Protective And Restorative Strategies In The Neurodegenerative Disorders 26

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26.2. Introduction

Investigations of free radical involvement in neuronal cell death seem to demonstrate a proliferating exponential (Coyle and Puttfarcken, 1993), and the observed neurotoxicity of various agents appears to have developed from the induction of defective energy metabolism (*e.g.*, Albin and Greenamyre, 1993; Tipton and Singer, 1993). Thus, the mechanisms of cell death which occur in neurodegenerative diseases are a topic of great current interest at morphological, neurochemical and, not least, functional levels. A large number of investigations have implicated both **necrotic** and **apoptotic** cell death with a variety of neurodegenerative diseases (*cf.* Smythies, 1999). Apoptosis and necrosis may either co-exist or be sequential events depending on the severity of the initiating insult (*e.g.*, excitotoxin). Apoptosis or programmed cell death is characterised by cell shrinkage, chromatin condensation with DNA fragmentation, whereas necrosis manifests itself with rapid energy loss, cellular edema, cell membrane rupture, nuclear pyknosis and release of intracellular content resulting in an inflammatory reaction (Tatton and Calmers-Redman, 1998; Tatton *et al.*, 1998). Apoptotic cell death is characterised by maintainence of membrane integrity until late in the process of cell death. Toxic agents can induce necrotic or apoptotic cell death.

26.2. Cellular destruction

Evidence implicating a critical role for cell mitochondria in both necrotic and apoptotic cell death is accumulating rapidly (Charriaut-Marlangue *et al.*, 1996; Beal, 1998). Any critical role assigned to cellular energy reserves implicates the two modes of cell death. Apoptosis is favoured under conditions of preserved ATP levels. Following administration of glutamate or N-methyl-D-aspartate (NMDA), there is a prominent and persistent depolarisation of mitochondrial membrane which then results in a depletion of energy reserves and typically results in necrosis. A requirement for mitochondrial calcium uptake in glutamate excitotoxicity has been described (Olney *et al.*, 1991). Increases in intracellular calcium, triggered by the activation of NMDA receptors, lead to much greater increases in free radical production than comparable increases produced by other types of receptor activation. There is also a direct link between activation of NMDA receptors and activation of neuronal nitric oxide synthase (nNOS) (*e.g.*, Dawson *et al.*, 1993). Regardless of the modes of cell death, there is increasing evidence that both energy depletion and free

radical production may play a critical role (Zhang and Piantadosi, 1992). Mitochondria are the largest inherited source of free radicals. Under conditions of cell damage or stress, the production of free radicals is increased due to leakage from the electron transport chain. The role of peroxynitrite ($ONOO^-$), as a key reactive oxygen specieis (ROS) escalating oxidative damage leading to Alzheimer's disease, is plausibly suggested by the over-production of inducible NOS (iNOS) and the appearance of nitrotyrosine residues in Alzheimer brain residues (Good *et al.*, 1996; Smith *et al.*, 1997; Hensley *et al.*, 1998). $ONOO^-$, formed by the direct chemical reaction of superoxide ($O_2^{\cdot-}$) with nitric oxide ($^{\cdot}NO$) (below) is one of the most potent free radical damaging agents.

$$O_2^{\cdot-} + {}^{\cdot}NO \rightarrow ONOO^-$$

The compound can induce immediate damage by producing either one or two electron oxidations, as well as by nitrating proteins. The nitration of critical enzymes, such as manganese superoxide dismutase (MnSOD) or creatine kinase can lead to their inactivation. $ONOO^-$ can damage glutamate metabolism through the inhibition of glutamate transporters (Trotti *et al.*, 1996) which predisposes a greater vulnerability to excitotoxic destruction.

