NEUROPHARMACOLOGY

Emilio Badoer

School of Medical Sciences, RMIT University, Melbourne, Victoria, Australia

Keywords: Glutamate, GABA, ligand gated receptors, metabotropic G protein coupled receptors, glutamate agonists, GABA agonists, glutamate antagonists, GABA antagonists.

Contents

Summary

Glutamate and GABA, respectively, are the most common excitatory or inhibitory neurotransmitters in the central nervous system. Post transcriptional and post translational modifications of glutamate and GABA receptors means there are many potential receptor subtypes and their distribution and abundance varies widely, and it is not clear whether all are functional normally.

There are four main subtypes of receptors, three of which are ligand gated ion channels, known as NMDA, AMPA and Kainate receptors, and the last is a G protein coupled receptor which are divided into three groups (I-III) based upon their sequence homology. NMDA receptors contribute to the symptoms of a number of diseases/conditions that result from neuronal excitability, excitotoxicity and neuronal death, including Alzheimer's disease, cerebral ishaemia/hypoxia and epilepsy. NMDA receptors are also important for anesthesia. Antagonists of the NMDA receptor subtype have been useful in reducing neuronal damage and loss following hypoxic/ischemic insults in preclinical testing. However, clinical studies have had disappointing outcomes to date. Some commonly used antagonists of NMDA receptors include (i) memantine, which is in clinical use for the treatment of Alzheimer's disease, and (ii) ketamine, which is a commonly used anesthetic. The role of AMPA receptors in disease are similar to that

ascribed to the NMDA receptors and is probably related to the functional interactions of these two different glutamatergic receptors. The role of kainate receptors in disease is not understood. Metabotropic glutamate receptors may be involved in psychiatric and neurological disorders.

For GABA receptors there are two ligand gated ion channels (GABA $_A$ and $_C$ receptors) and one group of G protein coupled receptors (GABA$_B$). Activation of GABA receptors results in the stabilization of the neuronal membrane, making it less likely for the neuron to be activated. Thus, conditions/diseases characterized by overexcitability of neurons, such as epilepsy, anxiety, insomnia and schizophrenia, have GABA receptors as the main therapeutic target. Commonly used drugs such as benzodiazepines, barbiturates, neurosteroids and alcohol act by enhancing the action of GABA (mainly at the GABA$_A$ receptor). Benzodiazepines, barbiturates and neurosteroids, each act on different sites of the GABA$_A$ receptor complex. Drugs that affect the GABA$_C$ and GABA$_B$ receptors have similar effects to those that act on GABA$_A$ receptors, and these may become therapeutically important as more selective agents are discovered.

1. Introduction

The discipline of neuropharmacology can be broadly defined as the effect of drugs on the nervous system. This topic is diverse and large and includes the effects of neurochemicals such as amines, neuropeptides, purines, acetylcholine, histamine and many others. For a discussion of neurochemicals, the reader is referred to recent reviews (Aston-Jones, 2005; Ballard *et al.*, 2005; Girault *et al.*, 2004; Hökfelt *et al.*, 2003). The topics on anaesthetics, pain and analgesic and Neuropsychopharmacology in this pharmacology theme cover focused neuropharmacological issues related to these systems. This article will be restricted to the discussion to the effects of the excitatory amino acid, glutamate, and the inhibitory amino acid, gamma-aminobutyric acid (GABA) on the central nervous system given their important role and also that they exhibit basic features which describe the general principals of neurotransitters including receptor systems, reuptake, and signal transduction. The role of glutamatergic and GABAergic receptors in disease, and the effects of agonists and antagonists on these receptors are also discussed.

2. Excitatory Amino Acids

Glutamate is the principal excitatory amino acid neurotransmitter in the central nervous system. It has a wide distribution and is made from the conversion of glucose via the tricarboxylic acid cycle or glutamine that is synthesized in astrocytes. Glutamine lacks pharmacological activity but can be taken up by neurons and converted into glutamate. Neurotransmitter glutamate is stored in vesicles and released via a Ca^{2+}-dependent mechanism. The actions of released glutamate can be terminated by its reuptake back up into neurons or into astrocytes. This process is carrier mediated involving $Na^+/Cl^-/H^+$ co-transport and carriers known as excitatory amino acid transporters (EAATs), of which there are several subtypes. Transport of glutamate into vesicles is accomplished by vesicular glutamate transporters (VGLUTs).

