriaud C., Hosseini H., Sampaio N., Morin D., et al. (2001) Specific caspase pathways are activated in the two stages of cerebral infarction. *J. Neurosci.* **21,** 7127–7134.
187. Genini D., Budihardjo I., Plunkett W., Wang X., Carrera C. J., Cottam H. B., et al. (2000) Nucleotide requirements for the in vitro activation of the apoptosis protein-activating factor-1-mediated caspase pathway. *J. Biol. Chem.* **275,** 29–34.
188. Dubois-Dauphin M., Pfister Y., Vallet P. G., and Savioz A. (2001) Prevention of apoptotic neu-

ronal death by controlling procaspases? A point of view. *Brain Res. Brain Res. Rev.* **36,** 196–203.
189. Schneider P. and Tschopp J. (2000) Apoptosis induced by death receptors. *Pharm. Acta Helv.* **74,** 281–286.
190. Ashkenazi A. and Dixit V. M. (1998) Death receptors: signaling and modulation. *Science* **281,** 1305–1308.
191. Mariani S. M., Matiba B., Armandola E. A., and Krammer P. H. (1997) Interleukin 1 beta-converting enzyme related proteases/caspases are involved in TRAIL-induced apoptosis of myeloma and leukemia cells. *J. Cell Biol.* **137,** 221–229.
192. Kischkel F. C., Hellbardt S., Behrmann I., Germer M., Pawlita M., Krammer P. H., et al. (1995) Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J.* **14,** 5579–5588.
193. Fernandes-Alnemri T., Armstrong R. C., Krebs J., Srinivasula S. M., Wang L., Bullrich F., et al. (1996) In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proc. Natl. Acad. Sci. USA* **93,** 7464–7469.
194. Medema J. P., Scaffidi C., Kischkel F. C., Shevchenko A., Mann M., Krammer P. H., et al. (1997) FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO J.* **16,** 2794–2804.
195. Muzio M., Stockwell B. R., Stennicke H. R., Salvesen G. S., and Dixit V. M. (1998) An induced proximity model for caspase-8 activation. *J. Biol. Chem.* **273,** 2926–2930.
196. Nagata S. (1997) Apoptosis by death factor. *Cell* **88,** 355–365.
197. Hsu H., Xiong J., and Goeddel D. V. (1995) The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell* **81,** 495–504.
198. Wang J., Chun H. J., Wong W., Spencer D. M., and Lenardo M. J. (2001) Caspase-10 is an initiator caspase in death receptor signaling. *Proc. Natl. Acad. Sci. USA* **98,** 13,884–13,888.
199. Kischkel F. C., Lawrence D. A., Tinel A., LeBlanc H., Virmani A., Schow P., et al. (2001) Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. *J. Biol. Chem.* **276,** 46,639–46,646.
200. Stennicke H. R., Jurgensmeier J. M., Shin H., Deveraux Q., Wolf B. B., Yang X., et al. (1998) Pro-caspase-3 is a major physiologic target of caspase-8. *J. Biol. Chem.* **273,** 27,084–27,090.
201. Harrison D. C., Roberts J., Campbell C. A., Crook B., Davis R., Deen K., et al. (2000) TR3 death receptor expression in the normal and ischaemic brain. *Neuroscience* **96,** 147–160.
202. Jin K., Graham S. H., Mao X., Nagayama T., Simon R. P., and Greenberg D. A. (2001) Fas (CD95) may mediate delayed cell death in hippocampal CA1 sector after global cerebral ischemia. *J. Cereb. Blood Flow Metab.* **21,** 1411–1421.
203. Buttini M., Appel K., Sauter A., Gebicke-Haerter P. J., and Boddeke H. W. (1996) Expression of tumor necrosis factor alpha after focal cerebral ischaemia in the rat. *Neuroscience* **71,** 1–16.
204. Martin-Villalba A., Herr I., Jeremias I., Hahne M., Brandt R., Vogel J., et al. (1999) CD95 ligand (Fas-L/APO-1L) and tumor necrosis factor-related apoptosis-inducing ligand mediate ischemia-induced apoptosis in neurons. *J. Neurosci.* **19,** 3809–3817.
205. Martin-Villalba A., Hahne M., Kleber S., Vogel J., Falk W., Schenkel J., et al. (2001) Therapeutic neutralization of CD95-ligand and TNF attenuates brain damage in stroke. *Cell Death Differ.* **8,** 679–686.
206. Herdegen T., Claret F. X., Kallunki T., Martin-Villalba A., Winter C., Hunter T., et al. (1998) Lasting N-terminal phosphorylation of c-Jun and activation of c-Jun N-terminal kinases after neuronal injury. *J. Neurosci.* **18,** 5124–5135.
207. Lukaszevicz A.-C., Sampaïo N., Guégan C., Benchoua A., Couriaud C., Chevalier E., et al. (2002) High sensitivity of protoplasmic cortical astroglia to focal ischemia. *J. Cereb. Blood Flow Metab.* **22,** 289–298.
