Excitatory amino acids in neonatal brain: contributions to pathology and therapeutic strategies

Glutamate, the principal excitatory amino acid, is considered to be the major mediator of excitatory signalling and fast synaptic transmission in the central nervous system. The special importance of these signalling processes in the developing brain is starting to be appreciated. Glutamate evokes neuronal responses through well characterised, cell-surface receptors which were originally classified pharmacologically and more recently by molecular cloning as the ion channel-gating (ionotropic) α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate (KA) receptor family, the N-methyl-D-aspartate (NMDA) family, and the metabotropic family which is linked via G-proteins to intracellular signalling mechanisms.1-3 The AMPA/KA receptors mediate fast information transfer while the NMDA receptors seem only to function once the postsynaptic membrane has been depolarised. Most neurons as well as some glia seem to have glutamate receptors.

The extent to which receptors are activated is determined by extracellular glutamate concentrations. Apart from nerve terminal release, the other major influence on extracellular glutamate is the activity of specific uptake transporters, found on nerve terminals and surrounding glia (astrocytes). Their purpose is to maintain low extracellular concentrations of glutamate to keep a balance between effective signalling and excitotoxicity.4 The conditions which cause glutamate to be released can also produce energy depletion and ion imbalances which can lead to failure of uptake transporters or even drive them in reverse,5 thus releasing more glutamate, raising extracellular concentrations even further, and incurring excitotoxicity and a chain of events leading to neuronal damage.

Excitotoxicity

Experimental studies have shown that excitotoxicity is triggered by excessive release and extracellular overflow of glutamate which causes overactivation of receptors. There is overwhelming evidence that an excessive concentration of extracellular glutamate and other excitatory amino acids has a crucial role in the pathogenesis of neuronal death following brain ischaemia, trauma, seizures or, especially in the developing brain, hypoglycaemia. The neonatal brain may be exposed to any of these. In fetal sheep brain extracellular glutamate concentrations rose several-fold during ischaemia and hypoglycaemia.6,7 A feature of the usual sites of perinatal neuronal injury – hippocampus, cerebellum, basal ganglia – is the presence of many glutamate neurons.8 Finding ways to overcome glutamate excitotoxicity is a major therapeutic goal irrespective of age, but the neonate presents special problems because of the immaturity of the glutamate system and the fact that glutamate has special functions in the developing brain.

The principal approach used to overcome glutamate excitotoxicity is to design glutamate receptor blocking agents. The AMPA/KA receptors mainly depolarise neurons through sodium influx rather than calcium influx, because this receptor family triggers few calcium dependent processes.9 Protracted glutamate release and intense AMPA/KA stimulation can cause enough sodium influx to produce neuron damaging oedema. Furthermore, the neuron depolarisation also provokes NMDA receptor activation and subsequent entry of calcium. This triggers a network of enzyme cascades and messengers, leading inevitably to cell death.10,11 Thus both AMPA/KA and NMDA receptor blockers may achieve similar therapeutic goals.

Glutamate as a growth regulator

Whenever a brain neurotransmitter takes on a developmental role, especially during periods of rapid synapse production, both the nerve terminals and postsynaptic receptors may be temporarily overexuberant. Early modulation of these so-called precocious receptors may influence neural growth.12 The glutamate system may influence neurite sprouting, synaptogenesis, dendrite pruning and whether neurons survive developmental or pathological attrition. NMDA receptors in particular may coordinate synaptogenesis and synaptic plasticity in developing brain.12 Thus NMDA receptor activation was essential to promote neurite outgrowth from cerebellar cells,13 and whereas AMPA/KA receptors regulated growth of young hippocampal cell cultures, NMDA receptors assumed control in mature cell cultures.14

The glutamate NMDA receptor

NMDA receptors in neuronal membranes control channels which allow calcium ions to enter neurons. The
channels are normally blocked by magnesium unless the neuron is depolarised — for instance, when other glutamate receptors (AMPA, metabotropic) are activated. Excessive release of glutamate is generally required before NMDA channels open to calcium.\(^1\) This regulation of cell calcium by glutamate lies behind the phenomenon of long term potentiation (LTP), a type of synaptic plasticity (allied to memory) that is normally observed in the glutamate rich hippocampus.\(^16\)

Nitric oxide (NO) is synthesised by NO synthase (NOS) following activation by glutamate of NMDA receptors and it can diffuse through brain membranes. These properties make NO a promising candidate for a retrograde messenger in brain. NO is believed to play a part in the induction of LTP, because NOS inhibitors block LTP by a process that can be reversed by L-arginine.\(^17\) Haemoglobin, which binds and inactivates NO, is also known to block LTP.\(^16\) One of the actions of NO as a retrograde messenger may be to facilitate glutamate release.\(^18\)