There are five different EAATs which show approximately 50% homology. Each

contains 500-600 amino acids making 6-8 transmembrane domains, re-entrant loops and cytoplasmic N- and C-terminal domains. The precise topology, however, remains in dispute. The EAATs differ in their specificity (aspartate is the preferred amino acid in some brain regions) and in their distribution within the brain. Drugs that alter the expression of these transporters, or proteins (eg kinases) that are critical in the localization of the transporter in the plasma membrane can interfere with the synaptic transmission of glutamate and, therefore, the responses to the excitatory amino acid.

There are three families of VGLUTs at present. They share about 70% homology with each other and each contains about 600 amino acids. VGLUTs contain 8-10 subunits which are all believed to be transmembrane domains. VGLUTs have a much lower affinity for glutamate, of approximately 100-1000 fold compared to EAATs, and they do not transport aspartate, in contrast to EAATs. Transport of glutamate into vesicles by VGLUTs is dependent upon a vesicular proton, ATPase, to generate a proton electrochemical gradient. The distribution of VGLUTs within the central nervous system differs; VGLUT 1 is more abundant in cerebral and cerebellar cortex and the hippocampus, VGLUT 2 is found predominantly in the diencephalon and rhomencephalon, and VGLUT 3 has been identified in the striatum, hippocampus and cortex. VGLUT 3 has also been identified in the brainstem and it has recently been hypothesized to be a marker of neurons in the brainstem that mediate thermoregulatory responses (Nakamura *et al.*, 2004). The development of new compounds capable of selectively inhibiting these carrier molecules may contribute to understanding the physiological functions of the carriers in synaptic function.

2.1. Receptors for Glutamate

Glutamate can act on four main subtypes of receptors; three are ion channels and referred to as ionotropic receptors, and one subtype is a G-protein coupled receptor referred to as a metabotropic receptor. The three ionotropic receptors have been named according to their specificity for agonists, namely N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5- methyl-4-isoxazole-propionate (AMPA) and Kainate. The ionotropic receptors consist of –4-5 subunits arranged to form a pore. Each subunit contains a pore loop (a hair pin loop within the plasma membrane, entering and exiting from the intracellular surface) and three transmembrane helical domains. The pore loop lies between the first and second transmembrane domains. A large extracellular region links the second and third transmembrane domains. This structure differs from the normal four transmembrane helical domains that characterize ionotropic receptors of classical neurotransmitters, such as nicotinic acetylcholine receptors.

2.1.1. NMDA Receptors

These receptors have been studied in greater detail than other glutamate receptors. There are two predominant subunits that contribute to the formation of NMDA receptors, NR1 and NR2. Recently, a third NR subunit has been described (NR3). This subunit can assemble with NR1 and NR2 subunits to depress NMDA receptor function or can assemble with NR1 alone to form a glycine receptor. The NR1 subunit consists of eight variants produced by splicing at three sites. NR2 subunits are formed by four different genes that encode the four different subunits (NR2 A-D). Each subunit can

also undergo post-transcriptional and post-translational modification, including glycosylation and phosphorylation. These changes alter the kinetics and ionic performance of the ion channel. Furthermore, different isoforms may be differentially expressed in different areas or developmental stages.

The NMDA receptor is unique among ionotropic receptors in that it requires the binding of two ligands (glutamate and glycine (paradoxically) and reduced affinity for Mg^{2+} ions (usually via prior depolarization) to open. The NR1 subunit contains the glycine binding site and the NR2 subunit contains the glutamate binding site. The majority of evidence suggests that the NMDA receptor is composed of tetramers, that is, two NR1subunits and two NR2 subunits. Thus, each receptor contains two glycine and two glutamate binding sites. There are numerous isoforms of the receptor, since different isoforms of the NR1 subunit can combine and assemble with different isoforms of the NR2 subunits. Assembly of the NMDA receptor occurs in the endoplasmic reticulum, which contains abundant NR1 subunits. Since these can contain retention motifs that delay/prevent assembly and trafficking to the cell plasma membrane, this is the likely reason for the abundance of NR1 in the endoplasmic reticulum.

