Cell Tissue Res (1999) 298:383–395

REVIEW

Pawel Kermer · Nikolaj Klöcker · Mathias Bähr

Neuronal death after brain injury

Models, mechanisms, and therapeutic strategies in vivo

Received: 13 January 1999 / Accepted: 8 April 1999

Abstract Neuronal damage in the central nervous system leads to primary cell death, induced directly by the trauma, and delayed secondary death of neurons, the latter depending on environmental changes, lack of metabolic and trophic supply, and altered gene transcription. While primary death of neurons occurring within a short time after trauma is not a realistic target for therapy, secondary cell death might be prevented by new neuroprotective strategies. Although there are increasing data concerning cell rescue after ischemic and traumatic brain injury through the last decade, the mechanisms that underlie secondary death of neurons following lesion are still incompletely understood and are now the subject of a more detailed investigation. In this review, we want to give an overview on what is known about the molecular mechanisms of delayed ischemic and traumatic neuronal death in vivo and about promising neuroprotective treatment strategies that might be of future clinical relevance or have already entered clinical trials.

Key words Neuronal death · Ischemia · Traumatic brain injury · Axotomy · Mechanisms · Neuroprotection

Animal models of ischemic and traumatic brain injury

For investigating the cellular and subcellular events occurring in ischemic or traumatic brain injury, a large number of animal models have been developed. However, their major aim, which is to approximate the clinical conditions and neuropathological consequences found in human ischemic and traumatic brain injury as closely as possible, has been achieved with variable degrees of success. Since discussing all the advantages and disadvantages of the various models is beyond the scope of this review (for reviews, see Biros 1991; Zwiener et al. 1991), we will focus on basic differences between the available models for ischemia and trauma.

Among the models for ischemic brain injury, one can distinguish between two major paradigms: global ischemia and focal ischemia. Global ischemia is supposed to reflect the cardiac arrest situation, eventually leading to hypo- or nonperfusion of the complete brain, whereas focal ischemia leads to circumscript brain infarction. While in global ischemia models the lack of perfusion is often permanent, focal ischemia models hold the advantage of being either permanent or reversible, allowing the investigation of secondary injury due to reperfusion.

Among the trauma models, one can also distinguish between two groups. One is represented by deformation injuries, either as head impact models, in which the skull of the animal is primarily left intact and thus absorbes some of the forces, or as brain deformation models, in which the impact is delivered directly onto the exposed dura producing rather diffuse injuries. The second group of models consists of head acceleration/deceleration models, having the general limitation of being confined to primates, which have ananatomical head-to-neck relations and brain-to-skull mass ratios comparable with the human anatomy.

Mechanisms underlying neuronal death in vivo

Morphologically, two different forms of cell death can be distinguished: necrotic and apoptotic cell death. While necrosis is accompanied by a breakdown of transmembranous ionic pumps caused by a lack of ATP, apoptosis – also known as programmed cell death – requires active protein synthesis. Despite its decisive role during neuronal development, apoptotic cell death remained unnoticed for a long time in the context of trauma or ischemia. Based on many experimental data from the last decade, it became more and more accepted that necrotic and apoptotic cell death cannot be separated as two totally different entities. Both types of cell death are believed

P. Kermer · N. Klöcker · M. Bähr (✉)
Neurologische Universitätsklinik Tübingen,
Hoppe-Seyler-Str. 3, D-72076 Tübingen, Germany
e-mail: mathias.baehr@uni-tuebingen.de
Fax: +49-7071-295957

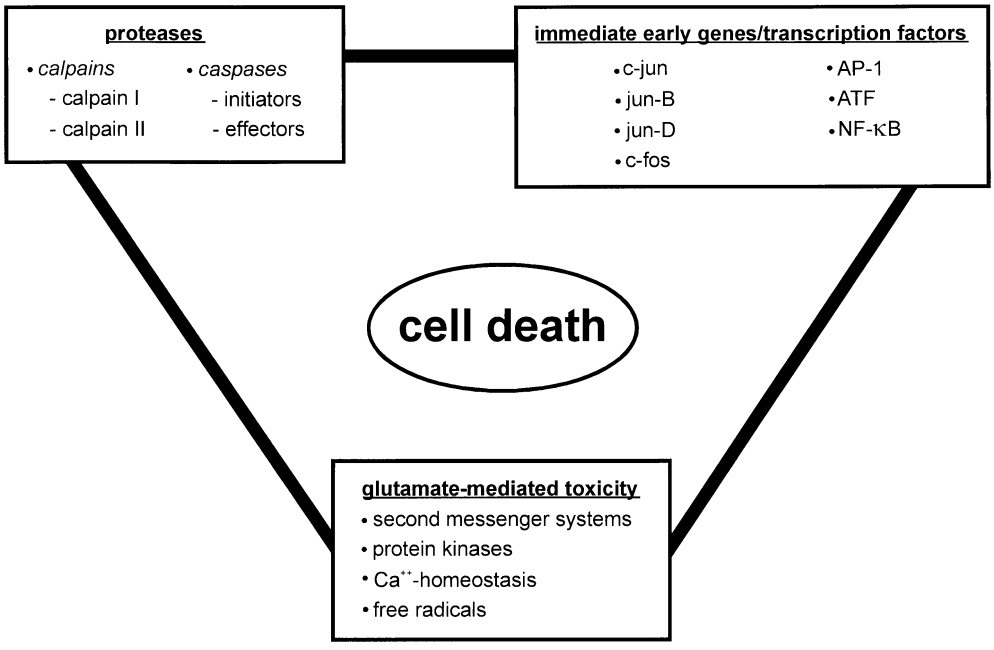

Fig. 1 Schematic illustration of the different pathways contributing to cell death after ischemia or traumatic brain injury. According to the text, the participants in neuronal death can be divided into three major groups: (1) proteases, (2) immediate early genes and transcription factors, (3) glutamate-mediated toxicity. Cross talk and intersections between these different groups have been described or are currently under investigation (*ATF* activating transcription factor, *AP-1* activator protein-1, *NF-κB* nuclear factor-κB)

to proceed in parallel in the lesioned tissue. There is even increasing evidence that, depending on the cellular environment and energy supply, a switch between the two types of cell death within a single cell is possible. Therefore, in the following, we will discuss the most important extra- and intracellular mechanisms contributing directly to secondary neuronal death after ischemia or neuronal trauma without a strict differentiation between necrosis and apoptosis. Related changes in the cellular environment enhancing the detrimental effects of neuronal trauma such as immune response and inflammation are reviewed elsewhere in greater detail (del Zoppo et al. 1997) and will not be a main topic of the following paragraph. A summary of the various pathways contributing to neuronal death as discussed below is given in Fig. 1.

Glutamate-mediated neurotoxicity and calcium homeostasis

In 1957, Lucas and Newhouse showed for the first time that sustained exposure to glutamate can destroy retinal neurons (Lucas and Newhouse 1957). Olney and coworkers discovered later that this neurotoxicity, which they termed "excitotoxicity," was not confined to glutamate but could also be caused by other excitatory amino acids (EAAs) acting on CNS neurons (Olney and Sharpe 1969; Olney et al. 1971). On the basis of these results, they hypothesized that glutamate and related compounds might be a cause of neuronal cell death in certain neurological diseases. Meanwhile, it has become evident that excitotoxicity is involved both in acute and delayed neuronal death in vascular, traumatic, and degenerative disorders of the CNS (Choi 1988).

In vitro experiments showed that glutamate neurotoxicity involves two components (Choi 1988). The first component is characterized by an influx of sodium, membrane depolarization, and subsequent influx of water leading to neuronal cell swelling. The second component, marked by delayed neuronal degeneration, depends on extracellular calcium and is produced by the toxic effects of excessive calcium influx.

Injured CNS neurons release glutamate (or other EAAs) in great amounts owing to depolarization after cell damage or energy failure, which are common events in cerebral ischemia and trauma. Furthermore, glutamate can leak through the damaged cell membranes. At the same time, energy failure stops reuptake mechanims for glutamate. All these events lead to excessive glutamate concentrations in the extracellular space, which can then damage other, still healthy cells in the proximity of the lesion. A vicious circle can evolve, since extracellular glutamate triggers its own release by depolarization of responsive cells.

The cellular effects of glutamate are mediated by two different classes of receptors: ionotropic and metabotropic glutamate receptors (iGluRs and mGluRs, respectively). Whereas iGluRs are directly coupled to ion channels, activation of mGluRs involves activation of guanosine triphosphate (GTP)-binding proteins (G-proteins) leading to subsequent activation or inhibition of second messenger cascades. The pharmacological classification of the iGluRs is based on three selective agonists: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid (AMPA), and kainate. Activation of NMDA receptors is particularly important for the above-described sodium and calcium influxes in excitotoxic events. Nevertheless, depending on the subunit arrangement of the non-NMDA receptors and thus on the neuronal cell type, intracellular rise in calcium can also be caused by activation of AMPA and kainate receptors. Among the mGluRs, at least eight types of receptor can

be distinguished. Their pharmacological properties and their subcellular localization differ greatly, which might account for their respective pro- and antiexcitotoxic effects.

The massive calcium influx elicited by glutamate triggers further calcium release from intracellular stores and thereby dramatically elevates cytosolic free calcium. The consequence is an overstimulation of physiological processes at the cell membrane, in the cytoplasm, and in the nucleus, which may result in neurotoxicity. Increased intracellular calcium can induce a great number of various enzymes, among them protein kinase C, phospholipases, proteases, endonucleases, phosphatases, and nitric oxide synthase, which when excessively activated can act as mediators of the detrimental effects of glutamate.