Much evidence points to the involvement of mitochondrial dysfunction in the more common neurodegenerative diseases (Tipton and Singer, 1993), including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral aclerosis (ALS) and Huntington's disease (HD). The evidence implicating altered redox metabolism as a common pathogenic mechanism in many neurodegenerative diseases is reviewed by Friedlich and Beal (Chapter 27) and putative neuroprotective strategies are described. Mitochondrial complex I deficiency was indicated in PD (Schapira *et al.*, 1990), whereas another focus of interest is protein aggregation in the pathogenesis of these disorders, *e.g.*, β-amyloid protein and its aggregation ability underlying the toxic effects (Pike *et al.*, 1991) in AD. A hallmark of AD is the presence of senile plaques and neurofibrillary tangles in the brain tissues of patients (Warzok *et al.*, 1998). Senile plaques are composed mainly of aggregated forms of a 4 kDa peptide, amyloid β peptide, that is derived from proteolytic processing of a large transmembrane amyloid precursor protein. Support for involvement of amyloid β protein in the pathogenesis of AD comes from the observation that certain forms of familial AD mutations result in the over-production of amyloid β protein, particularly in the longer form (Aβ 1-42) which aggregates more readily than the shorter form. β-Amyloid aggregation and the extent of cognitive impairment seem related (Cummings and Cotman, 1995). On the other hand, it has been demonstrated that Lewy bodies in PD are largely made up of α-synuclein (Spillantini *et al.*, 1997), and that α-synuclein mutations are associated with some familial cases of PD (Polymeropoulos *et al.*, 1997) and contribute to the pathogenesis of PD. Borden (1998) indicated that mutated α-synuclein may undergo conformational changes that precede protein-aggregation to insoluble fibrils. In ALS, point mutations in the enzyme SOD are responsible for the autosomal dominant inherited form of the disorder (Beal *et al.*, 1997). These point mutations may destabilise the enzyme giving either greater access to copper at the active site or leading to protein aggregates of the SOD. In HD, there is an accumulation of intranuclear inclusions which stain with antibodies to the N-terminal fragment of Huntingtin, the mutant protein (a polymorphic trinucleotide repeat) encoded by the disorder and normally restricted to the cytoplasm. It has been suggested that nuclear translocation of Huntingtin is a critical factor in the pathogenesis of HD (Reddy *et al.*,

1999). Whether these various protein aggregates play a direct role in disease pathogenesis or whether they are secondary effects of the pathologic process or are merely 'innocent bystanders' remains to be determined.

Accumulating evidence (*e.g.*, Gerlach *et al.*, 1994; Gerlach and Riederer, 1996; Double *et al.*, 1998) for the specter of iron as a factor in cellular destruction may be deduced from indications of iron involvement in the generation of ROS (Cohen, 1987). As described by Gerlach *et al.* (Chapter 32), there occurs in the parkinsonian brain a shift of the Fe(II)/Fe(III) ratio in the substantia nigra pars compacta (SNpc) from almost 2:1 (normal brain) to 1:2, accompanied by decreased glutathione reductase and catalase activities, increased SOD activity and decreased content of reduced glutathione (GSH). There are ten-fold increases of lipid hydroperoxides in SNpc of Parkinsonians (Dexter *et al.*, 1994). Clinical evidence implicates iron-accumulation and changes of form in PD and multiple system atrophy patients (Vymazal *et al.*, 1999). Injury to complex I-III demonstrates the role of mitochondrial damage in myocardial Fe toxicity. Link *et al.* (1999) induced iron loading by 24-hour incubation with 0.36 mM ferric ammonium citrate that caused decreased activity of complex I+III, complex II, complex III and complex IV. Inactivation of the respiratory transport chain was clearly a specific effect of iron toxicity since there was a complete restoration of the enzyme activities by *in vitro* iron chelation therapy. Sequential treatment with iron and doxorubicin caused a loss of complex I+III and complex II+III activity. Cardoso *et al.* (1999) indicated that the inhibition of the mitochondrial respiratory chain enzyme complexes was restored by antioxidants to different degrees. Indications of increased Fe(III) in SNpc of PD brains suggested iron-melanin interaction in oxidative neuronal damage, through accumulation of Fe(III) and continuous production of cytotoxic species (Bridelli *et al.*, 1999; Kienzl *et al.*, 1999). It has been demonstrated that melanin formed from DA chelates iron and inhibits radical formation (Youdim, 1994), and is accumulated within DA neurons of the SNpc (Jellinger *et al.*, 1992). Finally, Kropf *et al.* (1998) used X-ray absorption fine-structure spectroscopy to demonstrate the local environment of the iron site in natural (human) neuromelanin extracted from SN.

The toxic potency of glutamic acid underlying cell death and both acute and chronic neurodegenerative disorders has been addressed comprehensively by Olney (1990). Jhamandas *et al.* (Chapter 29) have reviewed and investigated the excitotoxic effects of quinolinic acid (Schwarcz *et al.*, 1983), which was implicated in the pathology of both neurodegenerative (Beal *et al.*, 1986) and inflammatory disorders (Heyes, 1993). In reviewing and analysing the cholinergic hypothesis of the cognitive deficits in AD, Beninger *et al.* (Chapter 17) utilized the differential sensitivity of cholinergic neurons of the basal forebrain nucleus basalis magnocellularis projecting to cortical and amygdaloid sites to different excitotoxins (*e.g.*, ibotenic acid versus quisqualic acid). They have applied the evidence from earlier studies indicating that the excitotoxins, quinolinic acid, quisqualic acid, ibotenic acid, α-amino-3-hydroxy-4 isoxazole propionic acid (AMPA) and NMDA applied to the nucleus basalis magnocellularis differentially affected cortical and amygdaloid choline acetyl transferase (Boegman *et al.*, 1992); functional concomitants have been described also (Mallet *et al.*, 1995).

