C5

Dopamine D1 receptors inhibit NMDA currents by modulation of receptor trafficking

H. Tong and A.J. Gibb

Pharmacology, University College London, London, UK

Striatal dopamine D1 receptors have been shown to inhibit NMDA currents without G-protein activation (Lee et al. 2002; Tong & Gibb, 2004). We hypothesise that this inhibition may be mediated by a change in NMDA receptor (NMDAR) trafficking. The non-receptor tyrosine kinase, Src, has been shown to be involved in NMDAR internalization (Dunah & Standaert, 2004) and in this study we tested the effect of a potent Src family-selective tyrosine kinase inhibitor (PP2) on D1 modulation of NMDARs. Dynamin also plays an essential role in clathrin mediated receptor endocytosis and we therefore also tested the effect of a dynamin inhibitory peptide (Kittler et al. 2000) on D1 inhibition of NMDA responses.

In striatal slices from 7-day-old rats, responses to 0.01 mM NMDA and 0.01 mM glycine in the presence of TTX (100 nM) were recorded with ATP (1 mM) and GTP (1 mM) in the pipette solution. In the presence of intracellular PP2 (10 μM), D1 inhibition of the NMDA current was significantly reduced from 38 ± 12% (n = 10 cells) to 1 ± 8.2% (n = 9 cells, unpaired t test, $P<0.05$) suggesting non-receptor Src tyrosine kinase activation is involved in D1 receptor inhibition of NMDA responses. Intracellular dynamin inhibitory peptide (QVPSRPNRAP, 0.05 mM) significantly attenuated the D1 inhibition to 3.08 ± 8.15% (n = 9 cells, unpaired t test, $P<0.05$). These results suggest dynamin-dependent endocytosis is important in D1 inhibition of NMDA responses.

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Supported by the Wellcome Trust. H.T. is funded by a UCL Graduate School Research Scholarship.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C6

NMDA receptor subunit requirements in the internalisation of AMPA receptors

C.M. Tigaret, A. Thalhammer and R. Schoepfer

Pharmacology, University College London, London, UK

The NMDA receptor (NMDAR)-dependent regulation of AMPA receptor (AMPAR) membrane trafficking is a key molecular mechanism in forms of synaptic plasticity where NMDAR activation alters the membrane density of AMPARs in parallel to the changes in synaptic strength (1). The signalling that underlies the bidirectional trait of the NMDAR-dependent plasticity is directed by the kinetics of the NMDAR-generated Ca^{2+} transients and also depends on the NR2 subunit composition of the NMDAR complexes (2,3). However, the early determinants of NMDAR signals leading to AMPAR internalisation are largely unknown.

Here, we investigated the requirements for NMDAR subtypes and NMDAR-mediated Ca^{2+} influx in GluR2 AMPARs endocytosis in primary murine hippocampal neurons (4).

Using a live antibody feeding assay and immunofluorescence imaging we found that activation of NMDARs (20 μM NMDA + 10 μM glycine, 2 min) enhanced the internalisation of endogenous GluR2-containing AMPAR as early as 5 min after agonist washout (2.52 ± 0.38, n=17; mean ± s.e.m. of internalised fraction normalised to unstimulated controls, $p<0.001$, two-tailed U test). The effect was comparable for recombinant AMPARs containing N-terminally EGFP-tagged GluR2 with short but not long C-termini, whether being expressed in cultures from wild-type or GluR2 knock-out mice. This indicates that NMDAR activation selectively triggered the internalisation of AMPAR containing GluR2 splice variants with short cytoplasmic domains.

The pharmacological block of NR2B-containing NMDARs with 10 μM ifenprodil completely inhibited the NMDAR-induced endocytosis of GluR2-AMPAR (1.16 ± 0.19, n=19, $p=0.5$ compared to unstimulated controls). In contrast, the NR2A-preferring antagonist NVP-AAM077 (1 μM) (3) did not affect NMDAR-induced GluR2 endocytosis (3.21 ± 0.5, n=20, $p<0.001$ compared to unstimulated controls). However, we found that at the stage when the internalisation assays were performed (14-21 days in vitro), NR2B-containing NMDARs contribute approximatively 25% of the NMDA-induced whole-cell currents. Our findings provide direct evidence that NR2B-containing NMDARs are selectively linked with the endocytosis of GluR2 AMPARs, although they make up a minor subpopulation of NMDARs.

