Neurotransmitters, Drugs and Brain Function.
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10 Amino Acids: Excitatory

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INTRODUCTION

For many years the amino acid glutamate (Fig. 10.1) has been known to play the major role in the transmission of excitatory signals via long axonal projections of neurons in the central nervous system. In fact, of the billions of long-axon neurons in the central nervous system, the majority use glutamate as their principal transmitter as do excitatory intrinsic neurons. A large proportion of peripheral sensory fibres conveying touch- and pain-related information contain glutamate and aspartate as do visual, auditory and other sensory afferent fibres. This is also the case for neurons in the CNS linking different areas of the brain and spinal cord. Due to the metabolic role of glutamate and the fact that it is the precursor for GABA, the inhibitory amino-acid, precise localisation studies have been fraught with difficulties. However, both release studies and, more importantly, electrophysiological recordings have shown that glutamate functions as a transmitter at many synapses. In the case of C-fibres, the co-existence of glutamate with peptides such as substance P and/or CGRP would make it highly likely that a noxious stimulus releases both peptides and excitatory amino acids from the afferent nociceptive fibres. Here the coincident actions of glutamate in concert with peptides have a functional importance that is discussed later.

While aspartic acid (aspartate) is also found in the CNS and has excitatory effects on neurons, little is known of its precise location and action although it may be released from intrinsic neurons and hippocampal pathways. It will not be discussed further.

NEUROCHEMISTRY

Due to the major role of glutamate, not only as a component of proteins but also as a key step in intermediate metabolism, the production and metabolism of the amino acid are compartmentalised in neurons. It may be that the transmitter pool of glutamate uses the amino acid from any source given that it can be produced from such diverse origins as glucose, aspartate, glutamine and oxoglutarate. Once release occurs there are high-affinity uptake sites in both terminals and glia that remove the transmitter from the synaptic cleft (Fig. 10.2).

These points have important functional implications. While neuronal glutamate may come from glucose via pyruvate, the Krebs cycle and transamination of alpha-oxoglutamate, it seems likely that most of the transmitter originates from the deamination of glutamine. After release, the high-affinity uptake sites (transporters)

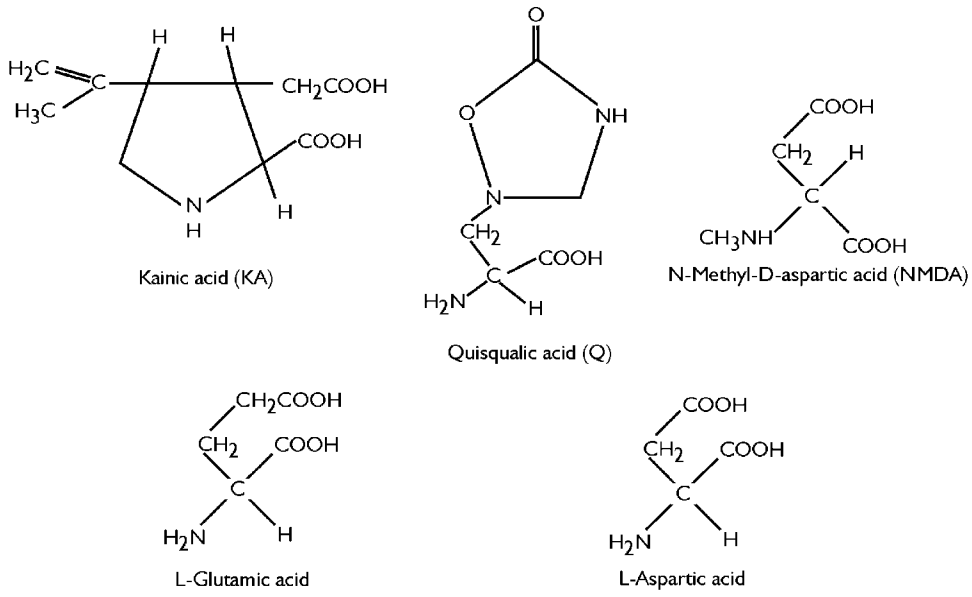

Figure 10.1 Structures of the transmitters and synthetic agonists at the various receptors for glutamate

remove glutamate from the synapse, partly back into the nerve terminal or more probably into adjacent glial cells. In the latter, it is converted by glutamine synthetase into glutamine which then passes back into the extracellular fluid (the CSF levels are high, of the order of 0.5 mM) to be taken up by the glutamate nerve terminal. Here it is deaminated to neurotransmitter available glutamate by mitochondrial glutaminase. Thus there is a conservation rather than a net synthesis of glutamate. This complex but very general biochemical process provides very little opportunity for drug modification of glutamate synthesis or metabolism.