26.3. Apoptosis due to mitochondrial oxidative stress

Recently, several neurodegenerative diseases have been linked directly to mitochondrial impairment, including HD, PD, hereditary spastic paraplegia and Friedreich's ataxia (see review by Schipper, 1999); the mitochondrial abnormalities thus exposed may converge on the function of the mitochondrion in apoptosis. Friedrich's ataxia,

the most common inherited ataxia, is well characterised as being caused by an expansion of a GAA repeat in a previously unknown gene which is encoded on chromosome 9. The GAA expansions lead to impaired transcription of the mitochondrial protein, frataxin, deficiency of which in turn is associated with an accumulation of iron within the cytoplasmic mitochondria. Frataxin has been found to be directly involved in iron-transport out of the mitochondria. Disruptions of a frataxin homolog in yeast causes increased sensitivity to oxidant stress and increased mitochondrial iron and respiration deficiency. This may lead then to oxidative damage and the evidence suggests that Friedreich's ataxia is a disease of mitochondrial oxidative stress. It has been demonstrated that enzymes containing iron-sulphur clusters, *e.g.*, aconitase, complex I and complex II and III of the electron transport chain, are particularly susceptible (Gelman, 1995); these enzymes are markedly decreased in endomyocardial biopsies from patients suffering from Friedreich's ataxia (Zouari *et al.*, 1998; Delatycki *et al.*, 1999). A clinical cardiomyopathy study in the disorder may be illustrative: Rustin *et al.* (1999) studied mechanisms of iron-induced injury in patients with Friedreich's ataxia. Fe^{2+}, but not Fe^{3+}, decreased complex II activity and increased lipoperoxidation in heart homogenate. Some of the iron-induced adverse effects were enhanced by ascorbate/deferoxamine whereas the scavenging agent, idebenone, gave protective effects. These data point to potentially harmful effects of iron chelators and antioxidant drugs. The fibroblasts taken from cell cultures of patients with Friedreich's ataxia were hypersensitive to iron-stress and significantly more sensitive to H_2O_2 than those taken from controls (Wong *et al.*, 1999). Evidence exists to implicate mitochondrial dysfunction in hereditary spastic paraplegia in which an autosomal recessive form has been linked to the mitochondrial protein paraplegia (Schapira, 1999), leading to an impaired mitochondrial protein import. In PD, families with inherited disease as an autosomal dominant trait show mutations in the α-synuclein gene (Kruger *et al.*, 1998); mutations in the "parkin" protein gene were found in patients with autosomal recessive juvenile parkinsonism (Kitada *et al.*, 1998). Finally, it has also been reported recently that the deafness dystonia syndrome may be linked to a defect in mitochondrial protein import (Koehler *et al.*, 1999; Wallace and Murdoch, 1999); the disorder involves the existence of a distinct neurodegenerative syndrome principally characterized by early-onset deafness and progressive dystonia (Hayes *et al.*, 1998).

26.4. Applications of transgenic mouse models

A major advance in the three disorders, ALS, HD and AD, has been the evolution and development of transgenic mouse strain models of the disease states. Hodgson *et al.* (1999) produced yeast artificial chromosome (YAC) transgenic mice expressing normal (YAC 18) and mutant (YAC46 and YAC72) huntingtin in a developmental and tissue specific manner identical to that observed in HD. At one year, YAC72 mice demonstrate selective degeneration of medium spiny neurons in the lateral striatum, associated with the translocation of N-terminal huntingtin fragments. The initial neuronal cytoplasmic toxicity of these mice is followed by the cleavage of huntingtin, nuclear translocation of huntingtin N-terminal fragments and selective neurodegeneration (Hodgson *et al.*, 1999). Furthermore, mice that over-express certain free radical scavenging enzymes show prolongation of survival when crossed with transgenic ALS mice (Reinholz *et al.*, 1999). An involvement of energy metabolism in transgenic mouse models of ALS and HD has been established (Reinholz *et al.*, 1999). Thus, dominant mutations in the copper/zinc SOD (SOD1) gene have been observed in 15-20% of familial ALS (fALS). In the