Furthermore, in neurons from mice expressing the mutant NR1(N598R) subunit (5) which renders NMDARs Ca^{2+}-impermeable we found that NMDAR activation failed to increase GluR2-AMPAR endocytosis. These findings indicate the requirement of Ca^{2+} influx directly through the activated NMDAR channels for the NMDA-dependent GluR2-AMPAR endocytosis.

In summary, our findings suggest that the NMDAR activation selectively induces the internalisation of short C-terminal GluR2-containing AMPARs through a mechanism that requires a NMDAR channel-carried Ca^{2+} signal processed in an NMDAR subtype-selective manner.

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This work was funded by a Wellcome Trust Senior Fellowship, a Wellcome grant and a BBSRC grant to RS. C.M.T. was supported in part by a Wellcome Trust Traveling Fellowship.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C7

Functional cross-inhibition between P2X4 and $GABA_c$ receptors

L. Jiang and Z. Mei

Faculty of Biological Sciences, University of Leeds, Leeds, UK

There is evidence indicating co-release of ATP and GABA neurotransmitters [1] and co-expression of P2X ATP-gated channels and GABA-gated channels, suggesting that these two structurally distinct channels may functionally communicate with each other. In this study, we expressed in rat P2X4 and rat rho1 subunits in human embryonic kidney 293 cells individually or in combination, and measured whole-cell agonist-evoked currents by patch-clamp technique at a holding potential of -60 mV in intracellular and extracellular solutions, both of which contained Na^+ and Cl^- as the major ion species. In cells co-expressing P2X4 and rho1 subunits, the mean ± SEM amplitude of peak currents evoked by co-application of ATP (100 μM) and GABA (10 μM) was 3066 ± 324 pA (n = 5), which was significantly reduced compared with that predicted from the sum of currents evoked by ATP and GABA separately (4554 ± 587 pA; $p < 0.01$, paired Student's t test). In cells expressing P2X4 subunit only, the currents evoked by co-application of ATP and GABA (1198 ± 117 pA, n = 3) were not significantly different from those by ATP alone (1263 ± 112 pA; $p > 0.1$, paired Student's t test). Similarly in cells expressing rho1 subunit only, the currents elicited by co-application of GABA and ATP (2649 ± 274 pA; n = 4) were similar to those by GABA alone (2704 ± 294 pA; $p > 0.1$, paired Student's t test). In addition, there were no observable alterations in activation and deactivation of P2X4 receptor by GABA and of $GABA_c$ receptor by ATP. In summary, our results indicate functional cross-inhibition between P2X4 and GABAc receptors and exclude the possibility that such cross-inhibition is due to functional modulation of P2X4 receptors by GABA or of $GABA_c$ receptor by ATP [2].

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We are grateful to the Royal Society for grant support and Dr Dongxian Zhang (Burnham Institute, La Jolla, USA) for providing the rat rho1 cDNA clone.

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C8

Functional NR2D- and NR2A-containing NMDA receptors are expressed in mouse Purkinje cells

M. Renzi, M. Farrant and S.G. Cull-Candy

Pharmacology, UCL, London, UK

NMDA-type glutamate receptors (NMDARs) are heteromultimeric channels assembled from NR1 subunits and at least one type of NR2 subunit (2A-D). Purkinje cells (PCs) from young rats contain mRNA for NR1 and NR2D subunits (1), and in these cells presumptive NR1/NR2D receptors in extrasynaptic membrane have been identified on the basis of their gating behaviour and low single-channel conductance (40 and 20 pS; 2). With development, functional NMDARs are lost from rat PCs. By contrast, PCs from adult mice display immunolabelling for NR1, NR2A, -2B and -2C/D subunits (3). Here we have used NR2D knock-out mice (NR2D -/-; 4) to investigate the identity of the NMDARs in mouse PCs. Further, we have examined the conditions under which PC NMDARs may be activated.