Unlike other transmitter systems, there are no obvious mechanisms for dampening glutamate release. Presynaptic autoreceptors for glutamate are mostly of the kainate type (see below) and appear to act as positive rather than negative influences on further release of the amino acid. Although poorly characterised at present, inhibitory autoreceptors of the metabotropic type of receptors may act to inhibit release of glutamate.

RECEPTORS — STRUCTURE AND FUNCTION

The extensive early literature on the idea of important excitatory roles for amino acids originated in work in the 1950s by Curtis and his group in Australia from iontophoretic studies which showed powerful excitatory effects of a number of dicarboxylic amino acids on a variety of CNS neurons. Thus despite any knowledge at that time of the receptors or the availability of selective antagonists, important roles were proposed for these transmitters in neuronal function. These early predictions have been confirmed by more recent approaches using selective agonists and antagonists which have now allowed the separation of the

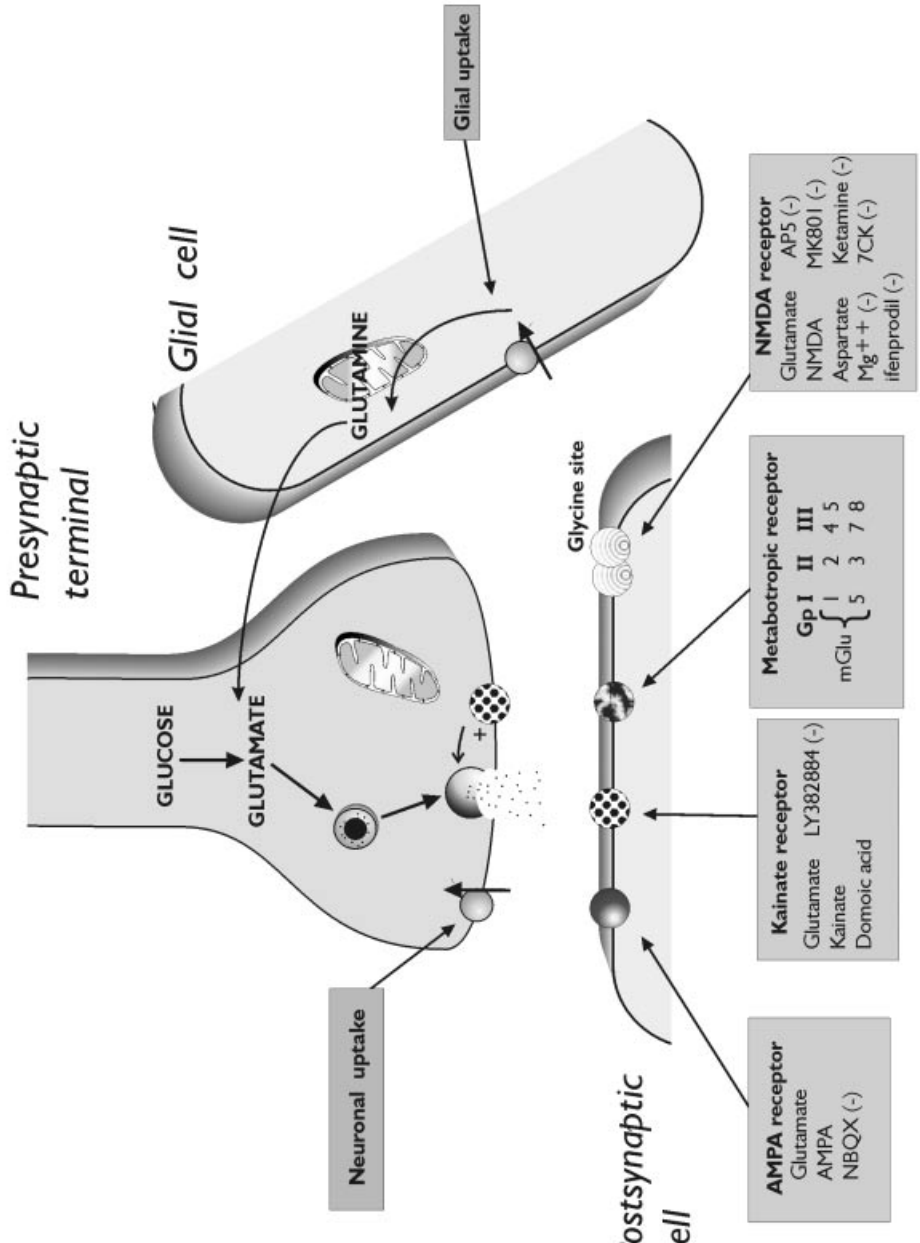

Figure 10.2 Site of action of drugs affecting glutamate synapses

receptors for glutamate into four main types. Block of a physiological response by an antagonist is good evidence for a functional role of any transmitter in CNS events and this has now been achieved for glutamate in many areas of the CNS.