mouse model of fALS over-expression of a fALS-linked Cu/Zn-SOD mutation leads to progressive MN loss and a clinical phenotype remarkably similar to that of human ALS patients (Kruman *et al.*, 1999). Using mice transfected with the normal human SOD1 gene and normal mice, Liu *et al.* (1999) showed that levels of H_2O_2 and .OH radical were significantly higher and that $O_2^{\cdot-}$ was lower in ALS mutant mice than in normals. In the transgenic ALS mice, there is a marked vacuolation of mitochondrial which directly correlates with the onset of a rapid phase of motor weakness; multiple lines of transgenic mice were generated expressing G86R mutant SOD1 restricted to astrocytes (Gong *et al.*, 2000). With maturation, significant hypertrophy and increased glial fibrillary acidic protein (GFAP) reactivity was evidenced in GFAP-m SOD1 mice whereas GFAP-mutant SOD1 transgenic mice developed normally and did not show spontaneous motor deficits with increasing age (Gong *et al.*, 2000). It has been demonstrated that mice deficient in copper maintain a retarded disorder progression in models of ALS (Ferrante *et al.*, 1997). In HD, it has been demonstrated that there is a marked depletion of NMDA, as well as increases in lactate in the brains of these mice, as assessed by magnetic resonance spectroscopy (Jenkins *et al.*, 1998). Although functional analyses are limited, Lione *et al.* (1999) studied the progressive cognitive decline through expression of the human HD mutation in mice (R6/2) which induces a progressive neurological phenotype with HD motor symptoms. Between 3.5 and 8 weeks-of-age, R6/2 mice showed progressive declines in specific aspects of learning in the circular water maze, visual cliff, two-choice swim tank and T-maze procedures. Similar to human HD patients, R6/2 mice develop progressive impairments on learning tasks sensitive to frontostriatal and hippocampal function. Spinocerebellar ataxia type 1, like HD, is caused by an expanded number of CAG repeats in the coding region of the respective gene (Kaemmerer and Low, 1999). Finally, the reciprocal interactions of nervous-immune system mechanics are becoming increasingly evident in neuropathological disease states: Bergquist *et al.* (1998) have demonstrated a model of catecholamine-induced suppression of lymphocytes whereby exposure to high exogenous levels of catecholamines exerted inhibitory effects on proliferation and differentiation within the nucleus (following catecholamine infiltration through the nuclear envelope), concomitant with induction of apoptosis. These authors found too that catecholamines were produced in the cell cytoplasm of normal lymphocytes, a synthesis blocked by α-methyl-p-tyrosine, and suggest that model implicates a plausible pathophysiological network of immune-related mechanisms contributing neuropsychiatrics disorders (Bergquist *et al.*, 1998).

26.5. Agents of neuroprotection

There are a number of neuroprotective, as well as neurorestorative, strategies which are becoming available and they reflect current notions on neuron destruction and protection. The former, neuroprotection, refers to the potential for reinforced defence against cell death (as assessed through diverse markers, such as neurotransmitter depletion, tyrosine hydroxylase, or dopamine-β-hydroxylase activity) whereas the latter, neurorestoration, refers to the transient recovery of functional variables, or symptoms, to levels comparable with pre-insult or 'steady-state' levels (as assessed by symptomatic markers, such as motor activity or learning performance). The parameters of the onset and course of the neuropathology of PD in relation to neuroprotective propensities is recipient of much current attention (Gerlach and Riederer, 1999). There is a large body of evidence implicating activation of glutamate receptors in contributing to cell death in a variety of neurodegenerative diseases (*e.g.*, Garthwaite and Garthwaite, 1987, 1989), bearing in mind that Ca^{2+} is all-important in mediating the transition to neuronal death in