The results of several studies, in both animals and humans, support glutamate-induced neurotoxicity in ischemic and mechanic injury of the CNS. Powerful evidence for its role in focal ischemia, for instance, derives from various neuroprotection studies using NMDA and non-NMDA antagonists (McIntosh et al. 1990; Meldrum 1990; Bullock and Fujisawa 1992; Kuroda et al. 1994). Intracerebral microdialysis studies have demonstrated a sixfold increase in glutamate levels in the extracellular fluid (ECF) following fluid percussion trauma in the rat (Faden et al. 1989; Katayama et al. 1990; Hayes et al. 1992). In humans, microdialysis studies suggest that the pattern of EAA release is determined mainly by the magnitude of the initial injury. Particularly, in patients with uncontrollable intracranial pressure and prolonged secondary events, the release of EAAs was shown to be massive and likely to cause the above-mentioned derangements in ionic fluxes (Zauner and Bullock 1995).

Nitric oxide synthase and free radical formation

The observation that activation of NMDA receptors can generate nitric oxide (NO) in a calcium-dependent manner has given rise to the hypothesis that NO, known as a cytotoxin, contributes to glutamate neurotoxicity (Garthwaite et al. 1988). NO is synthesized from L-arginine by the enzyme NO synthase (NOS). Three different isoforms have been characterized so far: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS; Griffith and Stuehr 1995). nNOS and eNOS are constitutively expressed and depend on intracellular calcium, whereas iNOS is calcium-independent and induced upon immunological stimuli, for instance. Depending on the availability of L-arginine and cofactors such as tetrahydrobiopterin, NOS produces not only NO but also superoxide anions, which can lead to the formation of highly cytotoxic peroxynitrite (Heinzel et al. 1992; Xia and Zweier 1997). Such reactions might particularly occur in cerebral ischemia and trauma, when substrate and cofactors are likely to become rate-limiting.

Despite the evidence that ischemia and trauma can have profound influences on NO synthesis, the role played by NO in these pathophysiologies is still a source of debate (Choi 1993; Pelligrino et al. 1993; Iadecola et al. 1994; Iadecola 1997). Inhibition of NOS has been found to reduce (Nowicki et al. 1991; Buisson et al. 1992; Nagafuji et al. 1992; Carreau et al. 1994), to not affect (Dawson et al. 1992; Buchan et al. 1994), or even to enhance (Yamamoto et al. 1992; Kuluz et al. 1993; Zhang and Iadecola 1993) cerebral ischemic damage. The reason for these apparently contradictory results could be the fact that, besides its cytotoxic effects, NO is a potent vasodilatator and an inhibitor of platelet aggregation, which might improve postischemic blood flow (Iadecola 1997). Furthermore, NO has been described to limit glutamate neurotoxicity by inhibiting calcium influx through the NMDA receptor (Manzoni et al. 1992; Lipton et al. 1993; Fagni et al. 1995). Finally, it has become clear that the redox status of NO has a profound impact on the biochemical effects of NO (Lipton et al. 1993; Mohr et al. 1997; Ogura et al. 1997; So et al. 1998).

Current understanding of NO effects in cerebral ischemia and trauma situations suggests that the point in time at which NO is produced after the insult is of great importance. At very early stages of ischemia, the beneficial vascular effects of eNOS activity seem to outweigh the potential neurotoxic effects of nNOS activity. A few hours later, the vascular effects are no longer protective and NO becomes predominantly toxic. In later stages, iNOS expression increases and causes a long-lasting, large production of NO, eventually contributing to the progression of cell damage.

Oxygen, while being essential for life, can also be part of the destructive cascade elicited by ischemia and trauma in brain tissue. Oxygen radicals are products of normal cellular metabolism. A number of cellular defense mechanims exist to keep the extent of free radical formation and their effects under strict control. In pathophysiological situations, however, normal cellular function may be disrupted to such a degree that oxygen radical production may be substantially increased and cellular defense mechanisms are turned over. Formation of superoxide, hydrogen peroxide, and hydroxyl radicals can then result in peroxidation injury of lipid membranes, protein, and DNA damage. Despite significant advances in methodology, techniques assessing the occurrence of lipid peroxidation and oygen radical formation in vivo are not entirely convincing. Most of the evidence for an important role of free radicals in cerebral ischemia and trauma comes from pharmacological studies demonstrating that antioxidants and free radical scavengers can prevent some of the pathophysiological and neurological sequelae of CNS trauma and ischemia (for review, see Braughler and Hall 1989).

Protein kinases, immediate early genes, and transcription factors

Activation of effector proteins causing secondary neuronal de- or regeneration after ischemia and traumatic

brain injury partly results from increased expression of so-called immediate-early genes (IEGs) and subsequent formation of transcription factors regulating a great variety of response genes (Akins et al. 1996; Herdegen et al. 1997).

Induction of IEGs by DNA binding proteins such as the serum response element (SRE) and the calcium/cAMP response element (Ca/CRE) is preceded by the activation of various second-messenger systems including protein kinases such as mitogen-activated protein kinases (MAPKs), protein kinase A, and protein kinase C (Cahill et al. 1996). Evidence is accumulating that all these signalling pathways can intersect and therefore "greatly alter a cell's response to a given stimulus" (Hale et al. 1996). Some examples of IEGs with increased expression after ischemia or traumatic brain injury include c-*fos*, c-*jun*, *jun-B* and *jun-D* (Kiessling et al. 1993; Hüll and Bähr 1994; Akins et al. 1996). c-*jun* as one of the most prominent IEGs can dimerize with *jun*, *fos*, activating transcription factor (ATF), and other transcription factors, which together form the activator protein-1 (AP-1), to control numerous target genes involved in cell cycle, organogenesis, cellular differentiation, apoptosis, cell survival, and regeneration (Angel and Karin 1991; Gass and Herdegen 1995; Hale et al. 1996; Herdegen et al. 1997). Following axotomy as an in vivo model of neuronal trauma, there is some correlation between c-*jun* expression and neuronal death. After intraorbital optic nerve transection, for example, c-*jun* is highly expressed in retinal ganglion cells that are supposed to die (Hüll and Bähr 1994; Isenmann and Bähr 1997). Moreover, c-*jun* has been found in neurons after ischemic insult (Dragunow et al. 1993). c-*jun* expression, predominantly investigated in nonneuronal cells, strongly depends on the activation of stress-activated protein kinases (SAPK), also known as Jun N-terminal kinases (JNKs), an alternative cascade of MAPKs. Immunocytochemistry and kinase assays have shown that activation of JNKs and phosphorylation of c-*jun* can last for weeks following neuronal damage and ischemia (Herdegen et al. 1997). Besides the control of target genes, activated JNK can interact with various crucial molecules regulating neuronal death or survival such as p53, *bax/bcl-2*, and caspases. The relation between the SAPK-JNK pathway and the activation of caspases as an up- or downstream event is under current investigation (Beyaert et al. 1997; Cardone et al. 1997; Huang et al. 1997; Lee et al. 1997).

Nuclear factor-κB (NF-κB), another transcription factor distinct from the AP-1 family, is also of interest concerning neuronal death and survival. Since NF-κB is a preformed factor with regulated activity, it can directly transduce an extracellular signal to the nucleus, ensuring a rapid response. Up to now, however, it remains unclear whether activation of NF-κB supports neuroprotection or neurodegeneration (O'Neill and Kaltschmidt 1997). An example for the complex function of NF-κB activation in neurodegeneration is provided by recent studies that suggest a neuroprotective role of NF-κB in a culture model of Alzheimer's disease (Barger et al. 1998; Mattson et al. 1997). In contrast, it was shown that NF-κB is activated by the neurotoxic peptide Aβ via the formation of reactive oxygen intermediates and can be found around early plaque stages in Alzheimer's disease patients, thus indicating a role for NF-κB in neurodegeneration (Kaltschmidt et al. 1997; O'Neill and Kaltschmidt 1997). More detailed investigations in various models of neuronal trauma are necessary to understand the regulation and interaction of signalling cascades determining the fate of injured neurons.

Caspases and calpains

Caspases

Besides many apoptosis-inducing or -preventing proteins that will not be discussed here in great detail, an increasing number of cysteine proteases termed caspases (*c*ysteinyl *asp*artate-specific protein*ases*) are central mediators of apoptotic cell death (Alnemri et al. 1996). Our knowledge about this family of proteases resides mainly in the fundamental work of Horvitz and colleagues (Ellis et al. 1991), who dealt with programmed cell death in the developing nematode *Caenorhabditis elegans*. During normal development of this worm, 131 cells of the 1090 originally generated cells die (Ellis et al. 1991). Three principal genes (*ced* genes) were identified to mediate or prevent this apoptotic death. While *ced-3* and *ced-4* mediate apoptosis in *C. elegans*, *ced-9* acts to antagonize these death genes and to suppress the death program (Hengarter et al. 1994). *Ced-9* bears sequence homology with the mammalian proto-oncogene *bcl-2*, one of a family of genes involved in the prevention or regulation of apoptotic death (for review, see Merry and Korsmeyer 1997). The mammalian homologue of *ced-4* is the recently identified "apoptotic protease activating factor" (*Apaf-1*), which seems to play an important role in cytochrome c-dependent apoptosis (Zou et al. 1997). *Ced-3* encodes for a cysteine protease related to the above-mentioned group of caspases in vertebrates (Alnemri et al. 1996). Interleukin-1β-converting enzyme (ICE; caspase-1) was the first caspase identified (Yuan et al. 1993), whereas CPP32 (caspase-3), also called Yama (the Hindu god of death) or apopain, is the caspase with the highest sequence homology to *ced-3* (Fernandes-Alnemri et al. 1994; Nicholson et al. 1995). Based on sequence homology, structure, and catalytic mechanism, the family of caspases meanwhile comprises at least 14 members (Cohen 1997; Hu et al. 1998; Thornberry and Lazebnik 1998), divided into the ICE and the CED-3 subfamily (Nicholson and Thornberry 1997).