The nomenclature for the glutamate receptors is confusing. Originally, the receptors were called N-methyl-D-aspartate (NMDA) and non-NMDA with the latter later subdividing into quisqualate and kainate. Now, the accepted classification is into AMPA, kainate, NMDA and metabotropic. This latter class of receptor is further divided into three groups (I, II and III) containing at least two subtypes. Figure 10.1 shows the agonists at the receptors.

NON-NMDA — AMPA AND KAINATE RECEPTORS

Non-NMDA ionotropic glutamate receptors (the majority sodium channel containing) can be subdivided into α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) (comprising cloned subunits GluR1–4) and kainate (GluR5–7, KA1–2) preferring receptors, with native receptors most likely to comprise either homo- or heteromeric pentamers of these subunits.

mRNA coding for GluR1–4 subunits is found throughout the brain and spinal cord, with differing patterns of expression of GluR2 and GluR1 in different regions. There is also evidence for both presynaptic AMPA and particularly kainate-preferring receptors comprising GluR5 subunits on neuronal terminals in various areas of the CNS.

There are competitive AMPA receptor antagonists (Fig. 10.3) of which NBQX (6-nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione) (which also displays micromolar affinity for the kainate-preferring GluR5 and GluR6 subunits3) is the most selective, and the recently developed selective GluR5 antagonist LY382884 [3S,4aR,6S,8aR-6-(4-carboxyphenyl)-methyl-1,2,3,4,4a,5,6,7,8,8a-deca-hydroisoquinoline-3-carboxylic acid]. These drugs are allowing the roles played by non-NMDA ionotropic glutamate receptors to be gauged.

The majority of AMPA receptors are impermeable to Ca^{2+}, although some AMPA receptors, as well as kainate receptors, have significant Ca^{2+} permeability. AMPA receptors are multimeric assemblies of four cloned subunits, GluR1–4, but it is the absence of the GluR2 subunit that determines the Ca^{2+} permeability of AMPA receptors, since editing out of this subunit following transcription into mRNA results in the introduction of a positive charge in the pore-forming region (Q/R site), which is not present in GluR1,3 or 4, 9 (see also AMPA receptors in Chapter 3). AMPA receptors lacking GluR2 have Ca^{2+} permeability ratios up to $P_{Ca}/P_{Na} = 3$. Since calcium is such a ubiquitous intracellular messenger any receptor that allows this ion to enter neurons is likely to be important in plasticity in the CNS. In a similar manner to the AMPA receptor, RNA editing in the pore region at the Q/R site controls the Ca^{2+} permeability of the kainate receptor subunits GluR5 and GluR6, with significant levels of the unedited (Ca^{2+} permeable) version of these receptors present in the adult CNS.

The AMPA receptor subunits are all found within many regions of the CNS but in differing numbers, and, in the spinal cord, have differing lamina distributions. GluR1 and GluR2 are generally the most abundant AMPA receptor subunits with lower levels of GluR3 and GluR4. The majority of AMPA receptors allow Na^+ to enter neurons and thus in most areas of the CNS studied, the initial stage in excitatory synaptic transmission is a fast-depolarising response due to the release of glutamate and subsequent activation of the AMPA receptor.