We recorded NMDA-activated channels in outside-out membrane patches from PCs in slices from wild-type (WT) and NR2D -/- mice of various ages. In P6-9 WT mice, NMDA (10 μM in nominally Mg^{2+}-free solution) activated single-channel openings of ~40 and ~20 pS (slope conductance: 41.2±1.7 and 21.0±0.5 pS; mean±S.E.M., n = 3). These low-conductance events were absent from patches taken from NR2D -/- mice (n = 10). These observations are consistent with the view that NMDARs in PC from young rodents are formed from NR1 and NR2D subunits. However, we also observed high-conductance NMDA-activated channels from NR2D -/- mice, suggesting the presence of NMDARs formed from other subunits. In one patch from a young mouse, NMDA-activated channels were found with a slope conductance of ~50 and ~40 pS, suggesting the presence of NR2A- or NR2B-containing NMDARs. Consistent with the formation of functional NR2D-lacking NMDARs in mice, we also observed similar high-conductance channels in a PC from an adult WT mouse. In this case, extracellular Zn^{2+} (5 and 200 nM; 10 mM tricine-buffered solution) blocked the openings with an efficacy that suggested the presence of NR2A-containing NMDARs (5). Although PCs in young mice express NMDARs, it is not clear if they are activated by endogenous glutamate. In this regard, we observed an AP5-sensitive whole-cell current upon application of the glutamate transport blocker (DL-threo-β-benzyloxyaspartate, TBOA 100 μM; 46±14 pA, n = 5; P6-9), suggesting that NR2D-containing NMDARs may be activated by ambient glutamate under certain conditions.

Our experiments confirm that the NR2D subunit is required for expression of most NMDARs in Purkinje cells of newborn animals. Our results also show that NMDARs lacking NR2D are also expressed, albeit rarely, in both young and adult mice. We are currently investigating a possible role for NMDAR activation in developing Purkinje cells.

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This work is supported by the Wellcome Trust (SGC-C; M.F.) and a Royal Society-Wolfson Research Award (S.G.C.-C.).

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SA1

Targeting of an ATP-gated ion channel to lysosomes in macrophages, and its retrograde delivery to phagosomes and the plasma membrane

O.S. Qureshi, A. Paramasivam and R.D. Murrell-Lagnado

Department of Pharmacology, University of Cambridge, Cambridge, UK

Trafficking of ligand-gated ion channels to lysosomes is generally thought of as an end-point for the receptor as lysosomes are cellular sites of degradation. We provide evidence that an ATP gated ion channel, the P2X4 receptor, is contained predominantly within lysosomes in freshly isolated mouse peritoneal macrophages. Lysosomal targeting was mediated by tyrosine and dileucine based motifs, and the receptor was protected from degradation by N-glycosylation. Upon stimulation of phagocytosis, P2X4 receptors were delivered to the phagosome membrane. To test the functionality of P2X4 receptors trafficked from lysosomes, we triggered lysosome exocytosis and measured ATP-evoked whole cell currents. Ivermectin-sensitive, P2X4-mediated currents increased ~5-fold following lysosome exocytosis and this increase was independent of delivery of newly synthesised receptors to the plasma membrane. Our results suggest that the targeting of P2X4 receptors to lysosomes ensures their low surface expression in unstimulated macrophages whilst providing a pool of receptors for rapidly up-regulating their expression at the phagosome and plasma membrane.

This work was supported by the Biotechnology and Biological Sciences Research Council. We thank Dr. D. Brough for general advice and help with peritoneal macrophage isolation and culture.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA2

Phosphorylation and PDZ-dependent control of ROMK channel trafficking in the kidney