208. Hemmer K., Fransen L., Vanderstichele H., Vanmechelen E., and Heuschling P. (2001) An in vitro model for the study of microglia-induced neurodegeneration: involvement of nitric oxide and tumor necrosis factor-alpha. *Neurochem. Int.* **38,** 557–565.
209. Velier J. J., Ellison J. A., Kikly K. K., Spera P. A., Barone F. C., and Feuerstein G. Z. (1999) Caspase-8 and caspase-3 are expressed by different populations of cortical neurons undergoing delayed cell death after focal stroke in the rat. *J. Neurosci.* **19,** 5932–5941.
210. Salvesen G. S. and Dixit V. M. (1997) Caspases: intracellular signaling by proteolysis. *Cell* **91,** 443–446.

211. Scaffidi C., Fulda S., Srinivasan A., Friesen C., Li F., Tomaselli K. J., et al. (1998) Two CD95 (APO-1/Fas) signaling pathways. *EMBO J.* **17,** 1675–1687.
212. Li H., Zhu H., Xu C. J., and Yuan J. (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* **94,** 491–501.
213. Shimizu S. and Tsujimoto Y. (2000) Proapoptotic BH3-only Bcl-2 family members induce cytochrome c release, but not mitochondrial membrane potential loss, and do not directly modulate voltage-dependent anion channel activity. *Proc. Natl. Acad. Sci. USA* **97,** 577–582.
214. Cerretti D. P., Kozlosky C. J., Mosley B., Nelson N., Van Ness K., Greenstreet T. A., et al. (1992) Molecular cloning of the interleukin-1 beta converting enzyme. *Science* **256,** 97–100.
215. Yuan J., Shaham S., Ledoux S., Ellis H. M., and Horvitz H. R. (1993) The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* **75,** 641–652.
216. Enari M., Talanian R. V., Wong W. W., and Nagata S. (1996) Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis. *Nature* **380,** 723–726.
217. Van de Craen M., Declercq W., Van den brande I., Fiers W., and Vandenabeele P. (1999) The proteolytic procaspase activation network: an in vitro analysis. *Cell Death Differ.* **6,** 1117–1124.
218. Bhat R. V., DiRocco R., Marcy V. R., Flood D. G., Zhu Y., Dobrzanski P., et al. (1996) Increased expression of IL-1beta converting enzyme in hippocampus after ischemia: selective localization in microglia. *J. Neurosci.* **16,** 4146–4154.
219. Rabuffetti M., Sciorati C., Tarozzo G., Clementi E., Manfredi A. A., and Beltramo M. (2000) Inhibition of caspase-1-like activity by Ac-Tyr-Val-Ala-Asp-chloromethyl ketone induces long-lasting neuroprotection in cerebral ischemia through apoptosis reduction and decrease of proinflammatory cytokines. *J. Neurosci.* **20,** 4398–4404.
220. Hayashi Y., Jikihara I., Yagi T., Fukumura M., Ohashi Y., Ohta Y., et al. (2001) Immunohistochemical investigation of caspase-1 and effect of caspase-1 inhibitor in delayed neuronal death after transient cerebral ischemia. *Brain Res.* **893,** 113–120.
221. Hara H., Fink K., Endres M., Friedlander R. M., Gagliardini V., Yuan J., et al. (1997) Attenuation of transient focal cerebral ischemic injury in transgenic mice expressing a mutant ICE inhibitory protein. *J. Cereb. Blood Flow Metab.* **17,** 370–375.
222. Schielke G. P., Yang G. Y., Shivers B. D., and Betz A. L. (1998) Reduced ischemic brain injury in interleukin-1 beta converting enzyme-deficient mice. *J. Cereb. Blood Flow Metab.* **18,** 180–185.
223. Yang G. Y., Schielke G. P., Gong C., Mao Y., Ge H. L., Liu X. H., et al. (1999) Expression of tumor necrosis factor-alpha and intercellular adhesion molecule-1 after focal cerebral ischemia in interleukin-1 beta converting enzyme deficient mice. *J. Cereb. Blood Flow Metab.* **19,** 1109–1117.
224. Schotte P., Van Criekinge W., Van de Craen M., Van Loo G., Desmedt M., Grooten J., et al. (1998) Cathepsin B-mediated activation of the proinflammatory caspase-11. *Biochem. Biophys. Res. Commun.* **251,** 379–387.
225. Nitatori T., Sato N., Waguri S., Karasawa Y., Araki H., Shibanai K., et al. (1995) Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *J. Neurosci.* **15,** 1001–1011.
226. Hill I. E., Preston E., Monette R., and MacManus J. P. (1997) A comparison of cathepsin B processing and distribution during neuronal death in rats following global ischemia or decapitation necrosis. *Brain Res.* **751,** 206–216.
227. Yamashima T., Kohda Y., Tsuchiya K., Ueno T., Yamashita J., Yoshioka T., et al. (1998) Inhibition of ischaemic hippocampal neuronal death in primates with cathepsin B inhibitor CA-074: a novel strategy for neuroprotection based on 'calpain-cathepsin hypothesis'. *Eur. J. Neurosci.* **10,** 1723–1733.