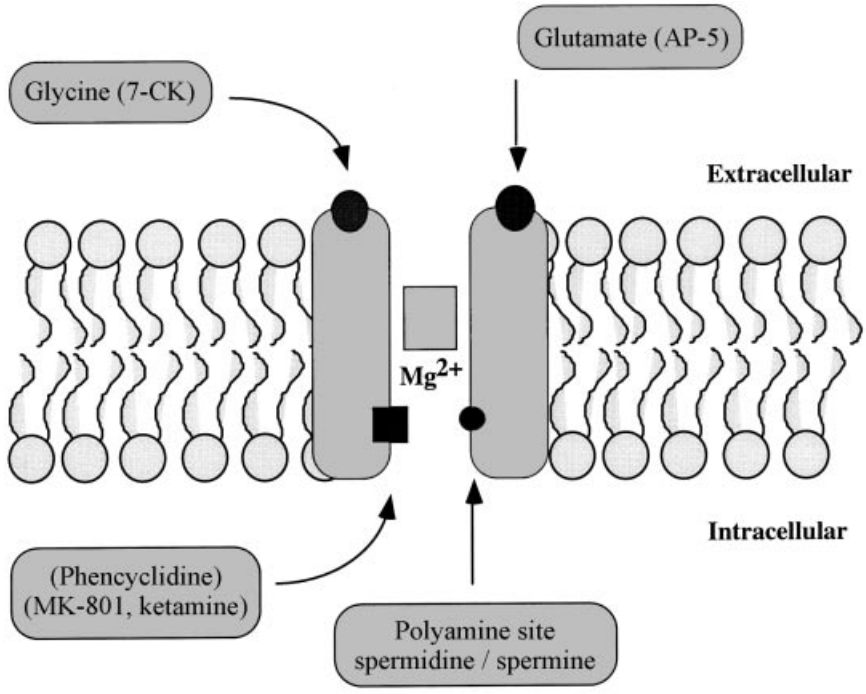

Figure 10.3 Structure of the NMDA receptor–channel complex. The receptor has a complicated structure and this is highlighted by the presence of many pharmacologically distinct binding sites through which the receptor activity can be modulated. The channel associated with the receptor is blocked by Mg^{2+} at resting potential (-70 mV). Receptor activation requires the removal of this Mg^{2+} block (voltage-gated) as well as the binding of glutamate and the co-agonist, glycine (ligand-gated). The different binding sites (glutamate, phencyclidine, polyamine, glycine) are illustrated, and together with antagonists which act at the various sites (in parentheses). The polyamine site is an intracellular site which modulates the affinity of other agonists and antagonists

mRNA coding for the kainate receptor subunits GluR5 and GluR7 is also found in isolated neurons in the CNS although many kainate GluR5 receptors are thought to be located presynaptically on terminals of neurons that release glutamate. Kainate receptors are therefore thought to be excitatory autoreceptors that enhance the release of glutamate. It could be predicted that the widespread distribution of AMPA receptors precludes the use of antagonists at this receptor in therapy since adverse effects are highly likely. By contrast, the kainate receptor might be an interesting target since its functional role will be linked to the level of glutamate release. Thus, antagonists at this receptor should reduce excessive glutamate release while having less effect on more normal functional synapses.

The role of the kainate receptor system in the brain is at an early stage since there are as yet few pharmacological tools to study its function. However, mutations in the kainate receptor genes have been made in mice and there is a GluR6 kainate receptor knock-out mouse. Kainate binding is absent in areas of the brain which normally have high levels such as the hippocampus. Here, in normal animals kainate receptors mediate a postsynaptic current which is absent in the GluR6 knock-out mouse. The mice have

reduced motor activity but can learn maze tasks. The knock-out mouse is resistant to kainate-induced seizures.

Studies have shown that neurons expressing high levels of GluR1 mRNA but lacking GluR2 are found in the superficial laminae of the spinal cord, an area where nociceptive primary afferents terminate, suggesting that a subpopulation of AMPA receptors in this region may have significant Ca^{2+} permeability. Calcium-permeable non-NMDA receptors have been demonstrated in spinal cord slices using kainate-induced cobalt loading. Studies performed using cultured neurons *in vitro* have suggested that Ca^{2+} entry through Ca^{2+}-permeable AMPA receptors in the spinal cord may provide a mechanism for the strengthening of transmission at synapses and enhancement of nociceptive transmission. Other studies have suggested a link between Ca^{2+}-permeable AMPA receptors and inhibitory systems since in the dorsal horn of the spinal cord many of these receptors are found on GABA neurons. Clearly, the functional role of Ca^{2+}-permeable non-NMDA receptors *in vivo* will depend on their location in the integrated circuitry of the CNS. Joro Spider Toxin (JSTx) has been reported to be a selective blocker of Ca^{2+}-permeable non-NMDA responses evoked by AMPA/kainate rather than those evoked by NMDA and so will be a useful tool for studying the roles of these receptors.

NMDA RECEPTORS

Much attention has been focused on the role of the N-methyl-D-aspartate (NMDA) receptor for glutamate, activation of which produces slow prolonged neuronal depolarisation. Thus unlike the AMPA receptor, it is not responsible for the fast transmission of excitation nor the initiation of impulses but has been shown to be critical for maintaining excitatory responses such as the manifestation of wind-up in spinal cord, long-term potentiation in the hippocampus, epileptiform activity and in neuroexcitotoxicity. Mechanisms of central amplification of a nociceptive input have been suggested to underlie aspects of the enhanced spinal transmission of nociceptive messages in protracted pain states, and in this case there is good clinical evidence to support the concepts that have arisen from animal studies.