P. Welling

Physiology, University of Maryland Medical School, Baltimore, MD, USA

The polarized location and cell surface density of different inwardly rectifying (Kir) channels is precisely controlled in the renal collecting duct for potassium balance. We have previously shown that Kir 1.1 (ROMK, KCNJ1) (1) and Kir 2.3 (2,3) channels interact with separate PDZ proteins to differently control polarized trafficking. Here we discuss our recent observations that a hierarchical trafficking program controls cell surface expression of the ROMK channel, involving PDZ-protein interaction and phosphorylation-dependent release from the endoplasmic reticulum. Protein-protein interaction studies indicate that NHERF-2, a PDZ protein, has the capacity to organize a multimeric protein complex, involving the ROMK channel, PKA, and the aldosterone-induced kinase, SGK-1 (4). As determined by in vivo and in vitro phosphorylation assays, serine 44 in ROMK1 is a substrate for PKA and SGK-1 phosphorylation. Phosphorylation of this residue absolutely required to drive traffic of newly synthesized channels to the plasma membrane. ROMK channels were found to acquire mature glycosylation in a serine 44-phosphorylation dependent manner, consistent with a phosphorylation-dependent trafficking step within the endoplasmic reticulum/Golgi. Serine 44 neighbours a string of three "RXR" motifs, reminiscent of basic trafficking signals involved in directing early transport steps within the secretory pathway. Mutational analysis revealed that the neighboring arginine residues are necessary for cell surface expression, identifying a structure that determines export in the biosynthetic pathway. Suppressor mutations in a putative dibasic ER retention signal, located within the cytoplasmic C-terminus (K370A, R371A), restored cell surface expression and activity of the phospho-null S44A channel to levels exhibited by the phospho-mimic S44D channel (5). In summary, we have found that phosphorylation of S44 drives an early export step within the secretory pathway by overriding an independent endoplasmic reticulum localization signal. Thus, a balance of intracellular retention and phosphorylation-dependent export controls Kir1.1 cell surface density. Phosphorylation of S44 by SGK-1 provides a mechanism to explain the requirement of aldosterone for maximal up regulation of the secretory channel observed upon dietary potassium loading. Phosphorylation by PKA offers an explanation for vasopressin-dependent regulation of potassium channel density. Efforts to elucidate the structural basis for the phosphorylation-dependent trafficking signal will be discussed.

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This work was support from grants from the NIH.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA3

Polarised sorting of the polycystins: defects in polycystic kidney disease

R. Sandford

Medical Genetics, University of Cambridge, Cambridge, UK

Autosomal dominant polycystic kidney disease (ADPKD) is one of the commonest monogenic diseases of man affecting 1:1000 of the worldwide population. In the UK, it is predicted that there are over 50,000 individuals with or at risk of inheriting this condition. It is characterised by a highly variable but progressive increase in the number and size of renal cysts that ultimately leads to renal failure in later life. Approximately 6-8% of patients receiving renal replacement therapy, such as dialysis or transplantation, have a diagnosis of ADPKD. It is also associated with vascular complications including hypertension, ischaemic heart disease and cerebral aneurysms.

ADPKD is caused by mutations in PKD1 (85%) and PKD2 (15%). PKD1 and PKD2 encode polycystin-1 and polycystin-2 respectively. Polycystin-1 is a novel >650 kDa membrane associated glycoprotein containing up to 11 transmembrane domains. Its unique arrangement of extracellular and intracellular protein domains and motifs suggests a role in mechanosensitive signal transduction pathways. Polycystin-2 is a highly conserved member of the transient receptor potential (TRP) channel family. They associate to form a large mechanosensitive calcium-regulated calcium ion channel complex although independent functions have been identified. In polarised renal epithelial cells, polycystin-1 localises to focal adhesion complexes, cell-cell adhesion complexes and to the renal primary cilium. Polycystin-2 is localised in the ER and the primary cilium. Loss of normal cilial function regulated by a polycystin complex is thought to be the main pathogenic mechanism in ADPKD. Several studies have demonstrated the need for polycystin-1 and polycystin-2 to form a complex for plasma membrane localisation but the precise mechanisms that control the polarised distribution of these proteins are poorly characterised.

Polycystin-1 has been technically difficult to study in many model systems. Chimeric proteins using the intracellular C-terminus of polycystin-1 have shown that this region contains specific targeting information directing ER/golgi export and polarised distribution to the lateral membrane. All 11 transmembrane domains and the intracellular C-terminus are required for cilial localisation. Deletion and mutagenesis studies have identified a short motif in the C-terminus of polycystin-1 responsible for ER/golgi export. As the majority of PKD1 mutations are truncating and 5' to the region encoding this motif, ADPKD is likely to result from a failure of normal polycystin polarised trafficking. Intracellular levels of polycystin-1 are frequently increased in ADPKD supporting this hypothesis.