Evidence for the in vivo relevance of caspases mainly derives from experiments in knockout mice. Caspase-1 knockout mice, apart from defective generation of mature interleukin-1β (IL-1β), develop normally and lack an in vivo phenotype indicating apoptotic failure (Kuida et al. 1995; Li et al. 1995). Thus, caspase-1 does not ap-

pear to play an essential, nonredundant role in neuronal apoptosis. Mice deficient in caspase-3, however, display a striking phenotype, with profound affection of brain development resulting in multiple hyperplasias, disorganized cell deployment, and failure in neuronal apoptosis detectable in the cortex, cerebellum, striatum, hippocampus, and the retina (Kuida et al. 1996). Surprisingly, other tissues and organ systems were not affected. These results suggest an important and predominant role for caspase-3 in neuronal apoptosis (Nicholson and Thornberry 1997). How caspase-3 mediates neuronal apoptosis and by which pathways caspase-3 is activated in vivo is under current investigation and not yet completely understood. It is believed that active caspase-3 leads to apoptotic death by cleavage of proteins implicated in DNA repair (poly(ADP-ribose)polymerase, PARP, DNA-dependent protein kinase, DNA-PK), mRNA splicing (U1–70 K), sterol biosynthesis (sterol regulatory element binding proteins, SREBPs), and cytoskeletal reorganization (Kumar 1997).

Despite an immense number of studies showing an activation of caspases during neuronal apoptosis in vitro, up to now little has been known about caspase activation following ischemia or traumatic brain injury in vivo. In 1996, for the first time, an increased expression of caspase-1 mRNA and protein in hippocampus after ischemia was shown (Bhat et al. 1996). This expression of caspase-1 was mainly localized in microglial cells, suggesting an indirect role in ischemic neuronal damage through mediation of an inflammatory response. One year later, Hara and colleagues (1997) were able to show an activation of caspase-1 by detecting the active p20 fragment after ischemia. An involvement of caspase-2 in ischemic cell death was suggested by increased mRNA expression without identification of active fragments (Kinoshita et al. 1997). Evidence for caspase-3 as mediator of neuronal death after ischemia rises from a study showing the active fragment of caspase-3 in TUNEL-positive neurons dying apoptotically by confocal microscopy (Namura et al. 1998). This is in line with findings after fluid percussion trauma (Yakovlev et al. 1997) and axotomy (Kermer et al. 1998), in which caspase-3 was identified as a crucial player in neuronal apoptosis. Together, these results suggest that activation of caspase-3 might be a general feature in neuronal apoptosis after various types of brain injury.

Calpains

Calpains are calcium-activated neutral proteases belonging to a papain family of cysteine proteases distinct from caspases. At least six members of the calpain family have been identified so far. Two isoenzymes are present in the brain: µ-calpain (calpain I) and *m*-calpain (calpain II; Melloni and Pontremoli 1989). Preferred substrates for proteolysis by calpains are: (1) cytoskeletal proteins such as fodrin (brain spectrin), microtubule-associated protein 2 (MAP2), tau, and neurofilaments; (2) enzymes involved in signal transduction such as protein kinase C and some phosphatases; (3) membrane proteins such as EGF receptors; (4) transcription factors (e.g., Jun and Fos); and (5) calmodulin-binding proteins such as G proteins (for review, see Wang et al. 1989; Saido et al. 1994; Suzuki et al. 1995). Interestingly, calpains share substrate specificity for the cleavage of fodrin with caspase-3.

The physiological role of calpains in vivo, however, is not completely understood yet, but there are reports demonstrating a role in neural differentiation (Hirai et al. 1991), cytoskeletal protein turnover (Giancotti et al. 1992), long-term potentiation (del Cerro et al. 1990), and physiological apoptosis (Squier and Cohen 1997; Zalewska 1996; Kampfl et al. 1997; Zhivotovsky et al. 1997).

Under pathological conditions, the disruption of calcium homeostasis, as it occurs in experimental traumatic brain injury and ischemia by excessive exposure to excitatory amino acids, leads to an unphysiological activation of calpains with a strong proteolytic activity and subsequent cleavage and degradation of intracellular proteins and membranes (Kampfl et al. 1997). Extensive cytoskeleton and plasma membrane damage can disrupt the integrity of the cell, cause increased membrane permeability to ions and even macromolecules, compromise transport of essential cell products, and induce signalling cascades, finally mediating secondary neuronal death (Zalewska 1996).

Pharmacological treatment strategies for prevention of neuronal death in vivo

The various processes and pathophysiological changes related to neuronal death after ischemia and trauma as outlined in the first section of this review initiated preclinical and/or clinical putative neuroprotective treatment strategies in humans. Below, we will give an overview focused on the most important classes of neuroprotectants. This information cannot be complete but may serve as a reference for the clinical relevance of basic neuroscience research.

NMDA antagonists

In the group of pharmacological agents known as NMDA antagonists, one can distinguish between noncompetitive NMDA antagonists, for instance binding to the phenylcyclidine (PCP) recognition site of the NMDA receptor, and competitive NMDA blockers. Most of these drugs proved to be effective in animal models of ischemia or trauma, but failed to show significant neuroprotection in humans.

Dizlocipine (MK-801), belonging to the group of noncompetitive antagonists, significantly reduced the infarct size in focal ischemia models in rats, cats, rabbits, and primates (Kochhar et al. 1988; Ozyurt et al. 1988; Park et al. 1988a, 1988b). In global ischemia models, re-

sults were conflicting, particularly when the treatment was started after the onset of ischemia (Gill et al. 1988). In trauma models such as optic nerve lesion, MK-801 led to a complete rescue of the subpopulation of β-ganglion cells in cats, whereas it was not effective, even showing adverse effects, in the rat retina (Russelakis-Carneiro 1996; Schmitt and Sabel 1996). The clinical development of dizlocipine was discontinued as a consequence of safety concerns, particularly psychotic side effects (Koek et al. 1988; Leung and Desborough 1988; Olney et al. 1989; Park et al. 1989; Buchan 1990).

Dextrorphan and dextromethorphan are also noncompetitive NMDA antagonists, interacting with the PCP binding site. In a rabbit model of ischemia, both agents reduced the infarct volume, when given before or after the onset of ischemia (George et al. 1988; Steinberg et al. 1988a, 1988b, 1989). In a small pilot study of ten patients, dextromethorphan did not cause any severe side-effects (Albers et al. 1991), whereas the clinical development of dextrorphan had to be stopped because of similar side effects to those elicited by dizolcipine (Albers et al. 1995).

Cerestat (CNS 1102; Reddy et al. 1994), another noncompetitive NMDA antagonist, significantly reduced infarct volume even when given 15 min after permanent occlusion of the MCA in rats (Meadows et al. 1994). A first dose-finding safety trial with Cerestat in over 60 patients approached neuroprotective dose levels without major side effects (Fisher 1994). A safety and efficacy trial of Cerestat in acute ischemic stroke patients is in progress (Turrini 1996).

Competitive NMDA antagonists include substances such as CGS 19755 (Selfotel), CPP, APH, MDL 100,453 (Boast et al. 1988; Hasegawa et al. 1994; Warkins and Olverman 1998). Selfotel is probably the most extensively studied drug in this group. Phase III clinical trials were terminated because of increased mortality (statistically not significant) in the treatment group and/or psychotic side effects (Wahlgren 1997). Eliprodil, an antagonist binding to the polyamine site of the NMDA receptor, was effective in animal models of ischemia (Gotti et al. 1990). However, a large phase III trial was recently terminated after interim analysis indicated a lack of efficacy (Wahlgren 1997).

Calcium channel antagonists

Opening of voltage-dependent calcium channels contributes largely to intracellular calcium overload during excitotoxic events. Glutamate-mediated depolarization leads to activation of voltage-dependent calcium channels, allowing extracellular calcium to enter the cell along its concentration gradient.

Nimodipine, an antagonist of L-type calcium channels, has been evaluated in controlled randomized trials for neuroprotection in acute ischemia using various treatment regimen. The Intravenous Nimodipine West European Stroke Trial (INWEST) had to be terminated due to worsened outcome and increased mortality (Wahlgren et al. 1994). Hypotension during intravenous nimodipine treatment might have been the reason for the worse functional outcome compared with placebo. Another study, with patients who had been given nimodipine orally, found a significant 38% reduction of the odds for an unfavorable outcome only in those patients who had received the treatment within the first 12 h after the onset of symptoms (Mohr et al. 1994). The early treatment approach is now being tested in a Dutch study (Very Early Nimodipine Use in Stroke, VENUS; Limburg 1996).

Glutamate release inhibitors

A known endogenous inhibitor of presynaptic EAA release is the nucleoside adenosine, which mediates its neuroprotective effects via four subtypes of G-protein-coupled receptors (Simpson et al. 1992; Olah and Stiles 1995). In the brain, EAA release is reduced predominantly via the A_1 adenosine receptor. Exogenous adenosine receptor agonists and enhancement of endogenous adenosine levels by metabolic inhibitors or uptake inhibitors have been discussed as promising therapeutic options ameliorating excitotoxic neuronal death. Reports reviewing the neuroprotective effects of such compounds following ischemia are numerous (Sweeney 1997). However, severe cardiovascular side effects of receptor agonist treatment such as bradycardia and hypotension have been reported (Sollevi 1986; von Lubitz et al. 1994) indicating that "the possible therapeutic value of treating acute stroke with systemically administered adenosine or one of the currently available metabolically stable analogs is dubious" (Sweeney 1997).

Another drug reducing neurotransmitter release, probably by interaction with presynaptic voltage-sensitive sodium channels, is BW619C89, a derivate of the anticonvulsive lamotrigin, which proved to be neuroprotective in experimental ischemia (Leach et al. 1993; Graham et al. 1994; Smith et al. 1997). Safety and tolerability was recently tested in a small group of stroke patients (Muir et al. 1998). Development of a phase III trial was stopped owing to detrimental effects.

