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Reviews

Towards an understanding of the role of glutamate in neurodegenerative disorders: energy metabolism and neuropathology

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Abstract. It is thought that impairment of energy metabolism that results in deterioration of membrane function, leading to loss of the Mg^{2+} block on NMDA receptors, and allowing persistent activation of these receptors by glutamate, might be a cause of neuronal death in neurodegenerative disorders. Studies in rodents using mitochondrial respiratory chain toxins, such as aminooxyacetic acid, 1-methyl-4-phenylpyridinium, malonic acid and 3-nitropropionic acid, suggest that such processes may indeed be involved in neurotoxicity. Striatal and nigral degeneration induced by mitochondrial toxins in rodents resembles the neuropathology seen in humans suffering from Huntington's or Parkinson's disease, and can be prevented either by decortication or by NMDA receptor antagonists. Such experimental observations suggest that glutamate may be involved in neuronal death leading to neurodegenerative disorders in humans. If so, glutamate antagonists may offer a therapeutic approach for retarding the progression of these disabling disorders.
Key words. Aminooxyacetic acid; 1-methyl-4-phenylpyridinium ion; malonic acid; 3-nitropropionic acid; rotenone; sodium azide; nitric oxide; N-methyl-D-aspartate; oxidative phosphorylation; calcium; cell death.

Mitochondrial energy metabolism and the concept of delayed and slow onset neurotoxicity – Where does it come from?

In vitro

In 1988 Henneberry and his associates[58] reported that impairment of energy metabolism in cell cultures increases vulnerability of neurons to the toxic action of excitatory amino acids and demonstrated that NMDA-gated ion channels are significantly involved. Subsequently, Zeevalk and Nicklas[92], experimenting with cultured chick embryo retina, showed that compromising the neuronal energy supply with cyanide triggers excitotoxic lesions sensitive to NMDA antagonists. Consistent with these observations was the demonstration that toxicity induced by metabolic inhibition can be mimicked by membrane depolarization with potassium or by relieving the Mg^{2+} block of the NMDA receptor[93].

In vivo

In 1989 we reported[86,88,89] that aminooxyacetic acid (AOAA), a non-selective inhibitor of transaminases, induces axon-sparing lesions in the rat striatum which can be blocked by NMDA antagonists and by prior decortication. The behavioral consequences of such lesions were similar to those reported following striatal lesions caused by quinolinate in rodents[89], a well-established and widely accepted animal model of Huntington's disease.

Two different facts led to the investigation of the neurotoxic potential of AOAA[87]. The first was the evidence that AOAA blocks in vitro kynurenate transaminase activity and decreases the endogenous concentrations of the glutamate antagonist kynurenic acid[70]. The second was the fact that AOAA blocks the malate-aspartate shunt across mitochondrial membranes, which leads to an inadequate supply of mitochondrial NADH in reducing equivalents for oxygenation[6,90]. The breakthrough in understanding the mechanisms involved in AOAA toxicity was achieved when data on the nigral neurotoxicity of the 1-methyl-4-phenylpyridinium ion (MPP^+; active metabolite of the parkinsonism-inducing compound MPTP) and the protective action of NMDA antagonists against it were published[82].

There is no a priori reason for a relationship between the actions of AOAA and MPP^+. However, it has been recognized that MPP^+ is a specific and irreversible mitochondrial complex I toxin[18,54]. AOAA, which inhibits the malate-aspartate shunt in the mitochondria, may indirectly produce transient but very similar effects to those of MPP^+ on the mitochondrial respiratory chain.