The mechanisms responsible for the polarised trafficking of polycystin-2 have been more clearly elucidated although some discrepancies remain. PIGEA-14 and PACS proteins have been shown to have a role in polycystin-2 localisation via interaction with C-terminal domains whilst an N-terminal motif has been shown to be responsible for polycystin-1 independent cilial localization. I will review the current literature and present some new data that characterises the abnormalities in polarised sorting of the polycystins in ADPKD.

RS is supported by a Wellcome Trust Senior Clinical Fellowship

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SA4

Transport protein trafficking in polarized cells: new partners and pathways

M. Caplan, T. Kimura, M. Morton, A. Duffield, V. Chauvet, M. Lal and C. Le Moellic

Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA

The physiological function of an ion transport protein is determined, in part, by its subcellular localization and by the cellular mechanisms that modulate its activity. We are interested in the molecular signals and interactions that control the sorting and regulation of ion transport proteins in polarized cells. Recently we have focused our attention upon members of the tetraspan family of interacting polypeptides. The tetraspan superfamily constitutes a large and growing collection of membrane proteins that appear to play a role in organizing membrane domains. As their name implies, these polypeptides span the membrane four times and share limited sequence homology. Tetraspan proteins form heteromeric complexes with one another and participate in a wide variety of interactions with other membrane proteins. We have recently found that several ion transport proteins engage in specific interactions with "subcellular domain-appropriate" tetraspans, both in heterologous expression systems and in situ. We have also found that these interactions can regulate the distribution and physiologic function of several critical ion transport systems. In addition, yeast two hybrid screens have revealed several novel partners for the Na,K-ATPase. The list of these partners includes phosphatases, transmembrane receptors and regulators of signaling complexes. Our data suggest that these interactions modulate the activity and trafficking of the Na,K-ATPase in heterologous expression systems as well as in situ.

Our studies of membrane protein trafficking extend to the polypeptides associated with autosomal dominant polycystic kidney disease (ADPKD). ADPKD is caused by mutations in the PKD1 or PKD2 genes, which encode the polycystin-1 and polycystin-2 proteins, respectively. Polycystin-1 is a plasma membrane protein that may be involved in signaling from sites of cell-cell contact, while polycystin-2 is a transmembrane protein that shares homology with some members of calcium channel families. Genetic and biochemical evidence suggests that these two proteins participate in the same signaling pathway. Physical interaction between both proteins has been demonstrated and a mutation in either of these two genes leads to the same phenotype. Nevertheless, the functions of these proteins and their common transduction pathway are largely unknown. A new signaling paradigm known as regulated intramembrane proteolysis (RIP) has been recently described. In this model, the intracytoplasmic portion of the transmembrane receptor is released after ligand interaction and enters the nucleus, where it directly acts as a modulator of gene expression, bypassing adaptor proteins and kinase cascades. We have found that polycystin-1 undergoes a RIP-like proteolytic cleavage that releases its C-terminal tail (CTT), which enters the nucleus and initiates signaling processes. The cleavage occurs in vivo in association with alterations in mechanical stimuli. Polycystin-2 modulates the signaling properties of the polycystin-1 CTT, and appears to serve as a cytoplasmic buffer that modulates the quantity of CTT that is available to enter the nucleus. In order to explore further the role that this cleavage plays in the normal functioning of the polycystin proteins and in the pathogenesis of ADPKD we are working to identify the enzyme responsible for the release of the polycystin-1 C terminal tail and to identify the stimuli and signaling pathways that induce or prevent the cleavage. In addition, we are conducting studies designed to identify the protein partners with which the C terminal tail fragment interacts in association with its nuclear translocation. We find that the CTT interacts directly with β-catenin. This interaction does not occur with CTT construct that lacks the putative nuclear localization sequence. Furthermore, expression of the CTT decreases the capacity of β-catenin to participate in WNT signaling, possibly by altering the ability of β-catenin to interact with the TCF transcription factor.

