Structure and trafficking of NMDA and GABA<sub>A</sub> receptors

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Abstract
The fidelity of synaptic function is dependent on the expression of the appropriate neurotransmitter receptor subtype, the targeting and trafficking of receptors to synapses as well as the regulation of the actual number of receptors at synapses. GABA<sub>A</sub> (γ-aminobutyric acid type A) receptors and NMDA (N-methyl-D-aspartate) receptors are both examples of ligand-gated, heteromeric neurotransmitter receptors whose cell-surface expression is dynamic and tightly regulated. NMDA receptors are localized at excitatory synapses. These synapses are highly structured but dynamic, with the interplay between NMDA receptors and NMDA receptor-associated scaffolding proteins regulating the expression of functional cell-surface synaptic and extrasynaptic receptors. Based on current information, inhibitory synapses seem to be less ordered, and a GABA<sub>A</sub> receptor equivalent of PSD-95 (postsynaptic density-95), the scaffolding molecule pivotal to the organization of NMDA receptor complexes at synapses, is yet to be validated. In the present paper, processes regulating the trafficking, assembly and molecular organization of both NMDA receptors and GABA<sub>A</sub> receptors will be discussed.

Introduction
NMDA (N-methyl-D-aspartate) receptors and GABA<sub>A</sub> [GABA (γ-aminobutyric acid) type A] receptors are two of the major classes of ionotropic neurotransmitter receptors of mammalian brain. GABA<sub>A</sub> receptors are the major inhibitory neurotransmitter receptors. They are chloride ion channels activated by the binding of the neurotransmitter, GABA. NMDA receptors are a subclass of the excitatory L-glutamate family of neurotransmitter receptors. They are unique in that they require the simultaneous binding of two neurotransmitters, the co-agonists L-glutamate and glycine, together with the alleviation of a voltage-dependent blockade by magnesium ions that is achieved by the activation of adjacent non-NMDA glutamate receptors in synaptic spines, for channel activation. Both GABA<sub>A</sub> receptors and NMDA receptors are not only pivotal for normal brain function but they are also important drug targets. NMDA receptors have potential as a therapeutic target post-ischaemia and in neuropathic pain; NMDA receptor channels are highly permeable to calcium ions; thus over-activation leads to excitotoxic neuronal cell death, which may be treated by NMDA receptor antagonists. Indeed, memantine, a non-competitive NMDA receptor antagonist, is licensed for use in the treatment of Alzheimer’s disease. NMDA receptor agonists, in line with the glutamate hypofunction hypothesis of schizophrenia, may also be important adjunct drug therapies for the treatment of this disorder. The major tranquillizer drugs, the benzodiazepines (e.g. valium), mediate their action via allosteric potentiation of GABA<sub>A</sub> receptors. Other drugs acting at GABA<sub>A</sub> receptors include hypnotics to aid sleep and anti-epileptics. Since all these classes of therapeutic agents either enhance or decrease receptor activity, it highlights the importance of the control of the trafficking and targeting of an appropriate number of functional neurotransmitter receptors to the cell surface of neurons. Recent advances have begun to unravel the mechanisms that control the assembly, trafficking and targeting of neurotransmitter receptors and indeed have shown that defects in these processes can result in neurological disease. The present paper will give an overview and comparison of our current understanding of the biogenesis of NMDA receptors and GABA<sub>A</sub> receptors and their molecular organization at glutamatergic and GABAergic synapses respectively. It is a short review, so for more in depth description and discussion of both neurotransmitter receptor systems, the following review papers are recommended: for NMDA receptor trafficking, see [1]; for NMDA receptor–PSD-95 (postsynaptic density-95) interactions, see [2]; for GABA<sub>A</sub> receptor structure and pharmacology, see [3,4]; for the organization of GABAergic synapses, see [5]; and for a general review discussing the role of receptor diffusion within the postsynaptic membrane, see [6].

The structures and assembly of NMDA and GABA<sub>A</sub> neurotransmitter receptors

The structure of NMDA receptors
NMDA receptors and GABA<sub>A</sub> receptors are both heteromeric, integral membrane proteins. Seven genes encode
NMDA receptor subunits NR1, NR2A–NR2D and NR3A–NR3B. The NR1 subunit undergoes extensive splicing to yield eight variants NR1-1a,1b to NR1-4a,4b. Functional NMDA receptors are formed from the co-assembly of what is termed the obligatory NR1 glycine-binding subunit with NR2 (glutamate binding) and/or NR3 subunits. The subunits probably assemble as tetramers with stoichiometry (NR1)2(NR2)2 although this has not been unequivocally proven and accepted. NMDA receptor diversity is generated by the association of the obligatory NR1 subunit with various NR2 subunits to yield four major subtypes, NR1/NR2A, NR1/NR2B, NR1/NR2C and NR1/NR2D. In minor subpopulations, two types of NR2 subunit are shown to co-associate within the same receptor, e.g. NR1/NR2A/NR2B. The role of the NR3 subunit is unclear. One report showed that NR1/NR3 subunits formed a novel, glycine-gated receptor but the existence of such an in vivo receptor is yet to be proven [7].