The NMDA receptor has a heteromeric structure composed of two subunit types; NR1 and NR2, the latter having four subunits (NR2A–NR2D) (Fig. 10.2). Molecular genetic techniques have demonstrated that native NMDA receptors are likely to be composed of a combination of the NR1 subunit (which can exist in eight different splice variants) and one or more of the four NR2 subunits which are the main determinants of functional diversity among the NMDA receptors (see Chapter 3 for further details). It has been shown that there are distinct developmental and spatial expression patterns of NMDA receptor NR1 subunit splice variants and NR2 receptor subunits in the CNS.

Although the exact subunit stoichiometry is not yet known for any NMDA receptor, heterologous expression studies suggest that they are likely to be tetramers composed of two NR1 subunits and two NR2 subunits providing the possibility for considerable structural diversity of NMDA receptors. The subtypes have been partially mapped in the CNS and show differing regional distributions. As the subunit composition imparts different physiological characteristics to the receptor, this would imply that different functional roles of the NMDA receptor could be separated — at present there are only antagonists for the NR2B subtypes. The NMDA receptor is a non-specific cation channel in that both sodium and calcium enter, but the latter ion appears to be the

predominant factor in the alteration of neuronal activity. This is not simply due to the large amounts of calcium that enter neurons and thus the degree of excitability that ensues but also simply that many intracellular pathways are calcium dependent.

The NMDA receptor is a complex entity. Functional modulation of the receptor can be achieved through actions at various recognition sites including the primary transmitter site, the site where glutamate binds (competitive), the phencyclidine (PCP) site (uncompetitive) situated in the channel of the receptor, the polyamine modulatory site and the strychnine-insensitive glycine site where glycine is a required co-agonist with glutamate (Fig. 10.3). Potentially, there are several ways in which the effect of released glutamate can be antagonised through NMDA receptor blockade. Numerous studies have investigated the potential use of antagonists acting through the different recognition sites. However, due to the ubiquitous nature of the receptor, it has often been difficult to achieve therapeutic effects at the target organ, in the absence of adverse side-effects. Prototypical antagonists for the various sites are shown in Fig. 10.3 — namely AP5 for the receptor, MK-801 for the channel (although the clinically used drugs ketamine and memantine also act at this site) and then there are other agents such as 7-chlorokynurante for the associated glycine site.

Alterations in the transmission of neuronal information via NMDA receptors arise due to two main factors, the first being that the calcium influx through the channel produces large depolarisations, and the second due to the unique profile of the receptor–channel complex, which requires various conditions for operation, and therefore is not necessarily involved in synaptic transmission at all times and under all circumstances. The release of the excitatory amino acids is obviously needed but in addition, glycine is required as a co-agonist, and this is of pharmacological and therapeutic interest, as antagonists of this site can produce inhibitions of NMDA-mediated events. The latter condition would appear to be ever present, due to the levels of glycine available in the brain and spinal cord. However, for the action of glycine on NMDA receptors to take on a clear physiological role, the concentration of glycine present at the synapse must normally be kept below saturating levels. Although it is not exactly clear if this occurs, it is thought that a glycine transporter is involved, whose distribution closely matches that of NMDA receptors in the CNS, so the glycine concentration present at glutamate synapses may be regulated by glycine uptake. The key role of glycine in activation of the receptor is borne out by the ability of antagonists at this site to produce inhibitions of NMDA-mediated transmission. Finally, an induced depolarisation of the neuron to relieve the resting voltage-dependent magnesium block of the channel is a prerequisite for activation of the complex. For these reasons, the NMDA receptor–channel complex is not a participant in 'normal' synaptic transmission, but when the correct conditions are achieved the complex will rapidly become activated and add a powerful depolarising or excitatory drive to synaptic transmission.

The NMDA receptor is an ionotropic receptor coupled to a cation channel, which is blocked by physiological levels of Mg^{2+} at the resting membrane potential — the sensitivity to magnesium block depends on the subunit composition as does the glycine sensitivity. The channel is blocked in a voltage-dependent manner so the receptor can only operate after sufficient repeated depolarisation. In the spinal cord, the removal of the Mg^{2+} block is mediated by peptides, including tachykinins, which are co-released with glutamate. After a brief acute stimulus, pain transmission from C-fibres is largely mediated by the action of glutamate on AMPA/kainate receptors. When the stimulus is

sustained or its intensity is increased, however, the action of substance P on NK-1 receptors produces sufficient membrane depolarisation so that the Mg^{2+} block can now be removed and the NMDA receptor activated. These events underlie central hyperexcitability and result in a significant amplification of the response. Substance P therefore plays an important role in this instance in recruiting NMDA receptors and contributes to the cascade of events leading to the enhancement and prolongation of the neuronal response. In other CNS areas, the NMDA receptor may be allowed to participate in synaptic events by glutamate acting on AMPA receptors. How AMPA/kainate receptors provide an excitatory drive of sufficient length to remove the block of the NMDA receptor channel while being fast ionotropic receptors is unclear. However, different subtypes of the NMDA receptor have differing sensitivity to both glycine and magnesium and the particular channel openings vary in both amplitude and duration. Thus regional specific conditions may control the receptor and determine its properties.