This inference has been experimentally confirmed by Beal and his associates[8] and by Schwarcz and his coworkers[49]. Beal et al.[8] have initially extended our obser-

vations on AOAA toxicity in the rat striatum, showing the similarity of its anatomical profile to that of quinolinate-induced lesions (sparing NADPH-diaphorase-containing neurons). They also demonstrated an inability of AOAA to activate NMDA receptors in electrophysiological tests[8]. Furthermore, these authors provided evidence that AOAA concentrations necessary to trigger striatal lesions do not significantly affect kynurenate transaminase activity in the rat brain[8]. Schwarcz and his associates[49] have shown that AOAA damages rat hippocampus with a pathological profile resembling that of quinolinate, and provided electrophysiological evidence that AOAA does not directly activate NMDA receptors on hippocampal pyramidal cells. Subsequently, it has been demonstrated that MPP^+ can induce striatal lesions with morphological and pharmacological profiles resembling lesions induced by AOAA[78]. Striatal lesions induced by MPP^+ in rats, as well as those triggered by AOAA in striatum or in hippocampus, were found to be sensitive to NMDA antagonists[8,49,79,89] and to depend upon intact corticofugal innervation[8,79,89]. Finally, it was demonstrated that AOAA may induce nigral lesions that are sensitive to NMDA antagonists[85].

Furthermore, 3-nitropropionic acid (3-NP), which irreversibly blocks succinate dehydrogenase (complex II), has been reported to be a selective striatal neurotoxin in rodents provided it is given to rats in sufficiently low doses and over period of weeks[3,12]. Chronic systemic administration of 3-NP to rats for as long as 1 month leads to low grade metabolic disturbances in the striatum and subsequently to striatal neuron damage (sparing NADPH-diaphorase containing neurons). This effect is blocked by prior decortication[3].

Malonic acid (MA), which reversibly inhibits succinate dehydrogenase (complex II), produces excitotoxic lesions in rat striatum that can be blocked by NMDA antagonists[4,24], glutamate release inhibitors[4], and prior decortication[4].

These observations supported the hypothesis that disturbances in mitochondrial energy metabolism can lead to axon-sparing glutamate-like toxicity in the brain and that such processes may be involved in the pathogenesis of chronic neurodegenerative disorders in humans.

Mitochondrial respiratory chain – How does it work?

Mitochondria supply cells, including neurons, with energy[69,90]. In the mitochondrial matrix energy carriers such as $NADH_2$ and $FADH_2$ are formed during the Krebs cycle. $NADH_2$ and $FADH_2$ donate electrons necessary for the generation of an electrochemical gradient across the inner membrane of the mitochondrion and thus for the synthesis of ATP. The enzymes responsible for the transport of electrons are located in the inner mitochondrial membrane. Two very important