228. Seyfried D. M., Veyna R., Han Y., Li K., Tang N., Betts R. L., et al. (2001) A selective cysteine protease inhibitor is non-toxic and cerebroprotective in rats undergoing transient middle cerebral artery ischemia. *Brain Res.* **901,** 94–101.
229. Tsuchiya K., Kohda Y., Yoshida M., Zhao L., Ueno T., Yamashita J., et al. (1999) Postictal blockade of ischemic hippocampal neuronal death in primates using selective cathepsin inhibitors. *Exp. Neurol.* **155,** 187–194.
230. Rao R. V., Hermel E., Castro-Obregon S., del Rio G., Ellerby L. M., Ellerby H. M., et al. (2001) Coupling endoplasmic reticulum

stress to the cell death program. mechanism of caspase activation. *J. Biol. Chem.* **276,** 33,869–33,874.
231. Yoneda T., Imaizumi K., Oono K., Yui D., Gomi F., Katayama T., et al. (2001) Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J. Biol. Chem.* **276,** 13,935–13,940.
232. Nakagawa T. and Yuan J. (2000) Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. *J. Cell Biol.* **150,** 887–894.
233. Deshmukh M. and Johnson E. M., Jr. (1997) Programmed cell death in neurons: focus on the pathway of nerve growth factor deprivation-induced death of sympathetic neurons. *Mol. Pharmacol.* **51,** 897–906.
234. Xu D., Bureau Y., McIntyre D. C., Nicholson D. W., Liston P., Zhu Y., et al. (1999) Attenuation of ischemia-induced cellular and behavioral deficits by X chromosome-linked inhibitor of apoptosis protein overexpression in the rat hippocampus. *J. Neurosci.* **19,** 5026–5033.
235. Namura S., Zhu J., Fink K., Endres M., Srinivasan A., Tomaselli K. J., et al. (1998) Activation and cleavage of caspase-3 in apoptosis induced by experimental cerebral ischemia. *J. Neurosci.* **18,** 3659–3668.
236. Chen J., Nagayama T., Jin K., Stetler R. A., Zhu R. L., Graham S. H., et al. (1998) Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. *J. Neurosci.* **18,** 4914–4928.
237. Endres M., Namura S., Shimizu-Sasamata M., Waeber C., Zhang L., Gomez-Isla T., et al. (1998) Attenuation of delayed neuronal death after mild focal ischemia in mice by inhibition of the caspase family. *J. Cereb. Blood Flow Metab.* **18,** 238–247.
238. Tamatani M., Ogawa S., and Tohyama M. (1998) Roles of Bcl-2 and caspases in hypoxia-induced neuronal cell death: a possible neuroprotective mechanism of peptide growth factors. *Brain Res. Mol. Brain Res.* **58,** 27–39.
239. Fink K., Linmin Z., Namura S., Shimizu-Sasamata M., Endres M., Ma J., et al. (1998) Prolonged therapeutic window for ischemic brain damage caused by delayed caspase activation. *J. Cereb. Blood Flow Metab.* **18,** 1071–1076.
240. Schulz J. B., Weller M., Matthews R. T., Heneka M. T., Groscurth P., Martinou J. C., et al. (1998) Extended therapeutic window for caspase inhibition and synergy with MK-801 in the treatment of cerebral histotoxic hypoxia. *Cell Death Differ.* **5,** 847–857.
241. Gillardon F., Bottiger B., Schmitz B., Zimmermann M., and Hossmann K. A. (1997) Activation of CPP-32 protease in hippocampal neurons following ischemia and epilepsy. *Brain Res. Mol. Brain Res.* **50,** 16–22.
242. Ni B., Wu X., Su Y., Stephenson D., Smalstig E. B., Clemens J., et al. (1998) Transient global forebrain ischemia induces a prolonged expression of the caspase-3 mRNA in rat hippocampal CA1 pyramidal neurons. *J. Cereb. Blood Flow Metab.* **18,** 248–256.
243. Gillardon F., Kiprianova I., Sandkuhler J., Hossmann K. A., and Spranger M. (1999) Inhibition of caspases prevents cell death of hippocampal CA1 neurons, but not impairment of hippocampal long-term potentiation following global ischemia. *Neuroscience* **93,** 1219–1222.
244. Ouyang Y. B., Tan Y., Comb M., Liu C. L., Martone M. E., Siesjo B. K., et al. (1999) Survival- and death-promoting events after transient cerebral ischemia: phosphorylation of Akt, release of cytochrome C and Activation of caspase-like proteases. *J. Cereb. Blood Flow Metab.* **19,** 1126–1135.
245. Himi T., Ishizaki Y., and Murata S. (1998) A caspase inhibitor blocks ischemia-induced delayed neuronal death in the gerbil. *Eur. J. Neurosci.* **10,** 777–781.
246. Li H., Colbourne F., Sun P., Zhao Z., Buchan A. M., and Iadecola C. (2000) Caspase inhibitors reduce neuronal injury after focal but not global cerebral ischemia in rats. *Stroke* **31,** 176–182.