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SA5

AMPA and kainate receptor trafficking

S. Martin[1], M. Ashby[2], A. Nishimune[1] and J.M. Henley[1]

[1]MRC Centre for Synaptic Plasticity, Bristol University, Bristol, UK and [2]NIH/NINDS, Bethesda, MD, USA

The precise and efficient transport, targeting and surface expression of AMPA receptors (AMPARs; GluR1-4) and kainate receptors (KARs; GluR5-7 and KA1-2) is fundamental to neuronal function. These processes are facilitated and regulated by spatially and temporally coordinated protein-protein interactions and considerable progress has been achieved in defining the exact roles of some of these interactions but much remains to be determined. An area of intense interest is assessment of the dynamic aspects of AMPAR and KAR trafficking in living neurones. In our lab we are using immunocytochemical techniques together with GFP variants to study receptor translocation, surface expression, lateral diffusion in the plasma membrane, endocytosis,recycling and exocytosis in live cells with high spatial and temporal resolution (Ashby et al. 2004b).

We use virus-mediated transduction to express fluorophore-tagged AMPAR and KAR subunits in cultured hippocampal neurones and hippocampal slices to directly visualize receptors. We then determine the rates, directions and extent of movement using photobleach procedures and confocal microscopy. Although conventional GFP (and its spectral variants) provide insight into subunit and receptor transport inside neurones (Perestenko & Henley, 2003), these GFP-tagged receptors are limited for study of real time changes in surface expression. To overcome this we use a GFP derivative called super ecliptic pHluorin (SEP) that alters in fluorescence according to pH. SEP does not fluoresce at pH <6.0 but the fluorescence intensity increases with pH up to a maximum at 8.5 (Ashby et al. 2004b). Since intracellular organelles are luminally acidified, a SEP tag placed on the extracellular side of a transmembrane protein will have very little fluorescence when the protein is inside the cell. This means that SEP fluorescence only comes from proteins on the cell surface. These pHluorin-receptor constructs have many uses, for example, when expressed in hippocampal neurones pHluorin-GluR2 fluorescence is particularly enriched in the heads of dendritic spines and we have tracked the movement of synaptic and extrasynaptic AMPAR receptors in response to NMDAR stimulation (chem-LTD) (Ashby et al. 2004a).

We have also shown that in cultured hippocampal neurones the surface expression of GluR6-containing KARs is dynamically regulated. Intriguingly, internalised KARs are sorted into recycling or degradative pathways depending on the endocytotic stimulus. Sustained kainate activation results in lysosomal targeting and degradation of the receptor whereas NMDAR activation evokes internalisation to early endosomes with subsequent recycling back into the plasma membrane. These processes provide mechanisms for both rapid and chronic changes in the number of functional receptors (Martin & Henley, 2004). Some of our current work is aimed at monitoring these trafficking events in near real-time using SEP-tagged KAR subunits.

In addition, we are also actively investigating the parameters for surface diffusion of SEP-GluRs and how surface receptor movement is influenced by membrane topology. It has been shown that individual AMPARs diffuse within the plasma membrane using single particle tracking (Borgdorff & Choquet, 2002) but it remains unclear how these movements affect the overall distribution of synaptic proteins. Furthermore, lateral diffusion in dendritic spines has not previously been directly assessed but our recent experiments suggest that lateral diffusion can account for a rapid exchange of AMPARs at spines.

Dendritic spines compartmentalize cytoplasmic molecules, a process suggested to underlie the synapse-specificity of plasticity. However, many of the proteins targeted for modification during plasticity are transmembrane (e.g. glutamate receptors) or membrane-associated (e.g. PSD95). The synapse-specificity of signalling would be compromised if these proteins laterally diffuse between synapses. Our data indicate that plasma membrane 3-D shape can control the redistribution of such proteins by physically limiting their movements at dendritic spines. This suggests that the spine neck acts to retain membrane proteins close to individual synapses.

In summary, recent and on-going work in our lab is directed at understanding the cellular processes that regulate constitutive and activity-dependent glutamate receptor trafficking in neurones. A key approach is to visualise, in near real-time, receptor movement both inside the cell and at the plasma membrane and to determine how the dynamics and the trafficking are altered by physiological, pharmacological and biochemical manipulations.