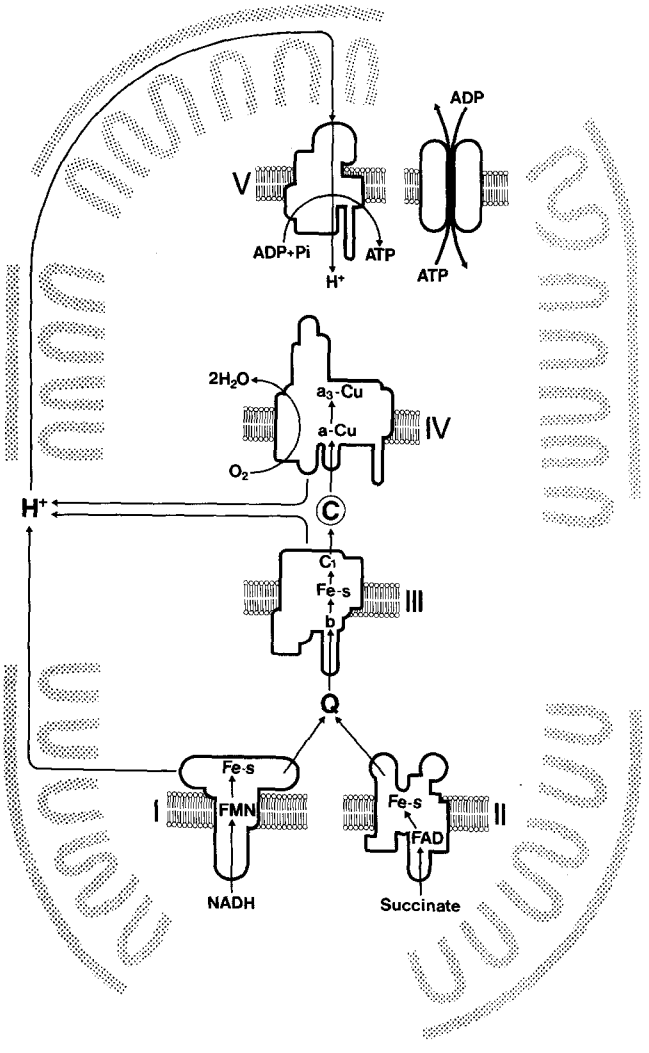

Figure 1. Schematic diagram of mitochondrial electron transport complexes. NADH, nicotinamide adenine dinucleotide-reduced; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; Fe-s, iron-sulphur center; Q, coenzyme Q; a, a_3, b, C, C_1, cytochromes.

members of this highly organized and hierarchical enzyme system are NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II) (fig. 1). The electrons entering the system are transported via flavin mononucleotides (FMN) and multiple iron-sulphur centers of complex I or II and passed to ubiquinone (coenzyme Q; CoQ). The electrons are then transported to the ubiquinol:cytochrome c oxidoreductase (complex III), containing cytochromes b, Rieske-iron-sulphur protein and cytochrome c_1. From complex III electrons are transferred via cytochrome c to cytochrome c oxidase (complex IV) containing cytochromes a and a_3 equipped with copper atoms and finally passed on to oxygen (fig. 1). As electrons travel through complexes I/II, III and IV, protons cross the inner mitochondrial membrane, creating an electrochemical gradient. This energy is used by ATP synthase (complex V) for the synthesis of ATP