NMDA receptors also interact with numerous proteins that influence the functional properties, as well as the trafficking and synaptic organization, of the receptor. Examples include membrane–associated guanylate cyclases, which include PSD-93 and PSD-95, that are important in the protein scaffolding interactions that are involved in the post synaptic density region, and in the trafficking of the receptor from the endoplasmic reticulum to the plasma membrane.

2.1.1.1. Physiological Effects Of NMDA Receptors

The NMDA receptor is highly permeable to Ca^{2+} and other cations and when activated results in 'slow' excitatory post synaptic potentials (EPSPs). Under physiological conditions, if the cell is at resting membrane potential, the NMDA receptors are blocked by normal physiological concentrations of Mg^{2+} ions, even in the presence of glutamate and glycine. However, the inhibitory actions of Mg^{2+} are voltage sensitive. Thus, when the cell is partially depolarized, e.g. by glutamate acting on AMPA receptors (see below), which results in sodium entering the cell, the inhibitory actions of Mg^{2+} can be overcome and the NMDA receptor can be activated (in the presence of glycine) and the entry of calcium into the cell occurs. The entry of calcium mediates most of the physiological actions of NMDA receptor activation. It should also be noted that, as well as the glutamate and glycine binding sites, the activity of NMDA receptors can be modified by drugs which are described in the sections below.

In general, NMDA receptors play an important role in long term plasticity (long term potentiation or depression) (Massey *et al.*, 2004). Long term potentiation is a long lasting enhancement of the effects of post synaptic transmission. It requires a short preconditioning burst of high frequency presynaptic activity and simultaneous post synaptic activity to occur, and is believed to be important in learning and memory. The role of NMDA receptors appears to be through the enhancement of AMPA receptors; that is, released glutamate activates AMPA receptors leading to the removal of the magnesium block of the NMDA receptor. This allows glutamate to activate NMDA

receptors to produce increased calcium entry into the cell, which ultimately facilitates AMPA receptor function (via protein kinase C activation and phosphorylation of AMPA receptors).

2.1.1.2. Role Of NMDA Receptors In Disease

In general, NMDA receptors are important in cerebral ischemic damage, memory formation and loss, neurotoxicity, pain and epilepsy, and also may play a role in diseases such as Huntingtons's disease and Parkinson's disease.

Brain damage due to cerebral ischaemic damage

Cerebral hypoxia or ischemia can lead to cellular damage that ultimately results in neuronal death. During hypoxia or ischemia there is a persistent elevation of glutamate that is believed to initiate the process of necrosis that is responsible for the cell death observed. These high levels of glutamate depolarize the cells and disturb their ability to maintain ionic and water balance, which can induce swelling within minutes, and ultimately lysis. The lysed cells result in further glutamate release, further exacerbating the neuronal cell death. However, apart from the immediate focal death of neurons that occurs, most cells that initially survived die after 24 hours of the initial insult. At that time, glutamate and calcium levels have returned to normal. Thus it would appear that multiple mechanisms are involved in the processes initiated by glutamate, however, there is clear evidence indicating that increased glutamate acting on NMDA receptors induces an increased intracellular concentration of calcium that leads to cellular death. Indeed, the concentration of calcium closely correlates with the degree of cell death.

The evidence that multiple mechanisms are involved in the process of necrosis seen after hypoxia and ischemia is further supported by the fact that elevated glutamate levels may not be necessary to initiate cell death, however, the activation of NMDA receptors is necessary to evoke excitotoxicity and cell death. The activation of NMDA receptors results in elevated intracellular calcium concentration. For excitotoxicity to occur, the elevated calcium levels need to take place in the vicinity of the NMDA receptor. This is believed to ultimately lead to downstream changes specific for the activated NMDA receptor and calcium level, since the elevation of calcium concentration induced by other means does not lead to excitotoxicity. For this reason, alterations in the downstream enzymes are believed to take place during excitotoxicity.