Lubeluzole is a benzothiazole compound that prevents the increase in extracellular glutamate concentrations and inhibits glutamate-induced nitric oxide-related neurotoxicity. Systemically administered lubeluzole reduced infarct size and sensorimotor deficits in animal models of ischemia (De Ryck 1996) and proved to be well tolerated in stroke patients in a dose-dependent manner (De Keyser et al. 1997). A single intravenous injection of 10 mg lubeluzole/day reduced stroke mortality in a phase II study (Diener et al. 1996). The North American phase III trial showed a reduction in mortality and a significant improvement in functional and neurological recovery in the lubeluzole group (Grotta 1997). In general, no such effect was found in the European and Australian phase III trial, but treatment benefit was related to stroke severity with a significant reduction of mortality

only in patients for whom stroke severity was mild to moderate (Diener 1998). A new trial with a modified protocol is in progress. It has to be noted that lubeluzole is the first and, to the authors knowledge, the only neuroprotectant that has led to positive results in acute ischemic stroke in large phase III trials so far.

GABA agonists

γ-Amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS modulating glutamate release via hyperpolarization of the cell membrane, suggesting a neuroprotective action via reversing the excitotoxic cascade (del Zoppo et al. 1997). While baclofen, a $GABA_B$ agonist, was ineffective, muscimol, a $GABA_A$ receptor agonist, as well as clomethiazole, another GABA agonist, proved to be protective in experimental cerebral ischemia (Lyden and Hedges 1992; Sydserff et al. 1995). The latter has already been tested in phase III (the Clomethiazole Acute Stroke Study, CLASS, unpublished data). However, the final analysis was not convincing (Bogousslavsky 1998).

Free radical scavengers

Potential agents reducing free radical damage include superoxide dismutase (SOD), catalase, alpha-tocopherol, glutathione, lazaroids, iron chelators, and phenyl-*t*-butylnitrones (PBN).

Tirilazad, a lipid peroxidation inhibitor, has been shown to significantly reduce infarct size in various stroke models (Xue et al. 1992). In clinical trials, however, Tirilazad was ineffective (Haley et al. 1995).

PBNs form stable adducts with various kinds of free radicals. In gerbils and rats, PBN reduced ischemia-induced forebrain edema and loss of hippocampal CA1 neurons (Yue et al. 1992; Sen and Phillis 1993; Cao and Phillis 1994). Excitotoxic lesions in the striatum, produced by injection of NMDA, AMPA, kainate, or malonate, were significantly attenuated by PBN and its derivative S-PBN (Schulz and Beal 1995). Interestingly, S-PBN was found either to add to the neuroprotective effect of the NMDA antagonist MK-801 or potentiate the neurotrophic effect of brain-derived neurotrophic factor (BDNF; Schulz et al. 1995; Klöcker et al. 1998). To our knowledge, there is no clinical trial testing PBN in the treatment of mechanical or ischemic CNS injury yet.

Gangliosides

EAAs unequivocally contribute to secondary neuronal death after injury as described above. Blocking downstream pathological events of EAA receptor stimulation without affecting physiological activation offers a therapeutic option avoiding the side-effects of direct receptor antagonism. This property, known as abuse-dependent antagonism (Manev et al. 1990), was shown for the exogenous monoganglioside GM_1 as a member of a family of glycosphingolipids. In vitro and in vivo treatment with GM_1 or GM_1-L (siagoside) attenuates EAA-related neuronal death in a dose-dependent manner even when administered systemically (for review, see Leon et al. 1990). First clinical trials in stroke patients were initiated in the early 1980s (Bassi et al. 1984; Battistin et al. 1985). However, two large trials were finished in 1994 without convincing evidence of efficiacy for this therapeutic approach (Alter et al. 1994; Lenzi et al. 1994)

Neurotrophic factors and other growth factors

Based on the neurotrophic factor hypothesis (Barde 1989), lack of trophic support was suggested to cause secondary neuronal death after neuronal trauma. During the last decade, regulation of mRNA as well as neuroprotective action of neurotrophic factors such as the neurotrophins (NGF, BDNF, NT-3, NT-4/5), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), glial-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF) was investigated in various models of ischemia and traumatic brain injury in vivo. To give only a few examples, the neurotrophins, CNTF, and IGF-I exhibit neuroprotective action on axotomized mouse motoneurons (Li et al. 1994). Following experimental cerebral or retinal ischemia in rat and mouse, several growth and neurotrophic factors rescued injured neurons, including NGF, BDNF, IGF-I, and FGF (Nakata et al. 1993; Siliprandi et al. 1993; Mattson and Scheff 1994; Unoki and LaVail 1994; Doré et al. 1997; Schabitz et al. 1997; Cuevas et al. 1998; Ferrer et al. 1998; Hicks et al. 1998; Ishimaru et al. 1998). After optic nerve transection, axotomized retinal ganglion cells could be rescued by neurotrophins, GDNF, and other factors (Mey and Thanos 1993; Mansour-Robaey et al. 1994; Peinado-Ramón et al. 1996; Klöcker et al. 1997, 1998). However, there are data suggesting that trophic factors rather delay neuronal death than provide permanent rescue (Naumann et al. 1994; Di Polo et al. 1998; Ishimaru et al. 1998).

Some of the factors mentioned above have already been clinically tested in amyotrophic lateral sclerosis and diabetic neuropathy, unfortunately only with moderate success (for review, see Ochs et al. 1997). A first American and European phase III trial has been launched to treat stroke in humans with bFGF. Neurotrophic effects of neurotrophins following traumatic brain injury in humans have not been investigated yet.

Calpain inhibitors

As stated above, calpains are activated after cerebral ischemia and traumatic brain injury (Bartus et al. 1998). There is an increasing number of studies verifying a neuroprotective effect of calpain inhibitors in vivo. Bartus et al (1994), for example, showed that administration of a

calpain inhibitor within 3 h after occlusion of the MCA (MCAO) in rats reduced the infarct volume by 75%. Following traumatic brain injury, inhibitors of calpains attenuated cytoskeletal protein loss in cortical neurons, leading to a reduced extent of contusion at the injury site (Postmantur et al. 1997). Since no clinical trials with these drugs are reported so far, future studies are needed to qualify this therapeutic approach for use after neuronal injury in humans.

Caspase inhibitors

By means of special synthetic inhibitors, the cascade of caspases can be blocked specifically and irreversibly with excellent neuroprotective effects in vitro (for review, see Martinou and Sadoul 1996; Cohen 1997). Up to now, data applying these promising compounds in vivo have been rare. One of the first in vivo papers in this context was the work of Milligan and coworkers (1995), who showed that caspase inhibition arrests developmental programmed cell death of motoneurons. Effectiveness of caspase inhibition after ischemia with reduced infarct region in mice was demonstrated by Hara et al. (1997). Evidence of caspase-3 as major mediator of neuronal apoptosis (Nicholson and Thornberry 1997) led to therapeutic approaches with a potent inhibitor of caspase 3-like proteases: z-DEVD-cmk (benzyloxycarbonyl-Asp-Glu-Val-Asp-chloromethylketone). This compound proved to be a highly neuroprotective agent in vivo after MCAO in rats (Namura et al. 1998), axotomy of adult rat retinal ganglion cells (Kermer et al. 1998), and fluid percussion trauma (Yakovlev et al. 1997). More studies are necessary to support this therapeutic strategy as a general neuroprotectant in various models of neuronal injury. Subsequently, after optimizing administration, this approach might be very promising in the treatment of cerebral trauma and even neurodegenerative disorders as already discussed elsewhere (Holtzman and Deshmukh 1997).

Immunomodulation and modulation of cell metabolism

After having realised that the brain is an immune-competent organ, a potential neuroprotective role for immune modulators after injury evolved. Indeed, pretreatment of rats with steroids provides protection against hypoxic-ischemic brain damage. The underlying mechanism, however, is unknown (Tuor 1997). Inhibition of protein synthesis, for example, with cycloheximide has been shown to prevent neuronal apoptosis and proved to be neuroprotective in experimental focal cerebral ischemia (Linnik et al. 1993; Ishitani and Chuang 1996). Moreover, tacrolimus (FK506) and cyclosporin, both immunosuppressive agents, were of benefit after ischemia in rats and gerbils (Shiga et al. 1992; Sharkey et al. 1996).

The only immune modulatory strategy that has reached clinical testing so far is the blockade of selected leucocyte and endothelial cell adhesion receptors by a murine antibody against the intercellular adhesion molecule ICAM-1 (enlimomab). Although the antibody was shown to reduce neurological deficits after embolic stroke in rabbits and rats (Bowes et al. 1993; Zhang et al. 1994), the clinical testing indicated no positive effects when compared with placebo (unpublished data; see Bogousslavsky 1998).

Others

Besides the described neuroprotective treatment strategies, few other approaches have been performed to reduce tissue damage following ischemia in animals and humans that are not acting via pathways described above. *Piracetam*, for instance, increases cyclic adenosine monophosphate in the brain. Heiss et al (1983) found that glucose metabolism in ischemic brain in acute stroke patients may be increased by piracetam. A randomized controlled study found no significant difference in clinical outcome between piracetam and placebo group (De Deyn 1998). Results of a large phase II trial are lacking so far.

Lifarizine is a sodium and calcium channel modulator with neuroprotective potential in experimental ischemia. A phase II trial reported a poorer outcome in the verum group (Squire et al. 1995), so that a phase III trial has been abandoned.

Citicholine raises the possibility of systemically administering cytidine and choline, both essential metabolites for cell membrane repair. Two smaller clinical stroke trials reported safety and efficiacy (Tazaki et al. 1988; Clark et al. 1997). The drug is already available in Japan and Europe. No results of a phase III trial are reported.