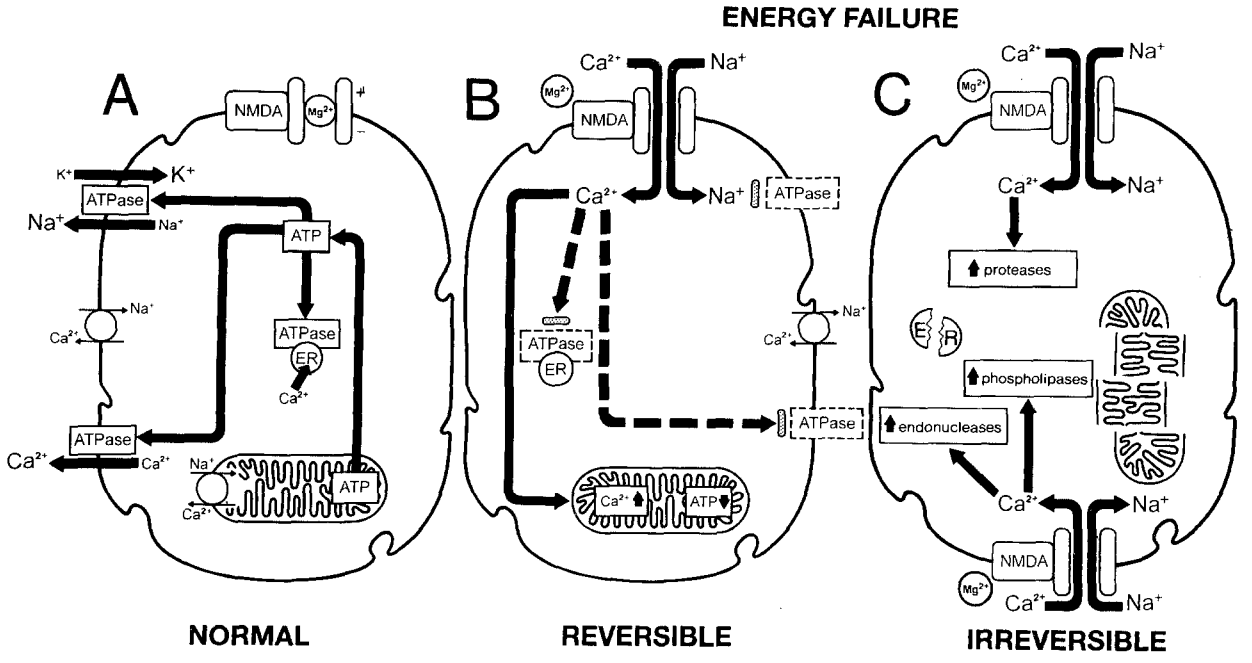

Figure 2. Schematic diagram of the excitotoxic mechanisms triggered by energy failure in neurons. *A* Energy metabolism under physiological conditions. *B* Reversible disturbances in energy metabolism and cell function triggered by energy failure. *C* Irreversible changes induced by sustained energy failure. ER, endoplasmic reticulum; NMDA, N-methyl-D-aspartate; ATPase, phosphatase.

(fig. 1). The adenine nucleotide translocator then exchanges ATP for cytosolic ADP (fig. 1).

Sites of action of mitochondrial toxins

AOAA prevents electron-supply to mitochondrial NADH, which leads indirectly to a relative insufficiency of complex I since reducing equivalents necessary for oxygenation do not enter the respiratory chain[8]. MPP^+ is a specific complex I toxin blocking one of the two major pathways for electrons entering the mitochondrial respiratory chain[18,54]. 3-NP is an inhibitor of the activity of complex II as is MA, and therefore both block another entry point for electrons into the mitochondrial respiratory chain[2,4,23,24,43]. The obvious consequence of the action of all four toxins is inadequate electron supply to CoQ and inhibition of its reduction to ubiquinol, which immediately leads to major dysfunction of complexes III and IV and loss of the electrochemical gradient across the inner mitochondrial membrane. Consequently, the energy-dependent synthesis of ATP is reduced and may cease completely. Such major changes in mitochondrial energy metabolism lead to disturbances of the functions of the entire cell and may result in cell death if the toxic insult persists.

How can energy failure trigger excitotoxic brain damage?

Chronic poisoning of oxidative phosphorylation disturbs the function of the mitochondrial respiratory chain (fig. 1). The resulting intracellular energy deprivation causes reduction of ATP stores in mitochondria and in the cytoplasm. This in turn interferes with Na^+/K^+-ATPase activity leading to disturbances in the maintenance of the membrane potential (figs 2B and 3). Defective function of Na^+/K^+-ATPase retards repolarization of the cell membrane, leading to inappropriate (prolonged) opening of voltage-dependent ion channels (fig. 2B). These initial disturbances will cause mild and reversible increases of the Ca^{2+} concentration in the cytoplasm, lessening the voltage-dependent Mg^{2+} block of NMDA channels (figs 2B and 3). As the number of activated NMDA channels increases, more and more Na^+ and Ca^{2+} will enter the cell (fig. 2B). Excessive elevation of intracellular Na^+ concentration critically disturbs Na^+/Ca^{2+}-exchange function and leads to additional increase in intracellular Ca^{2+} concentration because ATP-dependent Ca^{2+} extrusion is inhibited. Furthermore, the highly energy-dependent mechanism for Ca^{2+} storage in the endoplasmic reticulum fails because of insufficient cell ATP supplies. Similarly, Ca^{2+}-ATPase activity fails and Ca^{2+} accumulation in the cell increases further. At that stage, sustained energy deprivation may rapidly cause membrane depolarization and opening of still inactive NMDA channels (fig. 2C), thus exposing the neuron to the toxic action of endogenous glutamate. The resulting rapid increase in Ca^{2+} and Na^+ concentrations in the cell leads initially to the storage of Ca^{2+} in mitochondria, which further compromises energy supply because Ca^{2+} overload of mitochondria inhibits ATP synthesis[52,55]. The immedi-