The NMDA receptor is therefore unique in that it is not simply ligand-gated but also voltage-gated due to the channel block imparted by magnesium. No other receptor requires two ligands (e.g. glutamate and glycine) for receptor activation.

METABOTROPIC RECEPTORS

This fourth type of receptor for glutamate (mGluRs), so named as they are members of the seven transmembrane-spanning family, is the least well understood. The poor understanding of this class of receptor stems from the fact that there are eight receptors in the class which fall into three groups, divided by sequence homology, effector mechanisms and, to some extent, their pharmacology. We are presently lacking sufficiently potent and selective antagonists at all these metabotropic glutamate receptors to probe their roles. They are coupled through G-proteins to potassium and calcium channels and while Group I (mGluR 1 and 5) receptors interact with IP3 systems, both Group II and III inhibit adenyl cyclase. Thus broadly, the Group I receptors are therefore excitatory and Groups II and III are inhibitory. There is some evidence for both pre- and postsynaptic locations of all groups of receptors. Functionally, the mGluRs have been implicated in memory, pain, anxiety and neurodegeneration with few specific details due to the lack of antagonists.

FUNCTIONAL ROLES

EPILEPSY

There is much evidence that both the initiation and maintenance of epileptic seizures involves the release of glutamate even though there is clear evidence that reduced GABA function may be equally impaired. Drugs that block NMDA receptors are anticonvulsant experimentally whereas the clinically effective antiepileptic drug lamotrigine reduces glutamate release as part of its action (see Chapter 16). As yet no NMDA receptor antagonists have been tested clinically.

PAIN

The excitatory amino acids are found in most sensory fibres of both large- and small-diameter fibres and, in the latter, they are co-localised with peptides such as substance P. The co-existence of these two transmitters suggests that they are released together in

response to a noxious stimulus and hence contribute to the transmission of pain. While AMPA receptors are activated in response to brief acute stimuli and are involved in the fast events of pain transmission, NMDA receptors are only activated following repetitive noxious inputs, under conditions where the stimulus is maintained (for more details see Chapter 21). NMDA receptors have been implicated in the spinal events underlying 'wind-up', whereby the responses of dorsal horn neurons are significantly increased after repetitive C-fibre stimulation despite the constant input. Thus the activation of this class of receptors brings about a marked increase in neuronal excitability and is responsible for the amplification and prolongation of neuronal responses in the spinal cord. Substantial evidence exists for the involvement of NMDA receptors in various pathological pain states. Studies have demonstrated the effectiveness of NMDA receptor antagonists in animal models of inflammation, neuropathic pain, allodynia and ischemia. Both pre- and postsurgical administration of antagonists were shown to be effective, suggesting that the induction and maintenance of these ongoing pain states are dependent on NMDA receptor-mediated events.

Neuropathy may produce a prolonged activation of NMDA receptors, due to a sustained afferent input to the spinal cord, and this may result in a relatively small but continuous increase in the extracellular level of glutamate. As ketamine is a licensed drug which use-dependently blocks the NMDA receptor channel, the positive effects of this drug in patients with neuropathic pain (although not without side-effects) would strongly suggest that the NMDA receptor is as important in sensory processing of painful events in humans as suggested by the animal work.

MEMORY

It is generally accepted that long-term potentiation (LTP) is a key event in the processes that lead to the laying down of memories in the brain. LTP is a long-lasting enhancement of synaptic effectiveness that follows certain types of tetanic electrical stimulation to input pathways into the hippocampus. Although much of the work has been based on the hippocampus, where it was first documented, LTP has also been described in areas such as cortex, amygdala and spinal cord. The consensus would be that synaptic activation during high-frequency stimulation triggers a series of intracellular events that lead to the expression of synaptic potentiation with the release of glutamate being the first step. This persistent increase in synaptic efficacy is thought then to be critical for memory, presumably the acquisition. Much of the vast literature is based on electrophysiology, mainly *in vitro*, and so despite the conceptual appeal of LTP, the functional studies find it much harder to link LTP with memory. From a large clinical literature, the hippocampus appears to be a key structure in memory, and blocking glutamate receptors causes reduced memory-like behaviour in animals. Also the more recent description of activity-dependent long-term depression (LTD) could be associated with the processes of forgetting. However, this may be overly simple since LTD also occurs in the cerebellar cortex and might contribute to the motor aspects of learning in animals. LTD is reversibly blocked by NMDA receptor antagonists which suggests that postsynaptic Ca^{2+} entry through the NMDA receptor channel is critical for LTD induction.