NMDA neurotoxicity (Meldrum and Garthwaite, 1990). One potential avenue of drug therapy has been therefore to utilize excitatory amino acid receptor antagonists (Sonsalla *et al.*, 1989), although the neurotoxic potential of this class of compounds has been reviewed at some length (Olney *et al.*, 1991; Ellison, 1995). There are also antagonists which act at the glycine site of the NMDA receptor (Johnson and Ascher, 1987). However, the interactions between glutamatergic mechanisms mediated by receptors of the ionotropic and metabotropic classes in the brain are yet to be understood, particularly with regard to presynaptic *in vivo* functional expressions (Manahan-Vaughan *et al.*, 1999). Nevertheless, agonists of metabotropic type-2 or type-3 glutamate receptors which inhibit glutamate release (Wang *et al.*, 1999), as well as metabotropic glutamate receptor non-selective agonist [(1S, 3R)ACPD] and group I selective agonist (DHPG), demonstrated differential and complex anti-apoptotic actions (Kalda *et al.*, 2000). Other compounds, such as riluzole, also appear to block glutamate release. These compounds have demonstrated some degree of efficacy in a variety of models of neurodegenerative diseases. Danysz *et al.* (Chapter 28) describe comprehensively the actions of memantine, glutamate antagonist, in improving symptoms (*e.g.*, cognition) and retarding disease progression by assuming the physiological function of magnesium. Additionally, Hansson *et al.* (1999) have shown that transgenic HD mice, expressing the exon 1 of a human HD gene with an expanded CAG repeat (line R6/1), possessed remarkable neuroprotection against intrastriatal infusions of quinolinic acid, NMDA agonist. The presence of exon 1 of the mutant HD gene caused profound changes to striatal neurons by imparting a resistence to excessive NMDA receptor activation. Finally, in a transgenic model of spinocerebellar ataxia type 1, cerebellar grafting was carried out, whereby the mice were transplanted as the ataxic phenotype was becoming evident. Grafted mice showed better performance on tests of cerebellar function than sham-operated litter mates (Kaemmerer and Low, 1999). The results showed that the transplants may offer functional benefits and that the grafts could survive over long terms despite an ongoing pathological process.

The role of iron chelators in ROS damage has been illustrated by protectant effects of deferoxamine against functional and neurochemical damage by 6-hydroxydopamine (Ben-Shachar *et al.*, 1991). As indicated by Gerlach *et al.* (Chapter 32), since deferoxamine does not cross the blood-brain barrier, the "scavestrogens" J811 and J861, potent inhibitors of Fe-induced cell damage (Romer *et al.*, 1997) *in vitro* have received attention. In this regard, Gerlach *et al.* (Chapter 32) describe the putative protectant effects of monoamine oxidase-B (MAO-B) inhibitors, as seen from their actions against catecholamine neurotoxins (Gerlach *et al.*, 1992). It should be mentioned too that aspirin and salicylic acid gave some protection against DA depletions caused by of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice (Aubin *et al.*, 1998).

In AD, conditions affecting the deposition of β-amyloids, *e.g.*, ionic balance (Pike *et al.*, 1996) in the pathogenesis of the disease are critical to an understanding of its symptoms (Cummings and Kaufer, 1996) and progression (Knopman *et al.*, 1996). The paper by Nordberg (Chapter 34) outlines different classes of compounds with promise for a neuroprotective action in AD: (i) the cholinesterase inhibitors, *e.g.*, tacrine with preclinical efficacy (Svensson and Nordberg, 1998), (ii) nicotinic agonists, *e.g.*, nicotine (Zamani *et al.*, 1997), (iii) estrogens (Behl and Holsboer, 1999), (iv) the antioxidants, (v) anti-inflammatory agents (putative), (vi) anti-amyloidal substances that hasten removal of β-amyloid and retard its aggregation, and finally (vi) the neuroprotective neurotrophins. In the laboratory, Hefti *et al.* (1989) showed that the cognitive disruptions induced by cholinergic lesions were counteracted by neurotrophins. Using primary rat microglial cell cultures,

Rupniak *et al.* (Chapter 6) have described the effects of neuroprotectants on activation of microglial cells: *e.g.*, of the salen-manganese antioxidants, EUK8, EUK134 and EUK189 indicated significant SOD activity, the latter two catalase activity. These compounds were studied too, for effects on neuronal cells in culture of SIN-1, which is highly toxic to primary rat hippocampal neurons in culture.

NOS inhibitors offer another avenue of potentially efficacious therapeutic agents (Hantraye *et al.*, 1996; Chabrier *et al.*, 1999). It has been shown that inhibitors of nNOS are highly effective against mitochondrial toxin models of both PD and HD, including the MPTP model of PD (Schulz *et al.*, 1995) and the 3-nitropropionic acid model of HD (Schulz *et al.*, 1996). A variety of other free radical scavengers also show efficacy in these models, including free radical spin-trapping compounds and other antioxidants. The paper by Chiueh *et al.* (Chapter 31) outlines these neuroprotective interventions in PD: antioxidant scavengers, energizers, neurotrophins, hypothermia therapy, Ca^{2+} / Fe^{2+} antagonists, protease inhibitors, brain repair transplants and gene induction or therapy. De Yebenes and Mena (Chapter 35) describe neurotrophins enhancing neuronal survival and differentiation in degenerative disorders by highlighting the discrepancy between laboratory and clinical findings; these included in ALS, ciliary derived neurotrophic factor (CNTF) and brain derived neurotrophic factor (BDNF), in AD and peripheral neuropathy, NGF, and in PD, glial derived neurotrophic factor (GDNF).