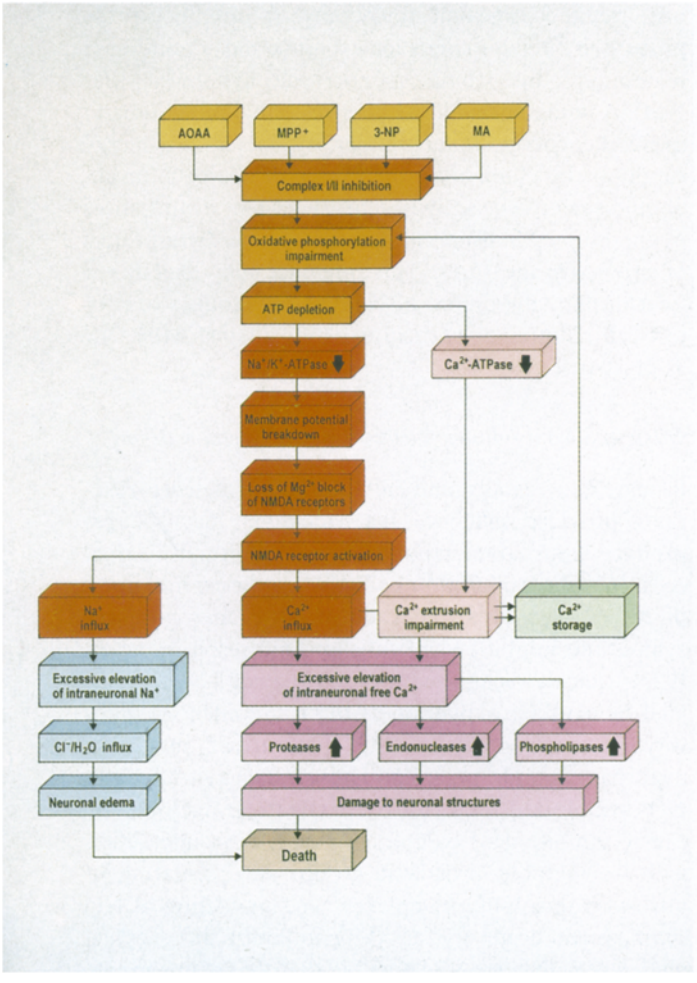

Figure. 3 Schematic diagram illustrating possible sequence of events triggered in neurons by mitochondrial toxins.

ate consequence of Ca^{2+} overload of mitochondria is irreversible blockade of the respiratory chain and energy metabolism leading to activation of mitochondrial phospholipases and mitochondrial damage. This sequence of events leads rapidly to additional elevation of Ca^{2+} in the cytoplasm of energy-deprived neurons, and consequently to self-destructive processes such as activation of phospholipases, endonucleases and proteases (figs 2C and 3). Intracellular influx of Cl^- and H_2O (fig. 3) may also contribute to cell death at the final stages of metabolic disturbance[55, 59].

Experimental evidence

In vivo

The question of whether glutamate receptors are involved in neurotoxicity induced by mitochondrial poisons has been addressed by Greenamyre and his associates using in vivo receptor autoradiography[1]. They show that immediately following intrastriatal administration of neurotoxic doses of MPP^+ in rats, NMDA-dependent ion channels, labeled by ^{125}I-MK 801, are activated (Greenamyre et al., unpublished data). These observations provide direct evidence that compounds which by themselves do not interact with NMDA receptor/ion channel complexes but which block entry pathways for electrons into the mitochondrial respiratory chain (complex I or II) lead to activation of NMDA-dependent ion channels before they elicit neurotoxic effects. These data demonstrate the crucial role of the NMDA receptor in neurotoxicity mediated by MPP^+ in vivo and explain the observations that temporary protection against MPP^+/MPTP and MA toxicity in rodents and primates can be provided by NMDA antagonists (refs[4, 11, 15, 24, 37, 38, 50, 64, 78–83, 94] (but see refs.[36, 75])