NMDA receptor subunits NR1 and NR2 confer localization and functionality to the receptor. These subunits link to specific enzymes and proteins docking to the post synaptic density zone associated with the post synaptic membrane. NMDA is intimately associated with PSD-95 and this is associated with enzymes and proteins including Pyr and trkB kinase, neuronal nitric oxide synthase, the enzyme responsible for the conversion of L-arginine to the gaseous neurotransmitter, nitric oxide. Other proteins associated with PSD-95 link the NMDA receptor to intracellular structural proteins such as alpha tubulin, and other transduction/second messenger systems such as CAM kinase II. The NR2A and NR2B subunits appear to be the crucial elements linking the NMDA receptor to the PSD-95 and associated proteins and it is now suspected that changes in NMDA activity, elevated calcium concentration and alterations in the proteins associated with PSD-95 are all required to induce excitotoxicity and cell death.

Alzheimer's disease
In Alzheimer's disease there is neurodegeneration, and with this is an increase in extracellular glutamate. As described above, this can lead to an overactivation of the NMDA receptor with the resultant increase in intracellular calcium that can lead to neuronal cell death. Memantine is an NMDA antagonist that has been approved for use in Alzheimer's disease (see below).

Pain and morphine tolerance
Pain pathways involve glutamate release in the dorsal horn. Enhanced glutamate release resulting in the activation of NMDA receptors can elevate intracellular calcium, resulting in the activation of kinases and ultimately morphine tolerance. Blockade of NMDA receptors can reduce pain and morphine induced tolerance. (The reader is also referred to the chapter entitled Pain Pharmacology and Analgesia).

Epilepsy
The NMDA receptor appears to be critical to the increased excitability seen in animal models of epilepsy. There is evidence to show that NMDA receptor density and function is altered in epilepsy. In some human forms of epilepsy, the expression of the subunits of the NMDA receptor is increased in the focal region of epileptic activity. In animal models of epilepsy, the activity of the NMDA receptor is increased and antagonists of the receptor can reduce the epileptic activity. Recent studies also suggest that epileptic activity can alter the function of the NMDA receptor by influencing its interactions with post synaptic density proteins via altered phosphorylation of residues in the NMDA receptor.

Huntington's disease
Huntington`s disease is a genetic disorder in which the protein huntingtin is mutated. This protein can bind to PSD-95 that can attach to the NMDA receptor. Huntingtin can reduce the excitotoxic actions of NMDA receptor activation through its interaction with PSD-95. The current view suggests that the mutated form of huntingtin is less capable of this action, thereby allowing the excitotoxic actions of NMDA receptor activation to be more dominant.

Parkinson`s disease
Parkinson`s disease is a progressive neurological condition in which the dopaminergic input to the striatum degenerates. The glutamatergic action on NMDA receptors within the striatum appears to be important in the pathogenesis of Parkinson`s. However, the mechanism is unclear at present, but there is evidence to suggest that the NR2 B subunit of the NMDA receptor is involved.

2.1.1.3. Agonists of NMDA Receptors

The most commonly used agonist of NMDA receptors is, not surprisingly, NMDA. It is, by definition, more selective for this glutamatergic receptor but less potent than glutamate. However, as NMDA is not a substrate for glutamate reuptake mechanisms, its action is more effective than glutamate. Other agonists of the NMDA receptor include L-aspartate, quinolinate and homocysteate. Activation of NMDA receptors may cause symptoms of depression, agitation and impaired cognition in animal models,

although activation of NMDA receptors has been postulated as a mechanism for positive benefits in schizophrenia.

-
-
-

TO ACCESS ALL THE **24 PAGES** OF THIS CHAPTER,
Visit: http://www.eolss.net/Eolss-sampleAllChapter.aspx

Bibliography

Aston-Jones, G (2005). Brain structures and receptors involved in alertness. Sleep Medicine 6. [A review on the mechanisms underlying alertness and the role of noradrenergic systems in the brain].

Ballard, C, Greig, N, Guillozet-Bongaarts AL, Enz A, S., D (2005). Cholinesterases: roles in the brain during health and disease. Current Alzheimer Research 3: 307-318. [A summary of the involvement of acetylcholine actions in the brain and its role in Alzheimer's disease].

