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Viewpoint

Excitatory amino acids and anoxic/ischaemic brain damage

The selective pattern of brain damage that follows cerebral anoxia or ischaemia is usually explained in terms of a failure of energy metabolism, or secondary disturbances of local blood flow, and the possible toxicity of free radicals However, recent experiments suggest that neurotoxic actions of synaptically-released excitatory amino acids play a crucial role in the development of such damage Antagonists of excitation due to acidic amino acids protect against anoxic/ischaemic damage in test systems in vitro *and* in vivo *This suggests a novel therapeutic or prophylactic approach to such conditions as stroke, perinatal asphyxia and the postoperative complications of cardiothoracic surgery*

In the hippocampus the pattern of neuronal loss is similar after an episode of ischaemia or of status epilepticus[1] Thus pyramidal neurons in regions CA_1 and CA_3 are preferentially affected whereas dentate granule cells are less vulnerable

The similar outcome in these physiologically very different circumstances has been tentatively explained in terms of an element of ischaemia occurring at some stage during or after status epilepticus, or of an insufficiency of energy metabolism in both circumstances (seizures being associated with enhanced energy requirements[2]) The suggestion that the common factor could be enhanced release of excitatory amino acid neurotransmitters[3] has recently received strong experimental support from both *in vitro*[4,5] and *in vivo* experiments[6,7]

Rothman[4] studying fetal rat hippocampal neurons in dispersed tissue culture sought to explain why the neurons are insensitive to exposure to cyanide (1 mM NaCN) or to an anoxic atmosphere (95% N_2 and 5% CO_2) during the first 48 h in culture but become highly vulnerable after 2 weeks in culture, so that exposure to cyanide for 1 h causes gross vacuolation and subsequent degeneration of the majority of neurons At 2 weeks the cultures have developed extensive synaptic contacts and show spontaneous spike activity Blockade of synaptic activity by high magnesium (10 mM $MgCl_2$) prevents the neuronal changes induced by 1 h of cyanide exposure[4]. That the factor involved is probably excitation induced by glutamate or aspartate is indicated by Rothman's subsequent experiments[5] γ-D-glutamyl glycine is an antagonist of the excitatory action of dicarboxylic amino acids, which lacks specificity for receptor subtype being slightly more effective against excitation due to *N*-methyl-D-aspartate than that due to kainate[14] Addition to the culture medium of γ-D-glutamyl glycine (10^{-2} M) blocks the neuronal degeneration induced by anoxia Addition to the culture medium of glutamate (10^{-4} M) or aspartate (10^{-4} M) for 1 h leads to neuronal degeneration similar to that induced by anoxia This toxic action of the excitatory amino acids is also blocked by γ-D-glutamyl glycine, indicating that the effect of glutamate and aspartate is probably via receptor sites rather than through a primary metabolic effect

Two questions arise concerning the possible clinical relevance of these findings Firstly, is anoxic neuronal degeneration in tissue culture equivalent, in its physiopathogenesis, to ischaemic cell change (and neuronal loss) *in vivo*? Secondly, does excitatory amino acid transmission play a comparably crucial role *in vivo*? A definite answer cannot yet be given to the first question. However, the second question can probably be answered positively.

A model for ischaemic damage is provided by bilateral occlusion of the common carotid artery in the rat combined with reduction of arterial pressure to 50 Torr This produces ischaemic changes in the forebrain the initial stages of which can be identified in vulnerable hippocampal neurons 2 h after a 30 min period of ischaemia[9] Local injections into one hippocampus directly prior to the ischaemia of 2-amino-7-phosphonoheptanoic acid produce an almost complete local protection against ischaemic changes evaluated after 2 h[7] 2-Amino-7-phosphonoheptanoic acid is an excitatory amino acid antagonist that is more potent than γ-D-glutamyl glycine and is highly selective for the *N*-methyl-D-aspartate preferring receptor. In animal models of epilepsy it possesses significant anti-convulsant activity given intracerebrally or systemically[10] Using the microdialysis

perfusion technique in the rat hippocampus[1], there is a dramatic increase in the extracellular concentration of glutamate and aspartate during transient cerebral ischaemia However, it is not yet clear to what extent the protective action of blockade of excitatory amino acid receptors relates to the pathological release of transmitters during ischaemia, or the synaptic release associated with burst firing during the reperfusion period

Selective damage to neurons following the systemic or focal intracerebral injection of glutamate and related excitotoxins, such as kainate and ibotenate, has been extensively studied in the last 25 years[11] The main clinical relevance of such studies has been thought to be either to epileptic brain damage secondary to status epilepticus, or more speculatively to chronic degenerative disorders such as Huntington's chorea and olivopontocerebellar atrophy[12]

The observations discussed here suggest that excitotoxic mechanisms play a role in the wide range of situations where cerebral hypoxia/-ischaemia leads to brain damage, including perinatal asphyxia, acute cerebrovascular accidents, and the neurological complications of cardiac arrest or cardiothoracic surgery

The selective patterns of neuronal damage may be explicable in terms of particular excitatory inputs or of receptor density on specific cell types Thus the fact that perinatal asphyxia frequently leads to basal ganglia pathology (with choreoathetosis and spasticity) whereas global ischaemia in adults damages the neocortex and hippocampus preferentially may relate to the greater density of glutamate receptors in the basal ganglia of infants and in the cortex of adults[13]

The possibility now revealed of pharmacological prophylaxis or therapy for hypoxic/ischaemic brain damage urgently requires further study It may be possible to test the therapeutic action of 2-amino-7-phosphonoheptanoic acid in man in certain special circumstances However, this compound is unlikely to be the optimal excitatory amino acid antagonist because of its limited penetration of the blood–brain barrier Future efforts must identify potent and selective antagonists with adequate penetration to the brain[14] Detailed study of side effects, long term toxicity and clinical efficacy will be required However, the potential for prevention or relief of neurological disorder is immense

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