Another piece of evidence implying that glutamate-dependent processes are involved in the toxicity of mitochondrial poisons is represented by the data of Beal and associates[79], who demonstrated that intrastriatal administration of MPP^+ in rats leads to nigral damage, which is sensitive to NMDA antagonists. Ikonomidou and Olney showed that damage in the substantia nigra pars compacta following intrastriatal MPP^+ administration in mice is of the axon-sparing type and resembles lesions induced by excitotoxins such as glutamate (unpublished data). A possible link between neurotoxicity triggered by poisoning of oxidative phosphorylation and glutamate is substantiated by observations on the dopaminergic toxicity of a complex I toxin, rotenone, in rodents[26]. As early as 1985, Heikkila and his colleagues postulated that the effects of rotenone and MPP^+ on oxidative phosphorylation in isolated mitochondria and in brain tissue in vivo are similar[26]. More recently, both rotenone (Greenamyre et al., unpublished data) and MPP^+[84] were shown to reduce the threshold for glutamate-mediated toxicity in vivo. Furthermore, metabolic impairment by 3-NP in rat striatum was shown to increase the likelihood of NMDA-induced neuronal death in this region several-fold[73]. Langston and his co-workers[14] demonstrated that impairment of energy metabolism by MPTP causes a selective decrease in glutamate and aspartate concentrations in the striatum and mesencephalon (nigrostriatal pathway) in mice, and concluded that excitatory amino acids may be involved in MPTP toxicity[14]. These observations support the view that changes in energy metabolism may modulate glutamate-mediated toxicity in vivo.

Other experimental evidence linking energy metabolism failure, disturbances in Ca^{2+} homeostasis, and glutamate-mediated toxicity comes from data on the protective action of growth factors against mitochondrial poisons. Basic fibroblast growth factor (bFGF) has a neuroprotective effect against MPTP-induced dopaminergic toxicity in mice[60]. bFGF also protects striatal neurons against NMDA toxicity in vitro[21], and protects neonatal rats against both striatal NMDA

toxicity and hypoxia-ischemia[57]. Moreover, bFGF protects gerbils against cerebral ischemia[53]. In vitro, bFGF prevents disturbances in Ca^{2+} homeostasis, prevents loss of mitochondrial transmembrane potential, and protects hippocampal neurons against hypoglycemia[48]. These observations suggest that growth factors may protect both against NMDA-mediated toxicity and against toxicity resulting from poisoning oxidative phosphorylation. Reduction of disturbances in Ca^{2+} homeostasis may explain the neuroprotective action of growth factors[57].

Protection by gangliosides against both NMDA and mitochondrial poison toxicity offers additional evidence linking the processes. Monosialoganglioside G_{M1} and semisynthetic sphingolipid II^{3}Nen-5-Ac-GgOse$_4$-2D-erythro-1,3-dihydroxy-2-dichloro-acetyl-amide-4-*trans*-octadecene (LIGA 20) protect mice[25,67] and primates[66] against MPTP toxicity. G_{M1} and LIGA 20 reduce NMDA toxicity in neonatal rats[40], protect rodents against cerebral ischemia[35] and trauma[46], and reduce glutamate and NMDA toxicity in vitro[45,46]. The neuroprotective effect of gangliosides is due to prevention of glutamate-induced disturbances in Ca^{2+} homeostasis rather than to interaction with glutamate receptors[46]. It is suggested that gangliosides act by modulating the function of the calmodulin-dependent Ca^{2+}–ATPase pump (fig. 2A) and therefore modulate intracellular Ca^{2+} concentration[29].

Altogether, it may be concluded that modulation Ca^{2+} homeostasis in the cell is crucial for the protective action of NMDA antagonists, growth factors and gangliosides against the toxicity of glutamate and that of mitochondrial poisons.