Nearly all mechanistic studies of LTP have been carried out in the CA1 region of hippocampal slices, where Schaffer collateral/commissural fibres make monosynaptic contacts with the dendrites of CA1 pyramidal cells. It is generally accepted that the

Table 10.1 Classification of NMDA receptors

	Metabotropic	Ionotrophic		
		NMDA	AMPA	Kainate
Channel		Sodium calcium	Sodium*	Sodium*
Endogenous agonist	Glutamate	Glutamate	Glutamate	Glutamate
Other agonists	Quisqualate	NMDA	AMPA Kainate	Kainate
Antagonists	L-AP3	MK-801 Memantine Ketamine Dextrophan CPP 7-CK (glycine site)	CNQX NBQX	LY382884

Abbreviations: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), L(+)-2 amino-3-phosphonopropionic acid (L-AP3), 6-cyano-7-nitroquinoxaline (CNQX), 2,3-dihydroxy-6-nitro-7-sulfamyl-benzo-f-quinoxaline (NBQX), 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 7 Chlorokynureic acid (7-CK).
*Some AMPA and kainate receptors are calcium permeable. Some of the antagonist structures are shown in Fig. 10.4.

induction of LTP at this synapse requires activation of postsynaptic NMDA receptors by synaptically released glutamate during adequate postsynaptic depolarisation. This results in the relief of the voltage-dependent block of the NMDA receptor channel by Mg^{2+}. Ca^{2+} then enters the postsynaptic neurons or dendrite as a necessary and perhaps sufficient trigger for LTP. Although the NMDA receptor channel may be the critical entry point for the Ca^{2+} involved in triggering LTP, activating voltage-dependent calcium channels during NMDA receptor blockade can also cause an increase in synaptic efficacy. Once induction of LTP has occurred, the maintenance of LTP is then non-NMDA receptor dependent, favouring the idea of intracellular mechanisms as key factors.

It has also been suggested that activation of mGluRs enhances NMDA receptor-mediated LTP and there is also good evidence that switching off GABA mechanisms is also a prerequisite. One of the most controversial areas in the study of the mechanisms of LTP has been the search for a so-called retrograde messenger, a factor that is released from the postsynaptic neuron and diffuses back across the synapse to modify neurotransmitter release from the presynaptic terminal. The necessity for the existence of such a messenger was first suggested by the finding that LTP was associated with an increase in the concentration of glutamate in perfusates. Although there is data that the candidate retrograde messenger could be arachidonic acid, most recent work indicates the gas NO (nitric oxide), although there is almost as much evidence against as for this molecule (see Chapter 13). Postsynaptic NMDA receptors are involved in the induction of both LTP and both forms of plasticity appear to need retrograde messengers, and use common intracellular events — what occurs when these mechanisms converge will then determine whether neurons become potentiated or depressed.

On the basis of the events that occur in pain and LTP, it is easy to see how the actions of glutamate relate to the excessive firing of neurons — as yet no NMDA receptor antagonist has been tested in human epilepsy.