The messenger or neuromodulator roles of NO may also include a role in the refining of neural connections during brain development. These processes, which may be related to LTP,\(^19\) have implications for memory and learning. Thus suppression of LTP may occur in post-traumatic head injury which damages hippocampal neurons rich in NMDA and causes profound memory and learning dysfunction.\(^20\)

The NMDA receptor complex (figure) is highly unusual in having multiple sites which, in different ways, can control channel opening and therefore calcium flux. These modulatory sites recognise glutamate (the NMDA site) as well as glycine and polyamines (spermine and spermidine). The channel lumen has other sites, some recognised by magnesium and others by phencyclidine (PCP) – like drugs, including the anaesthetic ketamine and the anticonvulsant MK-801 (Dizocilpine). Channels can therefore be opened by glutamate, glycine, or a polyamine. These may be most effective in concert, or blocked pharmacologically by glutamate, glycine, and polyamine antagonists (site blockers), or by MK-801 and similar channel site compounds. Experimentally, NMDA channel opening is assessed by how much \(^{3}H\)MK-801 binding can be measured.\(^21\) What is so special about the NMDA receptor is its multiple targets for potential therapeutic agents that can tone down channel opening.

Receptors are transmembrane proteins expressed by appropriate messenger RNA (mRNA). NMDA receptors are assembled from two molecular families: NMDA-R1 and NMDA-R2. The latter comprises four subunits (−2A to −2D).\(^22\)\(^23\) NMDA receptors in different parts of brain are assembled from different subunit combinations but NMDA-R1 may be obligatory for a functioning receptor.\(^22\) In vitro studies suggest that functioning NMDA receptors may not always possess all the recognition sites.\(^24\) Once it is known how human brain NMDA receptors are assembled, it may be possible to produce pharmacological agents which have some degree of regional selectivity for brain NMDA receptors.

Postnatal development of glutamate neurons
Some glutamate transporter sites are found on glutamate neurons and terminals and therefore provide an idea of the density of these elements. Term newborn infants had fewer transporter sites in areas of brain which in adults have many glutamate neurons.\(^25\)\(^26\) Reasons for this may be that glutamate nerve terminals in neonatal brain are sparse or functioning subnormally. Infant brain during development soon acquires more transporter sites, particularly in the cortex and cerebellum, which continue to appear until they are overabundant by the end of the first year.\(^25\)\(^26\) The apparent postnatal growth spurt in glutamate nerve terminals coincides with a rapid production of synapses. Brains of infants or children develop exuberant synapses which are slowly pruned up to adolescence.\(^27\)

Postnatal development of glutamate receptors
Developing mammalian brains may show brief and modest overexpression of glutamate receptors which do not necessarily coincide with synapse production and elimination. In human hippocampus AMPA receptors peak prenatally but decline by 26 weeks' gestation,\(^28\) long before the bulk of synaptogenesis. An early report described a transient postnatal rise in hippocampal NMDA receptors in the human infant.\(^29\) The glycine and MK-801 (channel) sites of the NMDA complex in term infant cortex start to increase at one week after birth and by about 20 weeks are overexpressed, greatly exceeding levels found in adult brain.\(^30\)\(^31\)
Examples of compounds in each class of NMDA receptor antagonists with potential neuroprotective properties

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>Selective antagonists</th>
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<tr>
<td>Competitive block of the transmitter recognition site</td>
<td>D-AP7, CGS 19755, CPP</td>
</tr>
<tr>
<td>Blocking the NMDA-coupled ion channel</td>
<td>Ketamine, phencyclidine, Diclopinine (MK-801)</td>
</tr>
<tr>
<td>Blocking the polyamine site</td>
<td>Anatox, DIPRODIL, IFENPRODIL</td>
</tr>
<tr>
<td>Blocking the glycine site</td>
<td>7-Chlorophenylglycine, (+)-HA-966, 5,7-dichloreuxymenuric acid, L-678,416</td>
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Furthermore, the ability of glutamate and glycine to increase [3H]MK-801 binding and thus stimulate opening of NMDA-coupled channels was most effective in 20 week old infant brain, suggesting that the NMDA receptor complex is also hyperresponsive.32

It may be a general rule that some NMDA regulatory sites in mammalian brain show differential development.12 In man, brains from older infants have excess glycine modulatory sites compared with adult brain.30-31 Thus, without doubt, growth of NMDA receptors in the human infant is far more pronounced than that in experimental animals.