In vitro

The in vitro experiments performed on primary cultures of fetal rat mesencephalic neurons show that the non-competitive NMDA antagonist MK-801 fails to protect against MPP^+ toxicity[20,51]. Similarly, MK-801 has only equivocal effects on 3-NP toxicity in fetal mouse cortical explants[44]. Such observations suggest either that a possible NMDA-dependent step does not account for toxicity of drugs affecting energy metabolism in vitro, or that under in vitro conditions the time-window for protection is short. In contrast, growth factors and gangliosides protect neurons against NMDA and MPP^+ toxicity in vitro[16,21,67,76]. Therefore, it would be important to test whether other mitochondrial toxins such as AOAA and MA, which transiently block the activity of complexes I and II, damage dopaminergic (or other) neurons in vitro and whether NMDA antagonists can protect against their toxicity. Another important issue concerns the determination of the protection time-window for NMDA antagonists against toxicity induced by mitochondrial toxins in vivo, provided concentrations of both the toxin and the NMDA antagonist are kept constant for weeks or months. If the protection offered under such experimental conditions is temporary, this would support the hypothesis that NMDA antagonism allows neurons only limited time to overcome energy failure either by increasing energy supply or by eliminating the causes of energetic insult. Should such rescue activities fail, persistent disturbances in energy metabolism will damage neurons irrespective of protective measures. This inference may explain, at least in part, the failure of NMDA antagonists to protect cell cultures or cortical explants against MPP^+ or 3-NP toxicity.

Nitric oxide: a mitochondrial respiratory chain toxin?

Research on cancer and immunological defense reactions provided evidence that activated macrophages produce unusual metabolic changes in tumor target cells, including inhibition of DNA synthesis, inhibition of protein synthesis, blockade of citric acid cycle enzymes, and inhibition of mitochondrial respiration[27,28]. It was quickly recognized that nitric oxide (NO) generated by cytokine activation of the L-arginine:NO pathway is responsible for this pattern of metabolic disturbances[28]. Drappier and Hibbs[19] demonstrated that NO synthesized in cytotoxic activated macrophages causes inhibition of complexes I and II by interaction with iron-sulphur prosthetic groups and formation of nitrosyl-iron-sulphur complexes. Since induction of NO synthase can occur in every cell containing this enzyme, including neurons[22], it is possible that the inappropriate synthesis of NO may result in nitrosylation of iron-sulphur centers in respiratory chain enzymes and cause metabolic inhibition resulting in neurotoxicity. In line with this, NO donors such as sodium nitroprusside or NO itself are toxic to rat striatal, cortical and hippocampal neurons in vitro[17,32] and the rat hippocampus in vivo[42]. Sodium azide, which in vivo is rapidly converted to NO[74] impairs oxidative phosphorylation in the rat striatum and induces striatal lesions by an excitotoxic mechanism following either systemic or intrastriatal administration[5]. Prior decortication attenuates the striatal lesions produced by sodium azide[5]. The toxicity induced by NMDA or glutamate can be limited by agents that decrease the activity of NO-synthase (NADPH-diaphorase)[17]. NO-synthase inhibitors protect rat brain against damage induced by ischemia[56]. Many NADPH-diaphorase/NO synthase-containing neurons are resistant to toxicity mediated by NMDA, several mitochondrial poisons, and NO[6,7,17]. This resistance may be explained by inhibition of Ca^{2+} influx through NMDA-gated ion channels by endogenous NO in NADPH-diaphorase/NO-synthase-containing neurons[31]. This interaction is suggested to take place at the redox-modulatory site of the NMDA receptor[39,41,47].