Ebselen is a lipophilic compound containing selenium and inhibiting lipid peroxidation. Its neuroprotective potential was tested in cats and in rats. Oral treatment of stroke patients in a small study ameliorated the patients' outcome 1 month following stroke (Yamaguchi et al. 1998). A phase III study will follow.

S*erotonin receptor agonists* have been considered to be neuroprotectants in cerebral ischemia, and opiate receptor antagonists such as *naloxone* have been shown to ameliorate neuronal injury (Hamilton et al. 1985; Andrews et al. 1988). Also *barbiturates* are suggested to be neuroprotective by reducing metabolic demands in the affected tissue. However, larger clinical trials were unsuccessful or are not yet available.

Conclusions

This review shows that significant advances have been made in the recent years in identifying the cellular and molecular mechanisms that underlie secondary neuronal loss after trauma and ischemia. However, the clinical setting and the human conditions are much more complex

than any animal model system available at present. Therefore, a therapeutic strategy possibly needs to be tested in several models and must proof efficacy also after delayed drug administration, which is closer to the clinical situation than online drug administration performed in many model systems. Furthermore, it seems that one single-treatment strategy will not be able to solve the whole problem. Instead, complex combination applications that target several cell death pathways need to be evaluated in the laboratory as well as in the hospital. This will pose major difficulties in clinical trials because of trial design and randomization of patients to treatment arms that have already proven to be not as effective as a single-treatment regimen. Further insight into the molecular regulation pattern of cell survival and death may help to design better targets for a pharmacological or gene therapy in the future.

References

Akins PT, Liu PK, Hsu CY (1996) Immediate early gene expression in response to cerebral ischemia. Friend or foe? Stroke 27(9):1682–1687

Albers GW, Saenz RE, Moses JA Jr, Choi DW (1991) Safety and tolerance of oral dextromethorphan in patients at risk for brain ischemia. Stroke 22(8):1075–1077

Albers GW, Atkinson RP, Kelley RE, Rosenbaum DM (1995) Safety, tolerability, and pharmacokinetics of the *N*-methyl-D-aspartate antagonist dextrorphan in patients with acute stroke. Dextrorphan Study Group. Stroke 26(2):254–258

Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J (1996) Human ICE/CED-3 protease nomenclature (letter). Cell 87(2):171

Alter M (1994) Ganglioside GM1 in acute ischemic stroke: SASS trial. Stroke 25:1141–1148

Andrews BT, McIntosh TK, Gonzales MF, Weinstein PR, Faden AI (1988) Levels of endogenous opioids and effects of an opiate antagonist during regional cerebral ischemia in rats. J Pharmacol Exp Ther 247(3):1248–1254

Angel P, Karin M (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072(2–3):129–157

Barde YA (1989) Trophic factors and neuronal survival. Neuron 2(6):1525–1534

Barger SW, Horster D, Furukawa K, Goodman Y, Krieglstein J, Mattason MP (1998) Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca^{2+} accumulation. Proc Natl Acad Sci USA 92(20):9328–9332

Bartus RT, Hayward NJ, Elliott PJ, Sawyer SD, Baker KL, Dean RL, Akiyama A, Straub JA, Harbeson SL, Li Z, et al. (1994) Calpain inhibitor AK295 protects neurons from focal brain ischemia. Effects of postocclusion intra-arterial administration. Stroke 25(11):2265–2270

Bartus RT, Dean RL, Mennerick S, Eveleth D, Lynch G (1998) Temporal ordering of pathogenic events following transient global ischemia. Brain Res 790(1–2):1–13

Bassi S, Albizzati MG, Sbacchi M, Frattola L, Massarotti M (1984) Double-blind evaluation of monosialoganglioside (GM1) therapy in stroke. J Neurosci Res 12(2–3):493–498

Battistin L, Cesari A, Galligioni F, Marin G, Massarotti M, Paccagnella D, Pellegrini A, Testa G, Tonin P (1985) Effects of GM1 ganglioside in cerebrovascular diseases: a double-blind trial in 40 cases. Eur Neurol 24(5):343–351

Beyaert R, Kidd VJ, Cornelis S, Van de Craen M, Denecker G, Lahti JM, Gururajan R, Vandenabeele P, Fiers W (1997) Cleavage of PITSLRE kinases by ICE/CASP-1 and CPP32/CASP-3 during apoptosis induced by tumor necrosis factor. J Biol Chem 272(18):11694–11697

Bhat RV, DiRocco R, Marcy VR, Flood DG, Zhu Y, Dobrzanski P, Siman R, Scott R, Contreras PC, Miller M (1996) Increased expression of IL-1beta converting enzyme in hippocampus after ischemia: selective localization in microglia. J Neurosci 16(13):4146–4154

Biros MH (1991) Experimental head trauma models: a clinical perspective. Resuscitation 22(3):283–293

Boast CA, Gerhardt SC, Pastor G, Lehmann J, Etienne PE, Liebman JM (1988) The *N*-methyl-D-aspartate antagonists CGS 19755 and CPP reduce ischemic brain damage in gerbils. Brain Res 442(2):345–348

Bogousslavsky J for European Ad Hoc Consensus Group (1998) Neuroprotection as initial therapy in acute stroke. Third report of an Ad Hoc Consensus Group meeting. Cerebrovasc Dis 8(1):59–72

Bowes MP, Zivin JA, Rothlein R (1993) Monoclonal antibody to the ICAM-1 adhesion site reduces neurological damage in a rabbit cerebral embolism stroke model. Exp Neurol 119(2): 215–219

Braughler JM, Hall ED (1989) Central nervous system trauma and stroke. I. Biochemical considerations for oxygen radical formation and lipid peroxidation. Free Radic Biol Med 6(3): 289–301

Buchan AM (1990) Do NMDA antagonists protect against cerebral ischemia: are clinical trials warranted? Cerebrovasc Brain Metab Rev 2(1):1–26

Buchan AM, Gertler SZ, Huang ZG, Li H, Chaundy KE, Xue D (1994) Failure to prevent selective CA1 neuronal death and reduce cortical infarction following cerebral ischemia with inhibition of nitric oxide synthase. Neuroscience 61(1):1–11

Buisson A, Plotkine M, Boulu RG (1992) The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia (Abstract). Br J Pharmacol 106(4):766–767

Bullock R, Fujisawa H (1992) The role of glutamate antagonists for the treatment of CNS injury. J Neurotrauma [Suppl 2] 9:S443–462

Cahill MA, Peter ME, Kischkel FC, Chinnaiyan AM, Dixit VM, Krammer PH, Nordheim A (1996) CD95 (APO-1/Fas) induces activation of SAP kinases downstream of ICE-like proteases. Oncogene 13(10):2087–2096

Cao X, Phillis JW (1994) Alpha-phenyl-*tert*-butyl-nitrone reduces cortical infarct and edema in rats subjected to focal ischemia. Brain Res 644(2):267–272

Cardone MH, Salvesen GS, Widmann C, Johnson G, Frisch SM (1997) The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. Cell 90(2):315–323

Carreau A, Duval D, Poignet H, Scatton B, Vige X, Nowicki JP (1994) Neuroprotective efficacy of *N*-omega-nitro-L-arginine after focal cerebral ischemia in the mouse and inhibition of cortical nitric oxide synthase. Eur J Pharmacol 256(3):241–249

Cerro S del , Larson J, Oliver MW, Lynch G (1990) Development of hippocampal long-term potentiation is reduced by recently introduced calpain inhibitors. Brain Res 530(1):91–95

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1(8):623–634

Choi DW (1993) Nitric oxide: foe or friend to the injured brain? (comment). Proc Natl Acad Sci USA 90(21):9741–9743

Clark WM, Warach SJ, Pettigrew LC, Gammans RE, Sabounjian LA (1997) A randomized dose-response trial of citicoline in acute ischemic stroke patients. Citicoline Stroke Study Group. Neurology 49(3):671–678

Cohen GM (1997) Caspases: the executioners of apoptosis. Biochem J 326(1):1–16

Cuevas P, Carceller F, Reimers D, Saenz de Tejada I, Gimenez Gallego G (1998) Acidic fibroblast growth factor rescues gerbil hippocampal neurons from ischemic apoptotic death. Neurol Res 20(3):271–274

Dawson DA, Kusumoto K, Graham DI, McCulloch J, Macrae IM (1992) Inhibition of nitric oxide synthesis does not reduce in-