Free radical scavenging and antioxidant properties have been assigned also to melatonin regarding ischemia-reperfusion, glutathione depletion, cyanide poisoning, and excitotoxicity induced by kainic acid (Giusti *et al.*, 1996; Reiter *et al.*, 1997; Floreani *et al.*, 1997). Though pharmacological concentrations of the compound protect against free radical damage, physiological levels of melatonin were beneficial against oxidative stress (Pozo *et al.*, 1994; Melchiorri *et al.*, 1995; Pappolla *et al.*, 1997). Several other neuroprotectant properies have been documented, including prevention of neuronal apoptosis triggered by ROS, brain injury by singlet oxygen (Cagnoli *et al.*, 1995; Knopf *et al.*, 1998), brain ischemia (Cho *et al.*, 1997), MPTP-induced lesions and methamphetamine-induced dopaminergic neurotoxicity (Acuña-Castroviejo *et al.*, 1997; Syed *et al.*, 1999), paraquat toxicity (Melchiorri *et al.*, 1996), NO-induced peroxidation (Escames *et al.*, 1997) and antioxidant enzyme expression (Antolin *et al.*, 1996). Thus, in highlighting the widespread neuroprotective applications of melatonin, Skaper *et al.* (1999) raise the issue of the sufficiency of endogenous productions of the compound under varying conditions of health, a consideration of broad consequence.

Several other promising strategies for protection may be reviewed briefly:

I. Inhibitors of the enzyme poly-ADP-ribose polymerase

Poly-ADP-ribose polymerase is activated whenever DNA damage occurs. It then leads to a depletion of both NADH and ATP concentrations which are involved in generating large poly ADP ribose complexes. Thus, it has been shown that mice with a knock-out of poly-ADP-ribose polymerase are highly resistent to focal ischemia as well as the neurotoxic effects of MPTP. Interestingly, a number of highly potent inhibitors of poly-ADP-ribose polymerase have demonstrated an efficacy in animal models of neurologic diseases.

II. Inhibition of apoptotic cell death

It has been demonstrated that mice which over-express Bcl-2 are resistent to both 3-nitro propionic acid and MPTP-induced neurodegeneration. Crossing these mice into

transgenic mouse models of ALS prolongs survival rate. Bcl-2, an oncogene protein (Merry and Korsmeyer, 1997), protects cells from apoptosis (Tatton *et al.*, 1997). Furthermore, Vukosavic *et al.* (1999) showed that over-expression of Bcl-2 attenuates the neurodegeneration produced by the fALS-linked SODI mutant G93A (mSODI). Mice possessing a dominant negative inhibitor of caspase 1, when crossed into both ALS and HD transgenic mouse models, show a marked improvement in survival rate. Caspase inhibitors that may prove efficacious in degenerative disorders are under development. Protease inhibitors that inhibit apoptosis (Kinloch *et al.*, 1999) implicate the role of protease activation in cell death.

III. Agents that may act on mitochondrial permeability transition

A pore in the inter mitochondrial membrane has been implicated in both excitotoxic and apoptotic cell death. Cyclosporine inhibits the actions at this pore site. Cyclosporine can block several different forms of cell death *in vitro* as well as hypoglycemic and ischemic cell death *in vivo*. A number of other agents are under synthesis that also may stabilise mitochondrial permeability. Some of these are analogues of L-deprenyl, which itself has shown both neuroprotective and restorative effects (see Archer and Fredriksson, Chapter 33).

IV. Anti-excitotoxin acting compounds

Agents that increase brain kynurenic acid levels both induce anticonvulsant (Connick *et al.*, 1992) and antineurodegenerative effects (Miranda *et al.*, 1999). Jhamandas *et al.* (Chapter 29) review the endogenous anti-excitotoxic effects of kynurenic acid (Boegman *et al.*, 1985), *e.g.*, in impairing the neurotoxicity induced by excitatory amino acid receptor activation (Salvati *et al.*, 1999) and its protectant neurochemical and functional effects (Wirsching *et al.*, 1989), as well as the endogenous neuroprotection offered by enzyme inhibitors, *e.g.*, nicotinylalanine, that were found to have neuroprotectant properties, and nitroglycerin (Kard *et al.*, 1998). Harris *et al.* (1998) showed that treatments enhancing endogenous brain kynurenic acid protected against the neurotoxic effects of quinolinic acid.