247. Harrison D. C., Davis R. P., Bond B. C., Campbell C. A., James M. F., Parsons A. A., et al. (2001) Caspase mRNA expression in a rat model of focal cerebral ischemia. *Brain Res. Mol. Brain Res.* **89,** 133–146.
248. Banasiaka K. J., Xiab Y., and Haddadbc G. G. (2000) Mechanisms underlying hypoxia-induced neuronal apoptosis. *Prog. Neurobiol.* **62,** 215–249.
249. Lindsay R. M., Wiegand S. J., Altar C. A., and DiStefano P. S. (1994) Neurotrophic factors: from molecule to man. *Trends Neurosci.* **17,** 182–190.

250. Lindvall O., Kokaia Z., Bengzon J., Elmer E., and Kokaia M. (1994) Neurotrophins and brain insults. *Trends Neurosci.* **17,** 490–496.
251. Abe K. (2000) Therapeutic potential of neurotrophic factors and neural stem cells against ischemic brain injury. *J. Cereb Blood Flow Metab.* **20,** 1393–1408.
252. Majdan M., Walsh G. S., Aloyz R., and Miller F. D. (2001) TrkA mediates developmental sympathetic neuron survival in vivo by silencing an ongoing p75NTR-mediated death signal. *J. Cell Biol.* **155,** 1275–1285.
253. Franke T. F., Kaplan D. R., and Cantley L. C. (1997) PI3K: downstream AKTion blocks apoptosis. *Cell* **88,** 435–437.
254. Datta S. R., Dudek H., Tao X., Masters S., Fu H., Gotoh Y., et al. (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* **91,** 231–241.
255. del Peso L., Gonzalez-Garcia M., Page C., Herrera R., and Nunez G. (1997) Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science* **278,** 687–689.
256. Zha H. and Reed J. C. (1997) Heterodimerization-independent functions of cell death regulatory proteins Bax and Bcl-2 in yeast and mammalian cells. *J. Biol Chem.* **272,** 31,482–31,489.
257. Rokudai S., Fujita N., Hashimoto Y., and Tsuruo T. (2000) Cleavage and inactivation of antiapoptotic Akt/PKB by caspases during apoptosis. *J. Cell Physiol.* **182,** 290–296.
258. Brunet A., Bonni A., Zigmond M. J., Lin M. Z., Juo P., Hu L. S., et al. (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96,** 857–868.
259. Bachelder R. E., Wendt M. A., Fujita N., Tsuruo T., and Mercurio A. M. (2001) The cleavage of Akt/protein kinase B by death receptor signaling is an important event in detachment-induced apoptosis. *J. Biol. Chem.* **276,** 34,702–34,707.
260. Dechant G., Tsoulfas P., Parada L. F., and Barde Y. A. (1997) The neurotrophin receptor p75 binds neurotrophin-3 on sympathetic neurons with high affinity and specificity. *J. Neurosci.* **17,** 5281–5287.
261. Barde Y. A. (1989) Trophic factors and neuronal survival. *Neuron* **2,** 1525–1534.
262. Snider W. D. (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77,** 627–638.
263. Bui N. T., Konig H. G., Culmsee C., Bauerbach E., Poppe M., Krieglstein J., et al. (2002) p75 neurotrophin receptor is required for constitutive and NGF-induced survival signalling in PC12 cells and rat hippocampal neurones. *J. Neurochem.* **81,** 594–605.
264. Griffiths E. J. and Halestrap A. P. (1991) Further evidence that cyclosporin A protects mitochondria from calcium overload by inhibiting a matrix peptidyl-prolyl cis-trans isomerase. Implications for the immunosuppressive and toxic effects of cyclosporin. *Biochem. J.* **274,** 611–614.
265. Lemaire C., Andreau K., Souvannavong V., and Adam A. (1998) Inhibition of caspase activity induces a switch from apoptosis to necrosis. *FEBS Lett.* **425,** 266–270.
266. Nicotera P., Leist M., and Ferrando-May E. (1999) Apoptosis and necrosis: different execution of the same death. *Biochem. Soc. Symp.* **66,** 69–73.
267. Hartmann A., Troadec J. D., Hunot S., Kikly K., Faucheux B. A., Mouatt-Prigent A., et al. (2001) Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. *J. Neurosci.* **21,** 2247–2255.
268. Deveraux Q. L. and Reed J. C. (1999) IAP family proteins—suppressors of apoptosis. *Genes Dev.* **13,** 239–252.
269. Deveraux Q. L., Takahashi R., Salvesen G. S., and Reed J. C. (1997) X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* **388,** 300–304.
270. Roy N., Deveraux Q. L., Takahashi R., Salvesen G. S., and Reed J. C. (1997) The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J.* **16,** 6914–6925.
271. Xu D. G., Crocker S. J., Doucet J. P., St-Jean M., Tamai K., Hakim A. M., et al. (1997) Elevation of neuronal expression of NAIP reduces ischemic damage in the rat hippocampus. *Nat. Med.* **3,** 997–1004.