Barnard, EA, Skolnick, P, Olsen, RW, Mohler, H, Sieghart, W, Biggio, G, Braestrup, C, Bateson, AN, Langer, SZ (1998). International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function. Pharmacological Reviews 50: 291-313. [A comprehensive summary of GABA A receptors].

Benneyworth, M, Xiang, Z, Smith, R, GArcia, E, Conn, P, Sanders-Bush, E (2007). A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. Molecular Pharmacology 72: 477-484. [Article presenting data in support of the targeting of mGlu2 receptors as a novel strategy for treating glutamatergic dysfunction in schizophrenia].

Chen, J, Naylor, D, Wasterlain, C (2007). Advances in the pathophysiology of status epilepticus. Acta Neurology Scandinavica 186. [A good review on recent advances in epilepsy].

Couve, A, Thomas, P, Calver, AR, Hirst, WD, Pangalos, MN, Walsh, FS, Smart, TG, Moss, SJ (2002). Cyclic AMP-dependent protein kinase phosphorylation facilitaes GABA-B receptor-effector coupling. Nature Neuroscience 17: 415-424. [An article discussing the signaling pathways that may have profound effects on GABA(B) receptor-mediated synaptic inhibition].

Creeley, C, Wozniak, D, Labruyere, J, Taylor, GT, Olney, JW (2006). Low doses of memantine disrupt memory in adult rats. Journal of Neuroscience 26: 3923-3932. [Article discussing neuroprotective action of memantine highlighting the accompanying intolerable side effects].

Gigler, G, Moricz, K, Agoston, M, Simo, A, Benedek, G, Kapus, G, Kertesz, S, Vegh, M, Barkoczy, J, Szabo, G, Matucz, E, Gacsalyi, I, Levay, G, Harsing, L, Szenasi, G (2007). Neuroprotective and anticonvulsant effects of EGIS-8332, a non-competitive AMPA receptor antagonist, in a range of animal models. British Journal of Pharmacology 152: 151-160. [Description of a novel AMPA receptor antagonist].

Girault, J, Greengard, P (2004). The neurobiology of dopamine signalling. Archives in Neurology 61: 641-644. [A broad review on dopamine actions in the brain].

Hökfelt, T, Bartfai, T, Bloom, F (2003). Neuropeptides: opportunities for drug discovery. Lancet Neurolology 8. [Reviews literature of neuropeptides in central nervous system].

Lavreysen, H, Dautzenberg, F (2008). Therapeutic potential of group III metabotropic glutamate receptors. Current Medicinal Chemistry 15: 671-84. [Reviews literature on metabotropic glutmate receptors]

Lees, KR, Asplund, K, Carolei, A, Davis, SM, Diener, HC, Kaste, M, Orogogozo, JM, Whitehead, J

(2000). Glycine antagonist (gavestine) in neuroprotection (GAIN International) in patients with acute stroke: a randomised control trial. GAIN International Investigators. Lancet 355. [Results of clinical trial highlighting major drawback in utilizing NMDA antagonists as therapeutically useful agents].

Lipton, SA (2006). Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nature Reviews Drug Discovery 5: 160-170.[Excellent review discussing novel approaches to NMDA receptor inhibition].

Loup, F, Wieser, H-G, Yonekawa, Y, Aguzzi, A, Fritschy, J-M (2000). Selective alterations in GABA A receptor subtypes in human temporal lobe epilepsy. Journal of Neuroscience 20: 5401-5419. [This discusses GABA receptors and epilepsy].

Massey, P, Johnson, B, Moult, P, Auberson, Y, Brown, M, Molnar, E, Collingridge, G, Bashir, Z (2004). Differential Roles of NR2A and NR2B-Containing NMDA Receptors in Cortical Long-Term Potentiation and Long-Term Depression. The Journal of Neuroscience 24: 7821-7828. [This provides evidence that NMDA receptors with different synaptic locations and subunit compositions are involved in various forms of synaptic plasticity in adult cortex].

Matrisciano, F, Panaccione, I, Zusso, M, Giusti, P, Tatarelli, R, Iacovelli, L, Mathe, A, Gruber, S, Nicoletti, F, Girardi, P (2007). Group-II metabotropic glutamate receptor ligands as adjunctive drugs in the treatment of depression: a new strategy to shorten latency of antidepressant medication. Molecular Psychiatry 12: 704-6. [Descibes potential use of Group II metabotropic receptor ligands for depression].