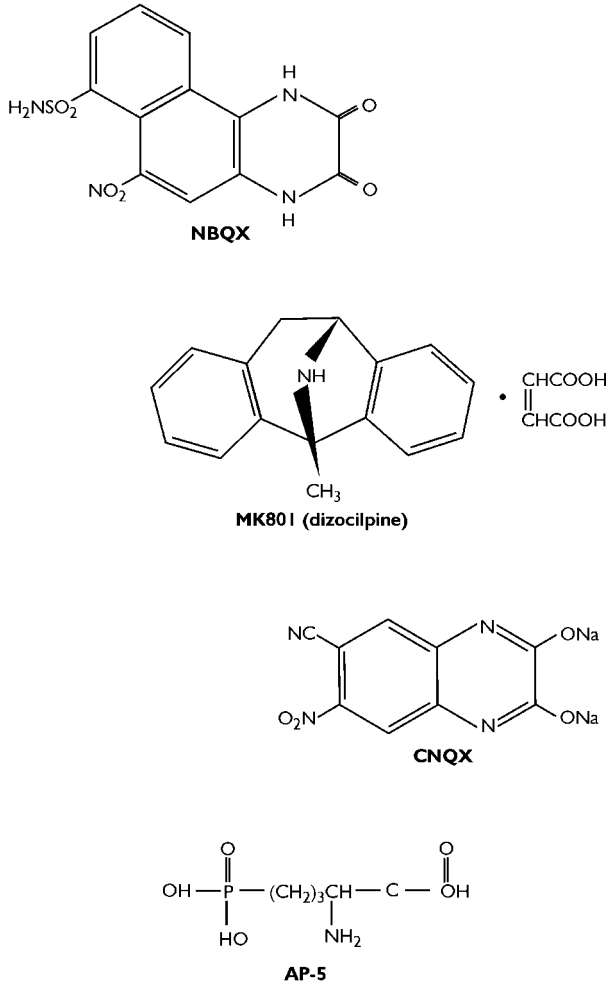

Figure 10.4 Structures of some antagonists at the various receptors for glutamate. CNQX is an AMPA antagonist but NQQX has greater selectivity. AP-5 is an NMDA receptor antagonist while MK-801 blocks the NMDA receptor channel (non-competitive)

EXCITOTOXICITY

The final issue relating to the function of the NMDA receptor is excitotoxicity. Briefly, the depolarisation may drive neurons into a state where large quantities of calcium enter the neuron. For this to occur, the release of glutamate would have to be excessive and in this context, cerebral ischaemic episodes are thought to disrupte the reuptake of glutamate into neurons and glia. The consequent influx of calcium, if excessive, can bring water into the neuron as a result of the cation entry. These osmotic changes can then lead to swelling and damage to the cell, although if the neuronal activity is reduced, then the osmotic stress is reversible. A second delayed phase of neuronal damage then occurs in that intracellular signalling is driven by the high calcium levels

leading to permanent destruction of the neuron. A number of culprits have been identified, including activation of kinases, phospholipases leading to the generation of arachidonic acid and free radicals, nitric oxide synthase and also lipases and proteases.

The overactivation of glutamate receptors is therefore thought to be a key initial step in the neuronal and glial cell loss following cerebral vascular accidents. Despite this, the trials of NMDA receptor antagonists in patients with brain ischemia had so far been disappointing with poor efficacy and marked side-effects. Both factors could be improved by targeting NMDA receptor subtypes but it may be that AMPA and kainate receptors also have key roles in excitotoxicity. Another issue is that even when NMDA receptors are blocked the influx of calcium through voltage-gated calcium channels may induce neuronal damage.

It is unclear as to what extent events such as these are responsible for the cell death seen in neurological disorders like Parkinson's, Huntington's and Alzheimer's diseases. However, a combination of motoneuron disease, dementia and a Parkinson-like syndrome was possibly triggered by a constituent of the cyclad seed, used in Guam in times of famine for which the most likely candidate appears to be an excitatory amino-acid agonist. Certainly, there is evidence for a defect in mitochondrial energy metabolism in PD which may lead to neuronal depolarisation and an easier removal of the voltage-dependent Mg^{2+} block of the NMDA receptor. The resulting excessive neuronal excitation may contribute to nigrostriatal cell death. Whatever the case, once PD is established, the corticostriatal and subthalamofugal pathways, that use glutamate, are overactive. In MPTP-treated monkeys both NMDA and non-NMDA antagonists have efficacy. In rats, NR2B antagonists stimulate motor function.

Finally, AIDS dementia has parallels with cerebral ischemia or stroke and again the key mechanism appears to involve overactivation of glutamate receptors, in particular the NMDA receptor, followed by excessive influx of calcium and the generation of free radicals.

Clearly there is much therapeutic potential for drugs acting on glutamate systems but much more progress is needed.

DEVELOPMENT

Glutamate receptor expression is developmentally regulated and glutamate-mediated neurotransmission is generally enhanced in the immature brain at ages when certain glutamate receptors are transiently overexpressed. Furthermore, receptor subunit composition differs compared to the adult. Glutamate receptors play a critical role in neuronal plasticity and activity-mediated growth during brain development and yet the immature brain is more vulnerable than the adult to excitotoxic neuronal injury, suggesting that the functional state of glutamate receptors modifies the response of the brain to injury. In view of the general role of glutamate in the development and plasticity of connections in the immature CNS it is perhaps surprising that as yet we know little of its function apart from a pivotal role in the organisation of sensory pathways. In the CNS, NMDA receptor binding is much more restricted in the adult than in young animals although in broad terms, affinity appears the same as in the adult. In the immature hippocampus, NMDA EPSPs are much greater in amplitude and significantly less sensitive to Mg^{2+} although glycine modulation appears the same as in the adult. In the spinal cord, during the first postnatal week, NMDA and NMDA induced elevations of $[Ca^{2+}]_i$ are markedly elevated, gradually declining to adult levels

although the AMPA response or resting $[Ca^{2+}]_i$ do not show these developmental changes. The neonatal brain represents a unique problem because any therapy based on glutamate receptors will somehow have to avoid adverse effects on the physiological roles of these receptors in plasticity and synaptic development.

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