farct volume in a rat model of focal cerebral ischaemia. Neurosci Lett 142(2):151–154
De Deyn PP, Orgogozo JM, De Reuck J (1998) Acute treatment of stroke. PASS group. Piracetam Acute Stroke Study (letter). Lancet 352(9124):326
De Keyser J, Van de Velde V, Schellens RL, Hantson L, Tritsmans L, Gheuens J, Van Peer A, Woestenborghs R, Franke CL, Gorp J van (1997) Safety and pharmacokinetics of the neuroprotective drug lubeluzole in patients with ischemic stroke. Clin Ther 19(6):1340–1351
De Ryck M, Keersmaekers R, Duytschaever H, Claes C, Clincke G, Janssen M, Van Reet G (1996) Lubeluzole protects sensorimotor function and reduces infarct size in a photochemical stroke model in rats. J Pharmacol Exp Ther 279(2):748–758
Diener HC (1998) Multinational randomised controlled trial of lubeluzole in acute ischaemic stroke. European and Australian Lubeluzole Ischaemic Stroke Study Group. Cerebrovasc Dis 8(3):172–181
Diener HC, Hacke W, Hennerici M, Radberg J, Hantson L, De Keyser J (1996) Lubeluzole in acute ischemic stroke. A double-blind, placebo-controlled phase II trial. Lubeluzole International Study Group. Stroke 27(1):76–81
Di Polo A, Aigner LJ, Dunn RJ, Bray GM, Aguayo AJ (1998) Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Müller cells temporarily rescues injured retinal ganglion cells. Proc Natl Acad Sci USA 95(7): 3978–3983
Doré S, Kar S, Quirion R (1997) Rediscovering an old friend, IGF-I: potential use in the treatment of neurodegenerative diseases. Trends Neurosci 20(8):326–331
Dragunow M, Young D, Hughes P, MacGibbon G, Lawlor P, Singleton K, Sirimanne E, Beilharz E, Gluckman P (1993) Is c-Jun involved in nerve cell death following status epilepticus and hypoxic-ischaemic brain injury? (published erratum appears in Brain Res Mol Brain Res 20(1–2):179). Brain Res Mol Brain Res 18(4):347–352
Ellis RE, Yuan JY, Horvitz HR (1991) Mechanisms and functions of cell death. Annu Rev Cell Biol 7:663–698
Faden AI, Demediuk P, Panter SS, Vink R (1989) The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science 244(4906):798–800
Fagni L, Olivier M, Lafon Cazal M, Bockaert J (1995) Involvement of divalent ions in the nitric oxide-induced blockade of *N*-methyl-D-aspartate receptors in cerebellar granule cells. Mol Pharmacol 47(6):1239–1247
Fernandes-Alnemri T, Litwack G, Alnemri ES (1994) CPP32, a novel human apoptotic protein with homology to *Caenorhabditis elegans* cell death protein Ced-3 and mammalian interleukin-1 beta-converting enzyme. J Biol Chem 269(49):30761–30764
Ferrer I, Lopez E, Pozas E, Ballabriga J, Marti E (1998) Multiple neurotrophic signals converge in surviving CA1 neurons of the gerbil hippocampus following transient forebrain ischemia. J Comp Neurol 394(4):416–430
Fisher M (1994) Cerestat (CNS 1102), a noncompetitive NMDA antagonist in ischemic stroke patients: dose escalating safety study (Abstract). Cerebrovasc Dis 4:245
Garthwaite J, Charles SL, Chess Williams R (1988) Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336(6197):385–388
Gass P, Herdegen T (1995) Neuronal expression of AP-1 proteins in excitotoxic-neurodegenerative disorders and following nerve fiber lesions (Abstract). Prog Neurobiol 47(4–5):257
George CP, Goldberg MP, Choi DW, Steinberg GK (1988) Dextromethorphan reduces neocortical ischemic neuronal damage in vivo. Brain Res 440(2):375–379
Giancotti FG, Stepp MA, Suzuki S, Engvall E, Ruoslahti E (1992) Proteolytic processing of endogenous and recombinant beta 4 integrin subunit. J Cell Biol 118(4):951–959
Gill R, Foster AC, Woodruff GN (1988) MK-801 is neuroprotective in gerbils when administered during the post-ischaemic period. Neuroscience 25(3):847–855
Gotti B, Benavides J, MacKenzie ET, Scatton B (1990) The pharmacotherapy of focal cortical ischaemia in the mouse. Brain Res 522(2):290–307
Graham SH, Chen J, Lan J, Leach MJ, Simon RP (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(2):854–859
Griffith OW, Stuehr DJ (1995) Nitric oxide synthases: properties and catalytic mechanism. Annu Rev Physiol 57:707–736
Grotta J (1997) Lubeluzole treatment of acute ischemic stroke. The US and Canadian Lubeluzole Ischemic Stroke Study Group. Stroke 28(12):2338–2346
Hale AJ, Smith CA, Sutherland LC, Stoneman VE, Longthorne VL, Culhane AC, Williams GT (1996) Apoptosis: molecular regulation of cell death (published erratum appears in Eur J Biochem 237(3):884). Eur J Biochem 236(1):1–26
Haley EC, Jr., Kassell NF, Alves WM, Weir BK, Hansen CA (1995) Phase II trial of tirilazad in aneurysmal subarachnoid hemorrhage. A report of the Cooperative Aneurysm Study. J Neurosurg 82(5):786–790
Hamilton AJ, Black PM, Carr DB (1985) Contrasting actions of naloxone in experimental spinal cord trauma and cerebral ischemia: a review. Neurosurgery 17(5):845–849
Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu Sasamata M, Yuan J, Moskowitz MA (1997) Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci USA 94(5):2007–2012
Hasegawa Y, Fisher M, Baron BM, Metcalf G (1994) The competitive NMDA antagonist MDL-100,453 reduces infarct size after experimental stroke. Stroke 25(6):1241–1244
Hayes R, Jenkins LW, Lyeth BG (1992) Neurotransmitter mediated mechanisms of traumatic brain injury: acetylcholine and excitatory amino acids (Abstract). J Neurotrauma 9:173
Heinzel B, John M, Klatt P, Bohme E, Mayer B (1992) Ca^{2+}/calmodulin-dependent formation of hydrogen peroxide by brain nitric oxide synthase. Biochem J 281(3):627–630
Heiss WD, Ilsen HW, Wagner R, Pawlik G, Weinhard K (1983) Positron emission tomography of the brain. In: Heiss WD, Phelps ME (eds) Remote functional depression of glucose metabolism in stroke and its alteration by activating drugs. Springer, Berlin Heidelberg New York, pp 162–168
Hengartner MO, Horvitz HR (1994) *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. Cell 76(4):665–676
Herdegen T, Skene P, Bahr M (1997) The c-Jun transcription factor – bipotential mediator of neuronal death, survival and regeneration. Trends Neurosci 20(5):227–231
Hicks D, Heidinger V, Mohand Said S, Sahel J, Dreyfus H (1998) Growth factors and gangliosides as neuroprotective agents in excitotoxicity and ischemia. Gen Pharmacol 30(3):265–273
Hirai S, Kawasaki H, Yaniv M, Suzuki K (1991) Degradation of transcription factors, c-Jun and c-Fos, by calpain. FEBS Lett 287(1–2):57–61
Holtzman DM, Deshmukh M (1997) Caspases: a treatment target for neurodegenerative disease? (news). Nat Med 3(9):954–955
Hu S, Snipas SJ, Vincenz C, Salvesen G, Dixit VM (1998) Caspase-14 is a novel developmentally regulated protease. J Biol Chem 273(45):29648–29653
Huang S, Jiang Y, Li Z, Nishida E, Mathias P, Lin S, Ulevitch RJ, Nemerow GR, Han J (1997) Apoptosis signaling pathway in T cells is composed of ICE/Ced-3 family proteases and MAP kinase 6b. Immunity 6(6):739–749
Hüll M, Bähr M (1994) Regulation of immediate-early gene expression in rat retinal ganglion cells after axotomy and during regeneration through a peripheral nerve graft. J Neurobiol 25(1):92–105
Iadecola C (1997) Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci 20(3):132–139
Iadecola C, Pelligrino DA, Moskowitz MA, Lassen NA (1994) Nitric oxide synthase inhibition and cerebrovascular regulation. J Cereb Blood Flow Metab 14(2):175–192

Isenmann S, Bahr M (1997) Expression of c-Jun protein in degenerating retinal ganglion cells after optic nerve lesion in the rat. Exp Neurol 147(1):28–36

Ishimaru H, Takahashi A, Ikarashi Y, Maruyama Y (1998) NGF delays rather than prevents the cholinergic terminal damage and delayed neuronal death in the hippocampus after ischemia. Brain Res 789(2):194–200

Ishitani R, Chuang DM (1996) Glyceraldehyde-3-phosphate dehydrogenase antisense oligodeoxynucleotides protect against cytosine arabinonucleoside-induced apoptosis in cultured cerebellar neurons. Proc Natl Acad Sci USA 93(18):9937–9941

Kaltschmidt B, Uherek M, Volk B, Baeuerle PA, Kaltschmidt C (1997) Transcription factor NF-kappaB is activated in primary neurons by amyloid beta peptides and in neurons surrounding early plaques from patients with Alzheimer disease. Proc Natl Acad Sci USA 94(6):2642–2647

Kampfl A, Posmantur RM, Zhao X, Schmutzhard E, Clifton GL, Hayes RL (1997) Mechanisms of calpain proteolysis following traumatic brain injury: implications for pathology and therapy: implications for pathology and therapy: a review and update. J Neurotrauma 14(3):121–134

Katayama Y, Becker DP, Tamura T, Hovda DA (1990) Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J Neurosurg 73(6):889–900

Kermer P, Klocker N, Labes M, Bahr M (1998) Inhibition of CPP32-like proteases rescues axotomized retinal ganglion cells from secondary cell death in vivo. J Neurosci 18(12): 4656–4662

Kiessling M, Stumm G, Xie Y, Herdegen T, Aguzzi A, Bravo R, Gass P (1993) Differential transcription and translation of immediate early genes in the gerbil hippocampus after transient global ischemia. J Cereb Blood Flow Metab 13(6):914–924

Kinoshita M, Tomimoto H, Kinoshita A, Kumar S, Noda M (1997) Up-regulation of the *Nedd2* gene encoding an ICE/Ced-3-like cysteine protease in the gerbil brain after transient global ischemia. J Cereb Blood Flow Metab 17(5):507–514

Klöcker N, Braunling F, Isenmann S, Bahr M (1997) In vivo neurotrophic effects of GDNF on axotomized retinal ganglion cells. Neuroreport 8(16):3439–3442

Klöcker N, Cellerino A, Bahr M (1998) Free radical scavenging and inhibition of nitric oxide synthase potentiates the neurotrophic effects of brain-derived neurotrophic factor on axotomized retinal ganglion cells in vivo. J Neurosci 18(3): 1038–1046

Kochhar A, Zivin JA, Lyden PD, Mazzarella V (1988) Glutamate antagonist therapy reduces neurologic deficits produced by focal central nervous system ischemia. Arch Neurol 45(2): 148–153