The structure of GABA<sub>A</sub> receptors

For GABA<sub>A</sub> receptors, there are 15 genes encoding receptor subunits that co-assemble in different pentamic combinations to form functional receptors. The major subtype expressed in the adult brain has α<sub>2</sub>(β<sub>3</sub>)<sub>2</sub>(γ<sub>2</sub>) stoichiometry. Receptor diversity is generated predominantly by α or β variant receptors, e.g. α<sub>2</sub>(β<sub>3</sub>)<sub>2</sub>(γ<sub>2</sub>), (α<sub>2</sub>)<sub>2</sub>(β<sub>3</sub>)<sub>2</sub>(γ<sub>2</sub>) and (α<sub>1</sub>)<sub>2</sub>(β<sub>3</sub>)<sub>2</sub>(γ<sub>2</sub>), or by the replacement of the γ<sub>2</sub>-subunit by e.g. δ-subunit in α<sub>2</sub>β<sub>3</sub>δ receptors. But, as for NMDA receptors, there are examples in minor receptor subpopulations of the co-existence of two types of subunit isoform such as in e.g. α<sub>1</sub>(γ<sub>2</sub>)<sub>2</sub>γ<sub>2</sub> receptors. The pertinent features of the families of NMDA receptors and GABA<sub>A</sub> receptors are summarized in Figure 1.

Both NMDA receptor and GABA<sub>A</sub> receptor subtypes undergo developmental and temporal expression and each subtype has characteristic functional and pharmacological properties. Thus the fidelity of synaptic function is dependent on both the expression of the appropriate neurotransmitter receptor subtype in the appropriate neuronal cell type at the right point in development and the targeting and trafficking of these defined receptors to the appropriate subcellular compartment, usually synapses, as well as the regulation of the actual number of receptors expressed in the plasma membrane. Further, evidence is now emerging that synapses are dynamic and the lateral mobility of receptors between synaptic and extrasynaptic membranes is an additional consideration in the understanding of the organization of the postsynaptic membrane.

The assembly of NMDA receptors

The expression of functional NMDA receptors and GABA<sub>A</sub> receptors is dependent on the transcription of the appropriate genes, the co-assembly of the appropriately folded subunits in the ER (endoplasmic reticulum), post-translational modifications and subsequent export to the cell surface. As described above, NMDA receptors are formed by the co-assembly of both NR1 subunits and NR2 subunits. It has been shown that there is a pool of unassembled intracellular NR1 (predominantly NR1-1a, the most abundantly expressed splice form) subunits now known to be a result of an ER signal retention signal in the NR1 C1 cassette found in NR1-1a, -1b, -3a and -3b splice variants (e.g. [8]). Transcription of appropriate NR2 subunit genes is therefore the first rate-limiting step in NMDA receptor biogenesis. The formation of functional receptors is then proposed to involve disulfide-linked NR1–NR1 dimer formation in the ER, followed by association of this dimer pairing with NR2–NR2 dimers to yield the mature quaternary receptor structure [9,10]. The N-terminal LIVBP (leucine/isoleucine/valine-binding protein) region distal to the membrane, NR1-1a-(1–380), has been identified as being a requisite for NR1-1a–NR2A subunit association [11]. It is within this domain that the NR1–NR1 inter-subunit disulfide bond via C-79 has been suggested to form [9]. Further, misfolding of this domain results in impaired cell-surface trafficking, showing that quality control mechanisms within the ER are operational during the biogenesis of NMDA receptors [9]. The NR1-2a, -2b, -4a and -4b splice variants do not have the C1 cassette and they do not therefore have an ER retention sequence and can be trafficked to the cell surface independently of NR2 subunits in both heterologous expression systems and in neurons (e.g. [8]). There is no evidence to show that these purported NR1 homomers are functional; thus their significance is unclear. As well as regulatory sequences within the NR1 subunit being important in the assembly of functional NMDA receptors, multiple regulatory sequences have been identified in the NR2B subunit [12]. These include again an ER retention sequence and additionally, immediately following the fourth membrane domain, an HLFF (His-Leu-Phe-Tyr) export sequence that contributes to the control of release from the ER of assembled, functional NMDA receptors.

The assembly of GABA<sub>A</sub> receptors

GABA<sub>A</sub> receptor subunits are also known to assemble in the ER. The assembly pathway of these functional pentamers is more complex compared with NMDA receptors because of their extensive subunit heterogeneity. Subunit assembly is mediated by defined motifs within the respective extracellular N-terminal domains that ensure that the appropriate subunits assemble in the correct orientation (e.g. [13,14]). Point mutations within these extracellular domains, as recently reported for certain inherited forms of epilepsy (reviewed in [15]), result in impaired cell-surface trafficking and/or subunit assembly, again highlighting the importance of quality control mechanisms in the expression of the requisite number of assembled functional neurotransmitter receptors. Assembly of GABA<sub>A</sub> receptors is discussed in more depth in the accompanying papers by Connolly and co-workers [15] and Sigel et al. [16].
Molecular organization of NMDA receptor macromolecular complexes and GABA<sub>A</sub> receptors in the postsynaptic membrane