Animal studies have shown that the relative proportions of the mRNA which express the NMDA subunits are different in neonatal and adult brains, implying that there is a postnatal reassembly of subunits to acquire the composition of adult brain NMDA receptors. The importance of this, in view of the fact that some NMDA subunits may not have their full complement of modulatory sites, is that the pharmacological properties as well as the functions of NMDA receptors alter during development. Thus NMDA blockers suited to adult brain may be less appropriate for neonatal brain in which NMDA receptors may have functions better suited to their developmental roles and may be guided into a molecular rearrangement as mature brain functions take over. The molecular reorganisation of NMDA receptors in man has yet to be worked out, but the fact that infant brain acquires plenty of NMDA receptors, as revealed by the MK-801 channel sites, as well as lots of glycine sites,30-32 suggests that receptor subunits with glycine sites are particularly overexpressed. NMDA receptors reach their highest densities around the end of the first year and decline in childhood until they slowly reach adult levels.30-33 This suggests that levels of the mRNA responsible for some NMDA subunits rise and fall during the process of molecular reconfiguration of NMDA receptors in human brain.

Conventional receptor binding studies on rat brains have confirmed that NMDA receptors change some of their properties in early postnatal life,34 35 probably at the same time as their molecular reorganisation.23 This may be to enable NMDA receptors in developing brain to lose whatever properties are required to guide neuronal migration and synapse elimination as these become less important in adult brain. NMDA receptor subunit switchover during development may be pre-programmed and also use dependent. One of the pieces of research still to be carried out is to discover whether intrauterine growth retardation, preterm birth, or neonatal complications affect the way NMDA receptors are assembled and how they subsequently function.

Glutamate receptors and their potential for treatment

Many experimental studies have reported how all kinds of glutamate receptor blockers can be neuroprotective in those conditions believed to involve excess glutamate (table), although many incur severe side effects.43 44 Most attention has focused on NMDA blockers which may offer better protection against focal rather than global brain ischaemia. Both ischaemia and seizures may induce high concentrations of glutamate, so substances which produce a non-competitive type of block may be best because such a block is harder to overcome. Furthermore, non-competitive channel site blockers have the extra benefit of affecting those sites which are exposed (for example, MK-801) by excess glutamate. However, a more effective strategy for protecting against glutamate excitotoxicity might combine an AMPA/KA receptor blocker with an NMDA blocker.

Any drug which may eventually be used to guard against excitotoxicity in the neonate may have to be both short acting and relatively mild in its effects on NMDA receptors, to avoid misdirecting the development of the glutamate system at a critical period. There is obvious concern that this might influence subsequent brain development. The possible consequences of such deviant development are not clearly understood.

NMDA channel blockers thought to have some therapeutic potential include, or may be based on, MK-801 and the dissociative anaesthetic ketamine. Both have known side effects which restrict their use, such as behaviour disorders and cognitive deficits, whilst MK-801 causes vacuolation of neurons and their destruction at high doses. Dextromethorphan is an established (antitussive) drug is a relatively weak NMDA blocker whose mechanism of action is uncertain. Although dextromethorphan may cause a reversible neuronal vacuolation with ataxia and neurobehavioural side effects at higher doses, it has proved valuable in overcoming the neurological consequences of hyperglycaemia in infants.35 Remacemide is an anticonvulsant, neuroprotective compound with antagonist activity on the NMDA receptor ion channel. It can induce hyperactivity, ataxia, and behaviour disorders and perhaps some neuronal vacuolation. Memantine, whose structure is different from other NMDA channel blockers, may have similar side effects to the dissociative anaesthetics, causing agitation, confusion, and psychotic symptoms.

Non-competitive AMPA/KA receptor blockers, including some 2,3 benzodiazepines, have recently been identified. The anticonvulsant and AMPA blocker NBQX was neuroprotective in brain ischaemia models. Barbiturates appear to block AMPA/KA receptors in a non-competitive way but they are obsolete. GYK 152466 is a non-competitive antagonist that is effective in animal models of global and focal brain ischaemia.

So far, animal studies have produced data which suggest that non-competitive NMDA and AMPA/KA receptor antagonists may protect against cellular damage. Dextromethorphan,45 remacemide, and memantine approach clinical acceptability and without doubt new compounds are being investigated.

NO and excitotoxicity

NO produced in brain as a consequence of glutamate NMDA receptor activation is a membrane diffusible free radical which, in high concentrations, is neurotoxic. Conditions such as ischaemia, which give rise to intense NMDA receptor stimulation, may also cause the production of excess NO and thus exacerbate excitotoxicity. NO may also interact with glutamate systems in various ways, including facilitating glutamate release18 which could raise extracellular glutamate concentrations even further. The role of NO in causing brain damage in cerebral ischaemia is therefore being investigated and early reports suggest that NOS inhibitors can be neuroprotective. It is too soon to predict whether any therapeutic agents will ever emerge
but research into NO will undoubtedly be a major source of important new findings.

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