Nakamura, K, Matsumura, K, Hubschle, T, Nakamura, Y, Hioki, H, Fujiyama, F, Boldogkoi, Z, Konig, M, Thiele, H-J, Gerstberger, R, Kobayashi, S, Kaneko, T (2004). Identification of sympathetic premotor neurons in the medullary raphe regions mediating fever and other thermoregulatory functions. Journal of Neuroscience 24: 5370-5380. [This describes the use of glutamate uptake transporter as a marker of neurons involved in fever].

Palucha, A, Pilc, A (2007). Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. Pharmacology and Therapeutics 115: 116-147. [This describes potential therapeutic actions of metabotropic glutamate receptor ligands].

Rodriguez, A, Williams, R (2007). Recent progress in the development of allosteric modulators of mGluR5. Current Opinion in Drug Discovory and Development 10: 715-722. [Describes evidence for selective agonists of group II metabotropic glutmate receptors as useful anxiolytic agents].

Rozzo, A, Armellin, M, Franzot, J, Chiaruttinni, C, Nistri, A, Tongiorgi, E (2002). Expression and dendritic mRNA localisation of the GABA C receptor rho1 and rho2 subunits in developing brain and spinal cord. European Journal of Neuroscience 15: 1547-1558. [Describes expression of GABA receptor subunits].

Saltarelli, M, Weaver, J, Hsu, C, Bednar, M (2004). Randomized double blind, placebo controlled study to evaluate the safety and efficacy of CP-101606 (traxoprodil), an NR2B selective N-methyl-D-aspartate antagonistin subjects with acute stroke. Stroke 35: 241. [Describes clinical results with ifenprodil analogue in the prognosis following stroke].

Stranger, H, Alford, R, Jane, D, Cunningham, M (2008). The Role of GLUK5-Containing Kainate Receptors in Entorhinal Cortex Gamma Frequency Oscillations. Neural Plasticity Published online 2008 November 17. doi: 10.1155/2008/401645. [Describes analogues that are selective for the kainate receptor and may show selectivity for specific isoforms].

Tretter, V, Ehya, N, Fuchs, K, Sieghart, W (1997). Stoichiometry and assembly of a recombinant GABAA recector subtype. Journal of Neuroscience 17: 2728-2737. [Describes arrangement of GABA A receptor subunits]

Wang, CX, Shuaib, A (2005). NMDA/NR2B selective antagonists in the treatment of ischaemic brain injury. Current Drug Targets. CNS Neural Disorders 4: 143-151. [Describes great promise of NMDA antagonists in laboratory tests in which infarct volume was significantly reduced following brain ischemia].

Biographical Sketch

Emilio Badoer BSc (Hons). PhD., Professor Badoer is head of the Neuropharmacology and Neuroinflammatory Laboratory, School of Medical Sciences, RMIT University. The laboratory consists of Post-Doctoral Fellows, PhD students and Honours students. Research assistants and international Postdoctoral Fellows are also part of the team. The group has received national and international recognition for their work, which has highlighted the role of specific subgroups in the brain and the role of the brain in the symptoms of chronic diseases/conditions. The group has a number of successful collaborative projects.

Professor Badoer is the Program Coordinator for the B Sc App Sci (Pharmaceutical Sciences) program. He is currently a member of the Science Engineering and Technology College Research committee of the University and Chair of the Research Committee of the School. He is also a research scientist representative on the University Animal Ethics Committee. He has been leader of the Postgraduate Research Program and the Honours Program for the School of Medical Sciences and convened the Research in Progress Seminar Program for the School.

Professor Badoer is an expert reviewer for numerous national and international grant funding agencies and journals. He has had grant and award successes that have included national competitive grants (including National Health and Medical Research Council, National Heart Foundation, Ramaciotti Foundation and Buckland Foundation) and international competitive grants and awards (including Wellcome and von Humboldt). Professor Badoer is also an Associate Research Fellow of two prestigious Research Institutes. His laboratory is currently funded by the National Health and Medical Research Council of Australia.