NMDA receptor macromolecular complexes
At synapses, NMDA receptors are tightly associated with a network of proteins that mediate downstream NMDA receptor-mediated signalling events that include activation of calmodulin kinase, calcineurin, protein kinases A and C, the tyrosine kinases Fyn, Src and Pyk2 (proline-rich tyrosine kinase 2), Ca<sup>2+</sup>-mediated gene regulation via activation of the CREB (cAMP-response-element-binding protein), the neuronal nitric oxide synthase neurotoxicity pathway and structural and trafficking proteins including stargazin, motor proteins, actin and myosin (reviewed in [17]). Pivotal to these downstream signalling pathways are the PSD-95, MAGUK (membrane-associated guanylate kinase) family of proteins (Figure 2). PSD-95 and the other members of this family, Chapsyn-110 (also termed PSD-93), SAP-102 (synapse-associated protein of 102 kDa) and SAP-97 are PDZ domain-containing scaffolding proteins. Association with PSD-95 at synapses has been shown to: (i) promote NMDA receptor clustering; (ii) facilitate appropriate regulation of NMDA receptor activity via phosphorylation/dephosphorylation mechanisms; (iii) regulate NMDA receptor cell-surface

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expression via surface delivery and endocytosis; (iv) inhibit NMDA-receptor-activated cleavage of NMDA receptor subunits by calpain; and (v) modulate NMDA receptor channel gating directly. In addition, this family of scaffolding proteins contributes to cell-surface trafficking of NMDA receptors, i.e. PSD-95 and SAP-102 traffic NR1/NR2B NMDA receptors to the cell surface by association either with KIF17 (kinesin family member 17) via the mLlin family (PSD-95) or with the exocyst complex via sec8 and sec6 (SAP-102); SAP-102 forms a complex with mPins to traffic NR1/NR2B independently of the exocyst complex. Thus the understanding of the molecular organization of NMDA receptor complexes at postsynaptic sites and the contribution that the PSD-95 MAGUK family of proteins makes with regard to the control of cell-surface receptor number is well advanced. Figure 2 is a schematic diagram summarizing the molecular components of postsynaptic NMDA receptor macromolecular signalling complexes.

Molecular organization of GABAergic synapses

The molecular components and organization of GABAergic synapses are less well defined. Despite extensive searches by many groups primarily using yeast two-hybrid cDNA screens, no GABA<sub>A</sub> receptor equivalent to the PSD-95 scaffold protein has, to date, been identified. The best candidate is the protein, gephyrin. Gephyrin is known to cluster glycine receptors at glycinerergic synapses, and evidence suggests that gephyrin also plays a role in the organization of GABA<sub>A</sub> receptors. Gephyrin is clustered at GABA<sub>A</sub> postsynaptic membranes, but in GABA<sub>A</sub> receptor γ<sub>2</sub>-knockout mice, these are diminished, suggesting that gephyrin clusters γ<sub>2</sub> subunit-containing GABA<sub>A</sub> receptors ([18] and reviewed in [5]). A direct association between gephyrin and GABA<sub>A</sub> receptors however has not been demonstrated, nor a possible intermediary linking protein identified. Figure 3 summarizes the major GABA<sub>A</sub> receptor-interacting proteins. Most of these proteins, e.g. GABARAP (GABA<sub>A</sub> receptor-associated protein) [19], HAP-1 (Huntington-associated protein 1) [20] and Plic-1 [21], probably play a role in regulating the surface expression of receptors rather than acting as clustering and scaffolding proteins; GODZ is Golgi-specific DHHC (Asp-His-His-Cys) zinc-finger protein, a palmitoyltransferase enzyme, that also contributes to the regulation of cell-surface receptor expression [22]. My group’s contribution to the field has been the identification of GRIF-1 (GABA<sub>A</sub> receptor-interacting factor 1) [23]. GRIF-1 [also termed TRAK2 (trafficking protein, kinesin-binding 2)] was identified as a GABA<sub>A</sub> receptor β<sub>2</sub> subunit-interacting protein. It is a member of a coiled-coil family of proteins that also includes TRAK1. GRIF-1 and TRAK1 are both kinesin-associated proteins [24]. Interestingly, the Trak1 gene was identified as the mutated gene in hyrt mice, an animal model of hypertonia [25]. Hyrt mice were shown to have a deficit of GABA<sub>A</sub> receptors, thus yielding another example of the importance of the trafficking of the appropriate numbers of functional, assembled neurotransmitter receptors to synapses for normal neuronal function.

Concluding remarks

In this brief paper, the NMDA and GABA<sub>A</sub> neurotransmitter receptor systems have been compared. Although there are significant differences between the two receptor structures and their synaptic organization, it is clear that the mechanisms that govern the assembly and the targeted, cell-surface trafficking of appropriate numbers of receptor proteins are highly regulated and thus important in the maintenance of normal neuronal function. Impairment in any part of these pathways can result in the reduced expression of cell-surface receptors, which then presents as neurological dysfunction.
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References

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