272. Xue D. and Horvitz H. R. (1995) Inhibition of the Caenorhabditis elegans cell-death protease CED-3 by a CED-3 cleavage site in baculovirus p35 protein. *Nature* **377,** 248–251.
273. Snipas S. J., Stennicke H. R., Riedl S., Potempa J., Travis J., Barrett A. J., et al. (2001) Inhibition of distant caspase homologues by natural caspase inhibitors. *Biochem. J.* **357,** 575–580.
274. Shibata M., Hisahara S., Hara H., Yamawaki T., Fukuuchi Y., Yuan J., et al. (2000) Caspases

determine the vulnerability of oligodendrocytes in the ischemic brain [In Process Citation]. *J. Clin. Investig.* **106,** 643–653.
275. Tschopp J., Irmler M., and Thome M. (1998) Inhibition of fas death signals by FLIPs. *Curr. Opin. Immunol.* **10,** 552–558.
276. Inohara N., Koseki T., Hu Y., Chen S., and Nunez G. (1997) CLARP, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis. *Proc. Natl. Acad. Sci. USA* **94,** 10,717–10,722.
277. Cheema Z. F., Wade S. B., Sata M., Walsh K., Sohrabji F., and Miranda R. C. (1999) Fas/Apo [apoptosis]-1 and associated proteins in the differentiating cerebral cortex: induction of caspase-dependent cell death and activation of NF-kappaB. *J. Neurosci.* **19,** 1754–1770.
278. Raoul C., Henderson C. E., and Pettmann B. (1999) Programmed cell death of embryonic motoneurons triggered through the Fas death receptor. *J. Cell Biol.* **147,** 1049–1062.
279. Rasper D. M., Vaillancourt J. P., Hadano S., Houtzager V. M., Seiden I., Keen S. L., et al. (1998) Cell death attenuation by 'Usurpin', a mammalian DED-caspase homologue that precludes caspase-8 recruitment and activation by the CD-95 (Fas, APO-1) receptor complex. *Cell Death Differ.* **5,** 271–288.
280. Deckwerth T. L., Elliott J. L., Knudson C. M., Johnson E. M., Jr., Snider W. D., and Korsmeyer S. J. (1996) BAX is required for neuronal death after trophic factor deprivation and during development. *Neuron* **17,** 401–411.
281. Shigeno T., Mima T., Takakura K., Graham D. I., Kato G., Hashimoto Y., et al. (1991) Amelioration of delayed neuronal death in the hippocampus by nerve growth factor. *J. Neurosci.* **11,** 2914–2919.
282. Andsberg G., Kokaia Z., Bjorklund A., Lindvall O., and Martinez-Serrano A. (1998) Amelioration of ischaemia-induced neuronal death in the rat striatum by NGF-secreting neural stem cells. *Eur. J. Neurosci.* **10,** 2026–2036.
283. Pechan P. A., Yoshida T., Panahian N., Moskowitz M. A., and Breakefield X. O. (1995) Genetically modified fibroblasts producing NGF protect hippocampal neurons after ischemia in the rat. *Neuroreport* **6,** 669–672.
284. Hefti F. (1994) Neurotrophic factor therapy for nervous system degenerative diseases. *J. Neurobiol.* **25,** 1418–1435.
285. Dijkhuizen P. A. and Verhaagen J. (1999) The use of neurotrophic factors to treat spinal cord injury: advantages and disadvantages of different delivery methods. *Neurosci. Res. Comm.* **24,** 1–9.
286. Eriksdotter-Jonhagen M., Nordberg A., Amberla K., Backman L., Ebendal T., Meyerson B., et al. (1998) Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* **9,** 246–257.
287. Trafermin (1999). CAB 2001, KCB 1, Fiblast. Drugs R.D. 1, 40–41.
288. Semkova I., Wolz P., Schilling M., and Krieglstein J. (1996) Selegiline enhances NGF synthesis and protects central nervous system neurons from excitotoxic and ischemic damage. *Eur. J. Pharmacol.* **315,** 19–30.
289. Seniuk N. A., Henderson J. T., Tatton W. G., and Roder J. C. (1994) Increased CNTF gene expression in process-bearing astrocytes following injury is augmented by R(–)-deprenyl. *J. Neurosci. Res.* **37,** 278–286.
290. Culmsee C., Stumm R. K., Schafer M. K., Weihe E., and Krieglstein J. (1999) Clenbuterol induces growth factor mRNA, activates astrocytes, and protects rat brain tissue against ischemic damage. *Eur. J. Pharmacol.* **379,** 33–45.
291. Waschek J. A., Dicicco-Bloom E. M., Lelievre V., Zhou X., and Hu Z. (2000) PACAP action in nervous system development, regeneration, and neuroblastoma cell proliferation. *Ann. NY Acad. Sci.* **921,** 129–136.
292. Lu N., Zhou R., and DiCicco-Bloom E. (1998) Opposing mitogenic regulation by PACAP in sympathetic and cerebral cortical precursors correlates with differential expression of PACAP receptor (PAC1-R) isoforms. *J. Neurosci. Res.* **53,** 651–662.