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We are grateful to the MRC, the Wellcome Trust and the EU (GRIPPANT) for financial support.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA6

Molecular mechanisms regulating glutamate receptor trafficking

K.W. Roche

NINDS, National Institutes of Health, Bethesda, MD, USA

NMDA receptors are widely distributed throughout the central nervous system and are critical for neuronal development. The precise trafficking and localization of NMDA receptors at excitatory synapses is essential for proper neurotransmission and synaptic plasticity. Endogenous NMDA receptors are tetramers composed of two NR1 subunits, which are essential components of NMDA receptors, combined with additional NR2 subunits (NR2A-D). Whereas the NR1 subunit is expressed throughout the brain, the NR2 subunits display unique spatiotemporal distributions and they confer distinct functional and pharmacological properties on NMDA receptors. We have investigated the molecular determinants encoded within the NR2A, NR2B, and NR2C subunits that regulate trafficking and surface expression of NMDA receptors. All NR2 subunits possess very long intracellular C-termini that contain important regulatory motifs

including protein phosphorylation sites and protein-protein interaction domains. We have examined the distal C-terminus of NR2A-C and characterized regulatory motifs in this region of each of these proteins. Although all three contain a PDZ binding domain and interact directly with the PSD-95 family of proteins, just upstream of the PDZ ligand, the sequences are quite divergent suggesting molecular specializations corresponding to these regions. The NR2A and NR2B subunits are highly expressed in the hippocampus and cortex. Interestingly, the expression of these subunits is developmentally regulated such that NR2B is expressed early in development, whereas NR2A expression is delayed. NR2B contains a strong consensus tyrosine-based endocytic motif just upstream of the PSD-95 binding site. We find this motif regulates a direct interaction with the medium chain of the AP-2 adaptor complex and is important for NR2B endocytosis (Lavezzari et al. 2003). Although the tyrosine is conserved in NR2A, the surrounding residues are distinct resulting in much lower affinity for the AP-2 medium chain. Furthermore, we find that NR2A and NR2B are differentially sorted following endocytosis, with NR2B-containing NMDA receptors preferentially trafficking through recycling endosomes (Lavezzari et al. 2004). These findings demonstrate that subunit-composition determines the intracellular sorting and surface expression of NMDA receptors. Unlike NR2A and NR2B, the NR2C subunit is specifically enriched in the cerebellum. The expression of NR2C is developmentally regulated and only appears in adult. Interestingly, NR2C does not have the tyrosine-based endocytic motif, but contains a strong prototypical PKA consensus motif just upstream from the PDZ binding site. We find that the serine within this consensus sequence (serine 1244) is robustly phosphorylated by PKA and PKC, both in vitro and in vivo, but that the phosphorylation does not affect binding of the PSD-95 family of proteins or surface expression of NR2C-containing receptors. However, we surprisingly find that modification of serine 1244 regulates the channel kinetics of NR1/NR2C NMDA receptors expressed in heterologous cells. In conclusion, the unique NR2 C-termini contain distinct substrates for the endocytic machinery and for protein kinases, demonstrating that this region of the NR2 subunits of NMDA receptors provides diversity in NMDA receptor regulation and trafficking. These defined regulatory motifs modulate a variety of NMDA receptor properties including receptor trafficking, surface expression, and, in the case of the NR2C subunit, channel kinetics.

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This work was supported by the NINDS Intramural Program, National Institutes of Health, USA.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA7

Surface diffusion of AMPA and NMDA receptors

L. Groc, M. Heine, C. Bats and D. Choquet

CNRS - UMR 5091- Universite Bordeaux 2, Bordeaux, France

Regulation of functional AMPA receptor (AMPARs) and NMDAR numbers at post-synaptic sites is a core mechanism for plasticity of excitatory synaptic transmission. AMPAR and NMDAR to a less extent can traffic in and out of synapses by lateral diffusion in the plane of the neuronal membrane. Understanding the processes that regulate AMPAR and NMDAR surface trafficking will likely shed new light on the mechanisms that control receptor concentration at synapses. In this lecture, I will present several lines of evidences showing that the AMPAR and NMDAR surface diffusion is dependent on the subunit composition, the scaffold protein, the neuronal activity status, and the synaptic transmission.

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