Koek W, Woods JH, Winger GD (1988) MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. J Pharmacol Exp Ther 245(3):969–974

Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA (1995) Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. Science 267(5206):2000–2003

Kuida K, Zheng TS, Na S, Kuan C, Yang D, Karasuyama H, Rakic P, Flavell RA (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. Nature 384(6607): 368–372

Kuluz JW, Prado RJ, Dietrich WD, Schleien CL, Watson BD (1993) The effect of nitric oxide synthase inhibition on infarct volume after reversible focal cerebral ischemia in conscious rats. Stroke 24(12):2023–2029

Kumar S (1997) The apoptotic cysteine protease CPP32. Int J Biochem Cell Biol 29(3):393–396

Kuroda Y, Fujisawa H, Strebel S, Graham DI, Bullock R (1994) Effect of neuroprotective *N*-methyl-D-aspartate antagonists on increased intracranial pressure: studies in the rat acute subdural hematoma model. Neurosurgery 35(1):106–112

Leach MJ, Swan JH, Eisenthal D, Dopson M, Nobbs M (1993) BW619C89, a glutamate release inhibitor, protects against focal cerebral ischemic damage (see comments). Stroke 24(7): 1063–1067

Lee FS, Hagler J, Chen ZJ, Maniatis T (1997) Activation of the I-kappaB alpha kinase complex by MEKK1, a kinase of the JNK pathway. Cell 88(2):213–222

Lenzi GL, Grigoletto F, Gent M, Roberts RS, Walker MD, Easton JD, Carolei A, Dorsey FC, Rocca WA, Bruno R, et al. (1994) Early treatment of stroke with monosialoganglioside GM-1. Efficacy and safety results of the Early Stroke Trial. Stroke 25(8):1552–1558

Leon A, Lipartiti M, Seren MS, Lazzaro A, Mazzari S, Koga T, Toffano G, Skaper SD (1990) Hypoxic-ischemic damage and the neuroprotective effects of GM1 ganglioside. Stroke [Suppl 11] 21:95–97

Leung LW, Desborough KA (1988) APV, an *N*-methyl-D-aspartate receptor antagonist, blocks the hippocampal theta rhythm in behaving rats. Brain Res 463(1):148–152

Li L, Oppenheim RW, Lei M, Houenou LJ (1994) Neurotrophic agents prevent motoneuron death following sciatic nerve section in the neonatal mouse. J Neurobiol 25(7):759–766

Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, McDowell J, Paskind M, Rodman L, Salfeld J, et al (1995) Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. Cell 80(3):401–411

Limburg M (1996) Very early nimodipine use in stroke – The first 100 patients – cooperation between GPs and neurologists (abstract). Stroke 27:172

Linnik MD, Zobrist RH, Hatfield MD (1993) Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. Stroke 24(12):2002–2008

Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HS, Sucher NJ, Loscalzo J, Singel DJ, Stamler JS (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds (see comments). Nature 364(6438):626–632

Lucas DR, Newhouse JP (1957) The toxic effect of sodium L-glutamate on the inner layers of the retina. Arch Opthalmol 58193–58201

Lubitz DK von, Lin RC, Melman N, Ji XD, Carter MF, Jacobson KA (1994) Chronic administration of selective adenosine A_1 receptor agonist or antagonist in cerebral ischemia. Eur J Pharmacol 256(2):161–167

Lyden PD, Hedges B (1992) Protective effect of synaptic inhibition during cerebral ischemia in rats and rabbits. Stroke 23(10):1463–1469

Manev H, Costa E, Wroblewski JT, Guidotti A (1990) Abusive stimulation of excitatory amino acid receptors: a strategy to limit neurotoxicity. FASEB J 4(10):2789–2797

Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci USA 91(5):1632–1636

Manzoni O, Prezeau L, Marin P, Deshager S, Bockaert J, Fagni L (1992) Nitric oxide-induced blockade of NMDA receptors. Neuron 8(4):653–662

Martinou JC, Sadoul R (1996) ICE-like proteases execute the neuronal death program. Curr Opin Neurobiol 6(5):609–614

Mattson MP, Scheff SW (1994) Endogenous neuroprotection factors and traumatic brain injury: mechanisms of action and implications for therapy. J Neurotrauma 11(1):3–33

Mattson MP, Goodman Y, Luo H, Fu W, Furukawa K (1997) Activation of NF-kappaB protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of manganese superoxide dismutase and suppression of peroxynitrite production and protein tyrosine nitration. J Neurosci Res 49(6):681–697

McIntosh TK, Vink R, Soares H, Hayes R, Simon R (1990) Effect of noncompetitive blockade of *N*-methyl-D-aspartate receptors

on the neurochemical sequelae of experimental brain injury. J Neurochem 55(4):1170–1179
Meadows MW, Fisher M, Minematsu K (1994) Delayed treatment with a non-competitive NMDA antagonist. CNS 1102, reduces infarct size in rats. Cerebrovasc Dis 4:26–31
Meldrum B (1990) Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission. Cerebrovasc Brain Metab Rev 2(1):27–57
Melloni E, Pontremoli S (1989) The calpains. Trends Neurosci 12(11):438–444
Merry DE, Korsmeyer SJ (1997) *Bcl-2* gene family in the nervous system. Annu Rev Neurosci 20:1978–1982
Mey J, Thanos S (1993) Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. Brain Res 602(2):304–317
Milligan CE, Prevette D, Yaginuma H, Homma S, Cardwell C, Fritz LC, Tomaselli KJ, Oppenheim RW, Schwartz LM (1995) Peptide inhibitors of the ICE protease family arrest programmed cell death of motoneurons in vivo and in vitro. Neuron 15(2):385–393
Mohr JP, Orgogozo JM, Harrison MJG, Wahlgren NG, Gelmers JH, Martinez-Vila E, Dycka J, Tettenborn D (1994) Meta-analysis of oral nimodipine trials in acute ischemic stroke. Cerebrovasc Dis 4:197–203
Mohr S, Zech B, Lapetina EG, Brune B (1997) Inhibition of caspase-3 by *S*-nitrosation and oxidation caused by nitric oxide. Biochem Biophys Res Commun 238(2):387–391
Muir KW, Hamilton SJ, Lunnon MW, Hobbiger S, Lees KR (1998) Safety and tolerability of 619C89 after acute stroke. Cerebrovasc Dis 8(1):31–37
Nagafuji T, Matsui T, Koide T, Asano T (1992) Blockade of nitric oxide formation by *N*-omega-nitro-L-arginine mitigates ischemic brain edema and subsequent cerebral infarction in rats. Neurosci Lett 147(2):159–162
Nakata N, Kato H, Kogure K (1993) Protective effects of basic fibroblast growth factor against hippocampal neuronal damage following cerebral ischemia in the gerbil. Brain Res 605(2): 354–356
Namura S, Zhu J, Fink K, Endres M, Srinivasan A, Tomaselli KJ, Yuan J, Moskowitz MA (1998) Activation and cleavage of caspase-3 in apoptosis induced by experimental cerebral ischemia. J Neurosci 18(10):3659–3668
Naumann T, Kermer P, Seydewitz V, Ortmann R, D'Amato F, Frotscher M (1994) Is there a long-lasting effect of a short-term nerve growth factor application on axotomized rat septohippocampal neurons? Neurosci Lett 173(1–2):213–215
Nicholson DW, Thornberry NA (1997) Caspases: killer proteases. Trends Biochem Sci 22(8):299–306
Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, Gareau Y, Griffin PR, Labelle M, Lazebnik YA, et al. (1995) Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis (see comments). Nature 376(6535):37–43
Nowicki JP, Duval D, Poignet H, Scatton B (1991) Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. Eur J Pharmacol 204(3):339–340
Ochs G, Schrank B, Toyka K, Sendtner M (1997) Neurotrophic factors in the treatment of neurologic diseases. Aktuelle Neurol 24(4):170–174
Ogura T, Tatemichi M, Esumi H (1997) Nitric oxide inhibits CPP32-like activity under redox regulation. Biochem Biophys Res Commun 236(2):365–369
Olah ME, Stiles GL (1995) Adenosine receptor subtypes: characterization and therapeutic regulation. Annu Rev Pharmacol Toxicol 35:581–606
Olney JW, Sharpe LG (1969) Brain lesions in an infant rhesus monkey treated with monsodium glutamate. Science 166(903): 386–388
Olney JW, Ho OL, Rhee V (1971) Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. Exp Brain Res 14(1):61–76
Olney JW, Labruyere J, Price MT (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs (see comments). Science 244(4910):1360–1362
O'Neill LA, Kaltschmidt C (1997) NF-kappa B: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci 20(6):252–258
Ozyurt E, Graham DI, Woodruff GN, McCulloch J (1988) Protective effect of the glutamate antagonist, MK-801 in focal cerebral ischemia in the cat. J Cereb Blood Flow Metab 8(1): 138–143
Park CK, Nehls DG, Graham DI, Teasdale GM, McCulloch J (1988a) The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. Ann Neurol 24(4):543–551
Park CK, Nehls DG, Graham DI, Teasdale GM, McCulloch J (1988b) Focal cerebral ischaemia in the cat: treatment with the glutamate antagonist MK-801 after induction of ischaemia. J Cereb Blood Flow Metab 8(5):757–762
Park CK, Nehls DG, Teasdale GM, McCulloch J (1989) Effect of the NMDA antagonist MK-801 on local cerebral blood flow in focal cerebral ischaemia in the rat. J Cereb Blood Flow Metab 9(5):617–622
Peinado-Ramon P, Salvador M, Villegas Perez MP, Vidal Sanz M (1996) Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study. Invest Ophthalmol Vis Sci 37(4):489–500
Pelligrino DA, Koenig HM, Albrecht RF (1993) Nitric oxide synthesis and regional cerebral blood flow responses to hypercapnia and hypoxia in the rat. J Cereb Blood Flow Metab 13(1):80–87
Posmantur R, Kampfl A, Siman R, Liu J, Zhao X, Clifton GL, Hayes RL (1997) A calpain inhibitor attenuates cortical cytoskeletal protein loss after experimental traumatic brain injury in the rat. Neuroscience 77(3):875–888
Reddy NL, Hu LY, Cotter RE, Fischer JB, Wong WJ, McBurney RN, Weber E, Holmes DL, Wong ST, Prasad R, et al. (1994) Synthesis and structure-activity studies of *N,N´*-diarylguanidine derivatives. *N*-(1-naphthyl)-*N´*-(3-ethylphenyl)-*N´*-methylguanidine: a new, selective noncompetitive NMDA receptor antagonist. J Med Chem 37(2):260–267
Russelakis-Carneiro M, Silveira LC, Perry VH (1996) Factors affecting the survival of cat retinal ganglion cells after optic nerve injury. J Neurocytol 25(6):393–402
Saido TC, Sorimachi H, Suzuki K (1994) Calpain: new perspectives in molecular diversity and physiological-pathological involvement. FASEB J 8(11):814–822
Schabitz WR, Schwab S, Spranger M, Hacke W (1997) Intraventricular brain-derived neurotrophic factor reduces infarct size after focal cerebral ischemia in rats. J Cereb Blood Flow Metab 17(5):500–506
Schmitt U, Sabel BA (1996) MK-801 reduces retinal ganglion cell survival but improves visual performance after controlled optic nerve crush. J Neurotrauma 13(12):791–800
Schulz JB, Beal MF (1995) Neuroprotective effects of free radical scavengers and energy repletion in animal models of neurodegenerative disease. Ann NY Acad Sci 765:100–110
Schulz JB, Matthews RT, Jenkins BG, Brar P, Beal MF (1995) Improved therapeutic window for treatment of histotoxic hypoxia with a free radical spin trap. J Cereb Blood Flow Metab 15(6):948–952
Sen S, Phillis JW (1993) *Alpha*-phenyl-*tert*-butyl-nitrone (PBN) attenuates hydroxyl radical production during ischemia-reperfusion injury of rat brain: an EPR study. Free Radic Res Commun 19:255–265
Sharkey J, Crawford JH, Butcher SP, Marston HM (1996) Tacrolimus (FK506) ameliorates skilled motor deficits produced by middle cerebral artery occlusion in rats. Stroke 27(12): 2282–2286
Shiga Y, Onodera H, Matsuo Y, Kogure K (1992) Cyclosporin A protects against ischemia-reperfusion injury in the brain. Brain Res 595(1):145–148