V. Application of neurotrophins

Levi-Montalcini (1987) described the critical role of nerve growth factor (NGF) for neuronal development and maintainance. Neurotrophins also have been shown to exert neuroprotective effects in different animal models (Alexi *et al.*, 1997; Connor and Dragunow, 1998) and in PD patients (Lopez-Lozano *et al.*, 1996). GDNF is particularly effective against MPTP neurotoxicity (Gash *et al.*, 1998), it enhances the survival of midbrain dopaminergic neurons *in vitro* and rescues degenerating neurons *in vivo* (Lapchak *et al.*, 1997). Among the profusion of therapeutic approaches instigated by the promise of the neurotrophins, some involve gene therapy techniques.

VI. Immunophilins

Immunophilins are small molecules which seem to possess growth factor-like effects and which readily cross the blood-brain barrier. Immunophilin ligands, *e.g.*, FK-506, FKBP-12 and GPI-1046, combine with immunophilin receptors to inhibit the calcium-

activated phosphatase, calcineurin. These molecules, *e.g.*, GPI-1046, have been found to be effective against the neurodegenerative effects of MPTP or 6-OHDA on surviving nigrostriatal neurons (Steiner *et al.*, 1997); FK-506 protected against DA loss after MPTP (Kitamura *et al.*, 1994). Both neurotrophins and immunophilins may exert also neuro-restorative effects following neuronal injury, an area of current intense proliferation.

VII. Agents influencing HO-1 expression and iron sequestration in aging astroglia

Stress-induced up-regulation of HO-1 in astroglia may be implicated in the abnormal patterns of brain iron deposition and mitochondrial insufficiency underlying AD and PD (Schipper, 1996; Schipper *et al.*, 1995, 1998, 1999). These studies demonstrated that HO-1 was significantly over-expressed in neurons and astrocytes of Alzheimer-diseased hippocampus and cerebral cortex relative to control brains. It has been argued that intracellular degradation of pro-oxidant heme to bile pigments with antioxidant properties, *e.g.*, biliverdin and bilirubin, may provide a restoration of the redox state under conditions of oxidative stress (Stocker *et al.*, 1987; Applegate *et al.*, 1991; Doré *et al.*, 1999).

VIII. Improvements in mitochondrial energy production or buffering

In considering creatine as a treatment of neurodegenerative disorders, two beneficial effects are known: (i) creatine can increase phosphocreatine levels in regions of the brain and spinal cord; and (ii) creatine can inhibit activation of the permeability transition pore. Thus, the compound is effective in preventing neuronal degeneration induced by both MPTP (Matthews *et al.*, 1999) and 3-nitropropionic acid (Docherty *et al.*, 1999). It has been demonstrated also that creatine dose-dependently improves the survival rate of transgenic ALS mice (Kalra *et al.*, 1999; Klivenyi *et al.*, 1999), and prevents neuronal degeneration of the anterior horn motor neurons in the model. Investigations of coenzyme Q10, which has the ability to increase ATP production as well as acting as a potent antioxidant, particularly in lipophilic membranes, have shown a neuroprotective propensity against both MPTP, malonate and 3-nitropropionic acid induced toxicity (Beal *et al.*, 1994). Coenzyme Q10 can extend also the survival rate of transgenic ALS mice; and oral administration reduced significantly the elevated lactate levels in HD patients (Beal, 1999). Other compounds, *e.g.*, lamotrigine and MK-801 (Lee *et al.*, 2000) and L-carnitine (Binienda *et al.*, 1999) possess neuroprotective efficacy against the toxicity of 3-nitropropionic acid. Pramipexole too (see below) inhibits the permeability transition pore induced by 1-methyl-4-pyridinium ion (MPP^+)(Cassarino *et al.*, 1998).