293. Vaudry D., Gonzalez B. J., Basille M., Yon L., Fournier A., and Vaudry H. (2000) Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol. Rev.* **52,** 269–324.
294. Banks W. A., Uchida D., Arimura A., Somogyvari-Vigh A., and Shioda S. (1996) Transport of pituitary adenylate cyclase-activating polypeptide across the blood-brain barrier and the prevention of ischemia-induced death of hippocampal neurons. *Ann NY Acad. Sci.* **805,** 270–277; discussion 277–279.
295. Arimura A., Somogyvari-Vigh A., Weill C., Fiore R. C., Tatsuno I., Bay V., et al. (1994) PACAP functions as a neurotrophic factor. *Ann. NY Acad. Sci.* **739,** 228–243.

296. Uchida D., Arimura A., Somogyvari-Vigh A., Shioda S., and Banks W. A. (1996) Prevention of ischemia-induced death of hippocampal neurons by pituitary adenylate cyclase activating polypeptide. *Brain Res.* **736,** 280–286.
297. Jones T. A. and Schallert T. (1994) Use-dependent growth of pyramidal neurons after neocortical damage. *J. Neurosci.* **14,** 2140–2152.
298. Defer G. L., Geny C., Ricolfi F., Fenelon G., Monfort J. C., Remy P., et al. (1996) Long-term outcome of unilaterally transplanted parkinsonian patients. I. Clinical approach. *Brain* **119,** 41–50.
299. Palfi S., Conde F., Riche D., Brouillet E., Dautry C., Mittoux V., et al. (1998) Fetal striatal allografts reverse cognitive deficits in a primate model of Huntington disease. *Nat. Med.* **4,** 963–966.
300. Dunnett S. B. and Bjorklund A. (1999) Prospects for new restorative and neuroprotective treatments in Parkinson's disease. *Nature* **399,** A32–A39.
301. Hagell P., Schrag A., Piccini P., Jahanshahi M., Brown R., Rehncrona S., et al. (1999) Sequential bilateral transplantation in Parkinson's disease: effects of the second graft. *Brain* **122,** 1121–1132.
302. Bjorklund A. and Lindvall O. (2000) Cell replacement therapies for central nervous system disorders. *Nat. Neurosci.* **3,** 537–544.
303. Svendsen C. N. and Smith A. G. (1999) New prospects for human stem-cell therapy in the nervous system. *Trends Neurosci.* **22,** 357–364.
304. Bjorklund A. and Svendsen C. (1999) Stem cells. Breaking the brain-blood barrier. *Nature* **397,** 569–570.
305. Eriksson P. S., Perfilieva E., Bjork-Eriksson T., Alborn A. M., Nordborg C., Peterson D. A., et al. (1998) Neurogenesis in the adult human hippocampus. *Nat. Med.* **4,** 1313–1317.
306. Altman J. (1962) Are new neurons formed in the brain of adult mammalians? *Science* **135,** 1127–1128.
307. Nottebohm F. (2002) Why are some neurons replaced in adult brain? *J. Neurosci.* **22,** 624–628.
308. Altman J. and Das G. (1965) Autoradiographic and histological evidence of postnatal neurogenesis in rats. *J. Comp. Neurol.* **124,** 319–335.
309. Kaplan M. S. and Hinds J. W. (1977) Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science* **197,** 1092–1094.
310. Cameron H. A., Woolley C. S., McEwen B. S., and Gould E. (1993) Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience* **56,** 337–344.
311. Gage F. H., Ray J., and Fisher L. J. (1995) Isolation, characterization, and use of stem cells from the CNS. *Annu. Rev. Neurosci.* **18,** 159–192.
312. Palmer T. D., Takahashi J., and Gage F. H. (1997) The adult rat hippocampus contains primordial neural stem cells. *Mol. Cell. Neurosci.* **8,** 389–404.
313. Richards L. J., Kilpatrick T. J., and Bartlett P. F. (1992) De novo generation of neuronal cells from the adult mouse brain. *Proc. Natl. Acad. Sci. USA* **89,** 8591–8595.
314. Reynolds B. A. and Weiss S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255,** 1707–1710.
315. Lois C. and Alvarez-Buylla A. (1994) Long-distance neuronal migration in the adult mammalian brain. *Science* **264,** 1145–1148.
316. Seki T. and Arai Y. (1993) Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. *J. Neurosci.* **13,** 2351–2358.
317. Altman J. (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J. Comp. Neurol.* **137,** 433–457.
318. Doetsch F., Garcia-Verdugo J., and Alvarez-Buylla A. (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J. Neurosci.* **17,** 5046–5061.
319. McKay R. (1997) Stem cells in the central nervous system. *Science* **276,** 66–71.
320. Seri B., Garcia-Verdugo J. M., McEwen B. S., and Alvarez-Buylla A. (2001) Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J. Neurosci.* **21,** 7153–7160.