Siliprandi R, Canella R, Carmignoto G (1993) Nerve growth factor promotes functional recovery of retinal ganglion cells after ischemia. Invest Ophthalmol Vis Sci 34(12):3232–3245

Simpson RE, O'Regan MH, Perkins LM, Phillis JW (1992) Excitatory transmitter amino acid release from the ischemic rat cerebral cortex: effects of adenosine receptor agonists and antagonists. J Neurochem 58(5):1683–1690

Smith SE, Hodges H, Sowinski P, Man CM, Leach MJ, Sinden JD, Gray JA, Meldrum BS (1997) Long-term beneficial effects of BW619C89 on neurological deficit, cognitive deficit and brain damage after middle cerebral artery occlusion in the rat. Neuroscience 77(4):1123–1135

So HS, Park RK, Kim MS, Lee SR, Jung BH, Chung SY, Jun CD, Chung HT (1998) Nitric oxide inhibits *c-Jun* N-terminal kinase 2 (JNK 2) via *S*-nitrosylation. Biochem Biophys Res Commun 247:809–813

Sollevi A (1986) Cardiovascular effects of adenosine in man; possible clinical implications. Prog Neurobiol 27(4):319–349

Squier MK, Cohen JJ (1997) Calpain, an upstream regulator of thymocyte apoptosis. J Immunol 158(8):3690–3697

Squire IB, Lees KR, Pryse Phillips W, Kertesz A, Bamford J (1995) Efficacy and tolerability of lifarizine in acute ischemic stroke. A pilot study. Lifarizine Study Group. Ann NY Acad Sci 765:317–318

Steinberg GK, George CP, DeLaPaz R, Shibata DK, Gross T (1988a) Dextromethorphan protects against cerebral injury following transient focal ischemia in rabbits. Stroke 19(9):1112–1118

Steinberg GK, Saleh J, Kunis D (1988b) Delayed treatment with dextromethorphan and dextrorphan reduces cerebral damage after transient focal ischemia. Neurosci Lett 89(2):193–197

Steinberg GK, Saleh J, Kunis D, DeLaPaz R, Zarnegar SR (1989) Protective effect of *N*-methyl-D-aspartate antagonists after focal cerebral ischemia in rabbits. Stroke 20(9):1247–1252

Suzuki K, Sorimachi H, Yoshizawa T, Kinbara K, Ishiura S (1995) Calpain: novel family members, activation, and physiologic function. Biol Chem Hoppe Seyler 376(9):523–529

Sweeney MI (1997) Neuroprotective effects of adenosine in cerebral ischemia: window of opportunity. Neurosci Biobehav Rev 21(2):207–217

Sydserff SG, Cross AJ, Green AR (1995) The neuroprotective effect of chlormethiazole on ischaemic neuronal damage following permanent middle cerebral artery ischaemia in the rat. Neurodegeneration 4(3):323–328

Tazaki Y, Sakai F, Otomo E, Kutsuzawa T, Kameyama M, Omae T, Fujishima M, Sakuma A (1988) Treatment of acute cerebral infarction with a choline precursor in a multicenter double-blind placebo-controlled study. Stroke 19(2):211–216

Thornberry NA, Lazebnik Y (1998) Caspases: enemies within. Science 281(5381):1312–1316

Tuor UI (1997)Glucocorticoids and the prevention of hypoxic-ischemic brain damage. Neurosci Biobehav Rev 21(2):175–179

Turrini R (1996) A pivotal safety and efficacy of Cerestat (Aptiganel HCL/CNS 1102) in acute ischemic stroke patients (abstract). Stroke 27:173

Unoki K, LaVail MM (1994) Protection of the rat retina from ischemic injury by brain-derived neurotrophic factor, ciliary neurotrophic factor, and basic fibroblast growth factor. Invest Ophthalmol Vis Sci 35(3):907–915

Wahlgren NG (1997) Neuroprotection in acute cerebral ischemia. In: Castillo J, Dávalos A, Toni D (eds) Management of acute ischemic stroke. Springer, Barcelona, pp 207–225

Wahlgren NG, MacMahon D, DeKeyser J, Indredavik B, Ryman T (1994) Intravenous Nimodipine West European Stroke Trial (INWEST) of nimodipine in the treatment of acute stroke. Cerebrovasc Dis 4:204–210

Wang KK, Villalobo A, Roufogalis BD (1989) Calmodulin-binding proteins as calpain substrates. Biochem J 262(3):693–706

Warkins JC, Olverman HJ (1998) Agonists and antagonists for excitatory amino acid receptors. Trends Neurosci 10:265–272

Xia Y, Zweier JL (1997) Direct measurement of nitric oxide generation from nitric oxide synthase. Proc Natl Acad Sci USA 94(23):12705–12710

Xue D, Slivka A, Buchan AM (1992) Tirilazad reduces cortical infarction after transient but not permanent focal cerebral ischemia in rats. Stroke 23(6):894–899

Yakovlev AG, Knoblach SM, Fan L, Fox GB, Goodnight R, Faden AI (1997) Activation of CPP32-like caspases contributes to neuronal apoptosis and neurological dysfunction after traumatic brain injury. J Neurosci 17(19):7415–7424

Yamaguchi T, Sano K, Takakura K, Saito I, Shinohara Y, Asano T, Yasuhara H (1998) Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. Stroke 29(1):12–7

Yamamoto S, Golanov EV, Berger SB, Reis DJ (1992) Inhibition of nitric oxide synthesis increases focal ischemic infarction in rat. J Cereb Blood Flow Metab 12(5):717–726

Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 75(4): 641–652

Yue TL, Gu JL, Lysko PG, Cheng HY, Barone FC, Feuerstein G (1992) Neuroprotective effects of phenyl-*t*-butyl-nitrone in gerbil global brain ischemia and in cultured rat cerebellar neurons. Brain Res 574(1–2):193–197

Zalewska T (1996) Calpain as proposed target for neuroprotective treatment of brain ischemia. Folia Neuropathol 34(3):121–127

Zauner A, Bullock R (1995) The role of excitatory amino acids in severe brain trauma: opportunities for therapy: a review. J Neurotrauma 12(4):547–554

Zhang F, Iadecola C (1993) Nitroprusside improves blood flow and reduces brain damage after focal ischemia. Neuroreport 4(5):559–562

Zhang RL, Chopp M, Li Y, Zaloga C, Jiang N, Jones ML, Miyasaka M, Ward PA (1994) Anti-ICAM-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in the rat. Neurology 44(9):1747–1751

Zhivotovsky B, Burgess DH, Vanags DM, Orrenius S (1997) Involvement of cellular proteolytic machinery in apoptosis. Biochem Biophys Res Commun 230(3):481–488

Zoppo GJ del , Wagner S, Tagaya M (1997) Trends and future developments in the pharmacological treatment of acute ischaemic stroke. Drugs 54(1):9–38

Zou H, Henzel WJ, Liu X, Lutschg A, 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(3):405–413

Zwiener U, Bauer R, Bergmann R, Eiselt M (1991) Experimental and clinical main forms of hypoxic-ischaemic brain damage and their monitoring. Exp Pathol 42(4):187–196