Clinical data

Clinical evidence suggests that cell death in chronic neurodegenerative disorders may be associated with defects in oxidative phosphorylation. In Parkinson's disease, complex I activity is significantly reduced in substantia nigra but not in other brain regions[33,65]. Complex I activity is also low in platelets[62] and muscles[71] of parkinsonian patients. Furthermore, defects in complexes III and IV can be detected in the muscles of such patients[71]. In Huntington's disease complex I activity is significantly reduced in platelets[61], while basal ganglia and cortex show decreased energy metabolism[10]. Complex IV defects can be seen in the basal ganglia of Huntington's disease patients[10]. In Alzheimer's disease reduced activity of cytochrome c oxidase (complex IV) in platelets has been reported[63,72]. Furthermore, dihydrorotenone binding is increased in the entorhinal cortex of Alzheimer's disease brains suggesting profound mitochondrial dysfunction[30]. There is also evidence that aging produces selective impairment of the function of complexes I and IV in primate frontoparietal cortex[9]. In degenerative ataxias, reduced complex I activity is observed in muscles[68]. There is also a number of disorders associated with point mutations in mitochondrial DNA, energy metabolism disturbances and several indices of neurodegeneration[69,90]. Leber's optic atrophy, myoclonus epilepsy with ragged red fibers (MERRF), and mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), represent the most common of such disorders[90].

Therapeutic implications

Experimental data suggest that disturbances in the function of mitochondrial respiratory chain may result in neurotoxicity resembling that triggered by the excitotoxin glutamate. The factors that disturb the normal function of mitochondrial respiratory chain enzymes are: 1) exogenous or endogenous toxins, such as AOAA, MPP^+, MA and 3-NP, or related toxins working at different levels of the respiratory chain such as azides[5], cyanide[92], manganese[13], or possibly NO[27,77,91], or 2) other processes preventing sufficient energy supply to neurons such as those present in genetic disorders of mitochondrial energy metabolism[69,90]. NMDA antagonists were found to protect temporarily against several mitochondrial toxins in vivo[4,5,8,11,15,24,37,43,49,50,64,78–80,82,87,89]. In animal studies, NMDA antagonists were found to be beneficial only when their pharmacokinetic properties and the timing of therapy allowed for a protective effect to take place. The half-life of toxins such as AOAA and MA is short, ranging between 1 and 2 h in rodents, while that of 3-NP and MPP^+ is longer, ranging from several hours to days. For this reason, NMDA antagonists of sufficiently long half-life such as CPP and, to a lesser extent, MK-801, protected against AOAA and MA toxicity since they efficiently blocked the transition between reversible and irreversible changes in cells undergoing energy failure and allowed the toxin to disappear from the cell and its concentration to decrease below a critical level. In the case of MPP^+ and 3-NP, although their mode of action is similar to that of other mitochondrial toxins, the situation is more complicated. Since the half-life of MPP^+ or 3-NP is much longer than that of available NMDA antagonists, it is necessary to continue administration of the antagonist for several hours or days in order to see any protective effect against in vivo toxicity. Such experiments are further complicated by the fact that in situations in which massive damage occurs within seconds or minutes, NMDA antagonists need to be administered in almost toxic doses. In several cases, therefore, studies with subchronic or chronic administration of NMDA antagonists were impossible due to systemic toxicity. Nevertheless, it was attractive to speculate that NMDA receptor blockade may offer efficient protection against toxicity related to energy metabolism failure. However, analysis of the sequential changes in cells undergoing sustained energy deficits shows that activation of NMDA receptor-gated channels represents only part of the complex process leading to cell death (fig. 2), and implies that NMDA receptor antagonism will not eliminate the cause of chronic neurological diseases associated with deficient mitochondrial energy metabolism (fig. 3). Antagonism at NMDA receptors will never normalize disturbed energy metabolism in the cell if other deleterious factors persist. What NMDA antagonism does is prolong the life-span of the cell undergoing energy failure. Such action might be expected to retard the transition between reversible and irreversible changes in the cell due to sustained energy failure. Thus, NMDA antagonism may provide valuable time for other therapeutic interventions that will normalize energy metabolism. Such therapies have already been developed for genetic disorders of mitochondrial energy metabolism[90]. Preliminary observations on oral ubiquinone supplementation (which may enhance complex I activity) in Huntington's disease patients suggest that such metabolic therapy may be beneficial and leads to a partial normalization of elevated lactate levels in occipital cortex[34]. If true for other neurodegenerative disorders, a metabolic approach could usefully retard the development of these diseases in patients who are desperately waiting for improved therapies.

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