321. Palmer T. D., Ray J., and Gage F. H. (1995) FGF-2-responsive neuronal progenitors reside in proliferative and quiescent regions of the adult rodent brain. *Mol. Cell. Neurosci.* **6,** 474–486.
322. Kondo T. and Raff M. (2000) Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* **289,** 1754–1757.

323. Shihabuddin L. S., Horner P. J., Ray J., and Gage F. H. (2000) Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. *J. Neurosci.* **20,** 8727–8735.
324. Benraiss A., Chmielnicki E., Lerner K., Roh D., and Goldman S. A. (2001) Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. *J. Neurosci.* **21,** 6718–6731.
325. Gould E. and Gross C. G. (2002) Neurogenesis in adult mammals: some progress and problems. *J. Neurosci.* **22,** 619–623.
326. Nilsson M., Perfilieva E., Johansson U., Orwar O., and Eriksson P. S. (1999) Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J. Neurobiol.* **39,** 569–578.
327. Rochefort C., Gheusi G., Vincent J. D., and Lledo P. M. (2002) Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. *J. Neurosci.* **22,** 2679–2689.
328. Gould E., McEwen B. S., Tanapat P., Galea L. A., and Fuchs E. (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J. Neurosci.* **17,** 2492–2498.
329. Tanapat P., Hastings N. B., Rydel T. A., Galea L. A., and Gould E. (2001) Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J. Comp. Neurol.* **437,** 496–504.
330. Galea L. A. and McEwen B. S. (1999) Sex and seasonal differences in the rate of cell proliferation in the dentate gyrus of adult wild meadow voles. *Neuroscience* **89,** 955–964.
331. van Praag H., Kempermann G., and Gage F. H. (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* **2,** 266–270.
332. Kempermann G., Kuhn H. G., and Gage F. H. (1997) Genetic influence on neurogenesis in the dentate gyrus of adult mice. *Proc. Natl. Acad. Sci. USA* **94,** 10,409–10,414.
333. Snyder E. Y., Yoon C., Flax J. D., and Macklis J. D. (1997) Multipotent neural precursors can differentiate toward replacement of neurons undergoing targeted apoptotic degeneration in adult mouse neocortex. *Proc. Natl. Acad. Sci. USA* **94,** 11,663–11,668.
334. Parent J. M., Janumpalli S., McNamara J. O., and Lowenstein D. H. (1998) Increased dentate granule cell neurogenesis following amygdala kindling in the adult rat. *Neurosci. Lett.* **247,** 9–12.
335. Parent J. M., Valentin V. V., and Lowenstein D. H. (2002) Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone-olfactory bulb pathway. *J. Neurosci.* **22,** 3174–3188.
336. Madsen T. M., Treschow A., Bengzon J., Bolwig T. G., Lindvall O., and Tingstrom A. (2000) Increased neurogenesis in a model of electroconvulsive therapy. *Biol. Psychiatry* **47,** 1043–1049.
337. Jin K., Minami M., Lan J. Q., Mao X. O., Batteur S., Simon R. P., et al. (2001) Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. *Proc. Natl. Acad. Sci. USA* **98,** 4710–4715.
338. Takagi Y., Nozaki K., Takahashi J., Yodoi J., Ishikawa M., and Hashimoto N. (1999) Proliferation of neuronal precursor cells in the dentate gyrus is accelerated after transient forebrain ischemia in mice. *Brain Res.* **831,** 283–287.
339. Jiang W., Gu W., Brannstrom T., Rosqvist R., and Wester P. (2001) Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. *Stroke* **32,** 1201–1207.
340. Liu J., Solway K., Messing R. O., and Sharp F. R. (1998) Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. *J. Neurosci.* **18,** 7768–7778.
341. Sharp F. R., Liu J., and Bernabeu R. (2002) Neurogenesis following brain ischemia. *Brain Res. Dev. Brain Res.* **134,** 23–30.
342. Park K. I. (2000) Transplantation of neural stem cells: cellular & gene therapy for hypoxic-ischemic brain injury. *Yonsei Med. J.* **41,** 825–835.
343. Lim D. A., Tramontin A. D., Trevejo J. M., Herrera D. G., Garcia-Verdugo J. M., and Alvarez-Buylla A. (2000) Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* **28,** 713–726.
344. Alvarez-Buylla A and Garcia-Verdugo J. M. (2002) Neurogenesis in adult subventricular zone. *J. Neurosci.* **22,** 629–634.
345. Dunnett S. and Björklund A. (1994) *Functional Neural Transplantation,* vol. 2, Raven, New York, pp. 103–138.
346. Bachoud-Levi A. C., Remy P., Nguyen J. P., Brugieres P., Lefaucheur J. P., Bourdet C., et al. (2000) Motor and cognitive improvements in

patients with Huntington's disease after neural transplantation. *Lancet* **356,** 1975–1979.
347. Aoki H., Onodera H., Yae T., Jian Z., and Kogure K. (1993) Neural grafting to ischemic CA1 lesions in the rat hippocampus: an autoradiographic study. *Neuroscience* **56,** 345–354.