IX. Dopaminemimetics with differential affinities for dopamine receptors subtypes

The effects of these compounds is directly upon dopamine receptors without previous enzymatic biotransformation and is over-and-above a direct antiparkinsonian effect on symptoms, as outlined by Grandas (Chapter 36) from clinical investigations. Apomorphine, the mixed D_1- D_2 agonist, with established neuroprotectant effects is a potent iron chelator, free radical scavenger and inhibitor of membrane lipid peroxidation *in vitro*, *in vivo* and in PC12 cell culture. The neuroprotectant potency of the compound has been compared with deferoxamine, DA, nifedipine, and the D_2 agonists: bromocriptine, lisuride, pergolide and pramipexole (Youdim *et al.*, 1999). In the laboratory, the neurorestorative of bromocriptine, D_2 agonist, by itself or in combination with the D_1

agonist SKF 38393 [(+/-)-1-phenyl-2, 3, 4, 5-tetrahydro-[1H]-3-benzazepine-7, 8-diol], but not SKF 38393 by itself, were shown in hypokinetic MPTP-treated mice (Fredriksson *et al.*, 1994). The neuroprotective potential of different dopamine D_2 agonists is the focus of recent attention (*e.g.*, Sethy *et al.*, 1997; Sawada *et al.*, 1998). The efficacy of several different DA agonists is reviewed also by Gerlach *et al.* (Chapter 32). For example, both pramipexole (Cassarino *et al.*, 1998) and pergolide (Opacka-Juffry *et al.*, 1998) reduced hydroxyl radical generation, following 6-hydroxydopamine (6-OHDA) or MPP^+, in the rat striatum, and the protectant scavenging effects of apomorphine and enantiomers against MPTP/6-OHDA/H_2O_2 induced oxidative stress were shown (Gassen *et al.*, 1996, 1998; Grunblatt *et al.*, 1999).

X. CREB transcriptional regulation

One mechanism to expain the selective death of neurons has been the differential activation of an internal termination program aimed at vulnerable nerve cells (Schreiber and Baudry, 1995). In exploring the regulation of cell survival, efforts to identify the key mediators of a 'survival cascade' have concentrated upon endogenous neuroprotective messengers, *e.g.*, neurotrophic factors and various cytokines (Mattson, 1997), as well as transcription factors (Dragunow and Preston, 1995; Watters and Dorsa, 1998). Recently, Walton and Dragunow (2000) have demonstrated that while a range of different molecules modulate the survival of neurons in the brain, the protective actions of the transcription factor cAMP-response-element-binding protein (CREB) are necessary. The activation of CREB, crucially dependent on phosphorylation of Ser133 by protein kinase A [PKA] (Gonzalez and Montminy, 1989), occurs in brain damage resistent dentate granule cells in the hippocampus, triggered by neuroprotective environmental stimulation. Since the Akt neuroprotective signalling pathway activates CREB, followed by CREB synthesis and phosphorylation, *in vitro*, CREB is implicated in programmed neuronal survival.

26.6. Conclusions

Mechanisms of neuronal cell death in apoptosis and necrosis are examined. Neurotoxic processes underlying cellular destruction may involve N-methyl-D-aspartate (NMDA) receptor activation and /or activation of neuronal nitric oxide synthase but the depletion of energy and generation of free radicals appears to be critical. In Alzheimer's disease the damaging effects of peroxynitrite and exposure to β-amyloid peptide is evident. Mitochondrial dysfunction is involved in several neurodegenerative diseases including Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease as well as Alzheimer's disease and in these disorders the innovations offered by techniques ranging from transgenic mouse models of the disorder to cell culture preparations are remarkable. Agents of neuroprotection and neurorestoration possess either characteristics specific to particular disorders or have a general applicability or both. The vast array of agents available are for the most part the objectives of laboratory examinations but an increasing selection of compounds are reaching the clinical necessities thereby influencing current strategic notions to modify tactical contingencies. Among the agents listed are included: inhibitors of the enzyme poly-ADP-ribose polymerase, inhibition of apoptotic cell death, agents acting on mitochondrial permeability transition, excitatory amino acid antagonists, applications of neurotrophins, immunophilins, agents influencing heme oxygensase-1 (HO-

1) expression and iron sequestration in aging astroglia, improvements in mitochondrial energy production or buffering, and finally dopaminemimetics with differential affinities for dopamine receptors.

Thus, the apparently unrestricted proliferation of novel therapeutic strategies for treatment of neurodegenerative diseases is both modulated and constrained by the elder classes of compounds that have stimulated identification of enitities critical for the understanding of apoptotic mechanisms. Many of these agents have been absorbed into the pragmatic realities of the clinical endeavours. The determination of which of these strategies eventually attain acceptable efficacy in slowing/halting disease progression will depend upon an accurate description of the underlying cause of neuronal cell death.

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