348. Hodges H., Nelson A., Virley D., Kershaw T. R., and Sinden J. D. (1997) Cognitive deficits induced by global cerebral ischaemia: prospects for transplant therapy. *Pharmacol. Biochem. Behav.* **56,** 763–780.
349. Szele F. G. and Chesselet M. F. (1996) Cortical lesions induce an increase in cell number and PSA-NCAM expression in the subventricular zone of adult rats. *J. Comp. Neurol.* **368,** 439–454.
350. Aubert I., Ridet J. L., Schachner M., Rougon G., and Gage F. H. (1998) Expression of L1 and PSA during sprouting and regeneration in the adult hippocampal formation. *J. Comp. Neurol.* **399,** 1–19.
351. Hayashi T., Seki T., Sato K., Iwai M., Zhang W. R., Manabe Y., et al. (2001) Expression of polysialylated neural cell adhesion molecule in rat brain after transient middle cerebral artery occlusion. *Brain Res.* **907,** 130–133.
352. Sinden J. D., Rashid-Doubell F., Kershaw T. R., Nelson A., Chadwick A., Jat P. S., et al. (1997) Recovery of spatial learning by grafts of a conditionally immortalized hippocampal neuroepithelial cell line into the ischaemia-lesioned hippocampus. *Neuroscience* **81,** 599–608.
353. Kondziolka D., Wechsler L., Goldstein S., Meltzer C., Thulborn K. R., Gebel J., et al. (2000) Transplantation of cultured human neuronal cells for patients with stroke. *Neurology* **55,** 565–569.
354. Morshead C. M., Reynolds B. A., Craig C. G., McBurney M. W., Staines W. A., Morassutti D., et al. (1994) Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* **13,** 1071–1082.
355. Laywell E. D., Rakic P., Kukekov V. G., Holland E. C., and Steindler D. A. (2000) Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc. Natl. Acad. Sci. USA* **97,** 13,883–13,888.
356. Duncan I. D. and Hoffman R. L. (1997) Schwann cell invasion of the central nervous system of the myelin mutants. *J. Anat.* **190,** 35–49.
357. Spedding M. (2002) Reasons why stroke trials underestimate the neuroprotective effects of drugs. *Stroke* **33,** 324–325.
358. Grotta J., Clark W., Coull B., Pettigrew L. C., Mackay B., Goldstein L. B., et al. (1995) Safety and tolerability of the glutamate antagonist CGS 19755 (Selfotel) in patients with acute ischemic stroke. Results of a phase IIa randomized trial. *Stroke* **26,** 602–605.
359. Davis S. M., Albers G. W., Diener H. C., Lees K. R., and Norris J. (1997) Termination of Acute Stroke Studies Involving Selfotel Treatment. ASSIST Steering Committed. *Lancet* **349,** 32.
360. Merchant R. E., Bullock M. R., Carmack C. A., Shah A. K., Wilner K. D., Ko G., et al. (1999) A double-blind, placebo-controlled study of the safety, tolerability and pharmacokinetics of CP-101,606 in patients with a mild or moderate traumatic brain injury. *Ann. NY Acad. Sci.* **890,** 42–50.
361. Muir K. W. and Lees K. R. (1995) A randomized, double-blind, placebo-controlled pilot trial of intravenous magnesium sulfate in acute stroke. *Stroke* **26,** 1183–1188.
362. Dyker A. G. and Lees K. R. (1999) Remacemide hydrochloride: a double-blind, placebo-controlled, safety and tolerability study in patients with acute ischemic stroke. *Stroke* **30,** 1796–1801.
363. Albers G. W., Clark W. M., Atkinson R. P., Madden K., Data J. L., and Whitehouse M. J. (1999) Dose escalation study of the NMDA glycine-site antagonist licostinel in acute ischemic stroke. *Stroke* **30,** 508–513.
364. Lees K. R., Asplund K., Carolei A., Davis S. M., Diener H. C., Kaste M., et al. (2000) Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke: a randomised controlled trial. GAIN International Investigators. *Lancet* **355,** 1949–1954.
365. Limburg M. and Hijdra A. (1990) Flunarizine in acute ischemic stroke: a pilot study. *Eur. Neurol.* **30,** 121–122.
366. Kassell N. F., Haley E. C., Jr., Apperson-Hansen C., and Alves W. M. (1996) Randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in Europe, Australia, and New Zealand. *J. Neurosurg.* **84,** 221–228.

367. (1996) A randomized trial of tirilazad mesylate in patients with acute stroke (RANTTAS). The RANTTAS Investigators. *Stroke* **27,** 1453–1458.
368. Haley E. C., Jr. (1998) High-dose tirilazad for acute stroke (RANTTAS II). RANTTAS II Investigators. *Stroke* **29,** 1256–1257.
369. Wahlgren N. G., Ranasinha K. W., Rosolacci T., Franke C. L., van Erven P. M., Ashwood T., et al. (1999) Clomethiazole acute stroke study (CLASS): results of a randomized, controlled trial of clomethiazole versus placebo in 1360 acute stroke patients. *Stroke* **30,** 21–28.