EDITORIAL

Neurophysiology of inhibitory and excitatory amino acid receptors

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Progress in understanding the workings of the vertebrate brain has been unprecedented over the last half century. Much of this advancement has paralleled our understanding of the myriad roles fulfilled by inhibitory and excitatory amino acid neurotransmitter receptors. It seems surprisingly now that interest in amino acid neurotransmitter receptors started with little fanfare. Instead, there was a gradual appreciation amongst the scientific community that the small, endogenous amino acids, L-glutamic acid (L-Glu), γ-aminobutyric acid (GABA) and glycine (Gly), act on pharmacologically distinct neurotransmitter receptor families (Krnjevic, 2010). With the introduction of better pharmacological tools, the pace of progress quickened as it became possible to systematically map out their distribution in the CNS (Fagg & Foster, 1983). By the end of the 1980s, cloning studies gave our first peek at the molecular diversity of the many subunits that make up each receptor superfamily (Grenningloh *et al.* 1987; Schofield *et al.* 1987; Hollmann *et al.* 1989). With their identity known, an entirely new era ensued as more and more molecular techniques and disciplines were brought to bear on deconstructing inhibitory and excitatory neurotransmission. Now, at the close of these last two decades of rapid progress, we are challenged with putting it all back together if the nature of behaviour and disease is to be finally understood.

To reflect upon the past, present and future of amino acid receptor research, a one-day symposium was organized at the 11th International Congress on Amino Acids, Peptides and Proteins held on 3 August 2009 in Vienna, Austria (Fig. 1). The symposium was divided into two parts, the first to examine advances in structure–function relationships of inhibitory and excitatory amino acid receptors and the second to explore their impact on neurophysiology. To address these issues, ten speakers who have made significant contributions to our understanding of these receptors over the last few years were invited to present their individual perspectives. This issue of *The Journal of Physiology* collects the symposium reviews from seven of these invited speakers (Allene & Cossart, 2010; Bowie, 2010; Khatri & Weiss, 2010; McKinney, 2010; Rebola *et al.* 2010; Sivilotti, 2010; Tyagarajan & Fritschy, 2010). As a special treat, Dr Krešimer Krnjevic, emeritus Professor of Physiology at McGill University, Montréal, was invited to open the symposium with a personal account of the early years and experiments that established the predominance of GABA and L-Glu as neurotransmitters. This was particularly fitting since his contribution to this field of research has been significant, culminating in his receipt of the 1984 Gairdner Foundation International Award. Unfortunately, unforeseen circumstances prevented his attendance, but he has provided a review of this subject (Krnjevic, 2010) which, when taken together with all the other invited reviews, provides a unique snapshot of this exciting field of research and a glimpse of the new frontiers that lie ahead.

The symposium's first speaker was Lucia Sivilotti from University College London (UCL) who reviewed some of her recent findings on two Cys Loop receptor families, namely the glycine (GluR) and nicotinic acetylcholine (nAChR) receptors. Over the last few years, the Sivilotti lab in collaboration with David Colquhoun (also of UCL) has made a number of seminal findings on how Cys loop receptors respond to agonist binding. Their work has focused on detailed kinetic analysis of single-channel behaviour to explain why some agonists are more effective activators than others (Sivilotti, 2010). Specifically, they have proposed that full and partial agonists differ not in their ability to gate, as has been assumed since pioneering work by del Castillo & Katz (1957), but rather in their ability to enter into a short-lived 'flipped' state – a concept that is gaining support by work from other labs (Mukhtasimova *et al.* 2009). From a structural perspective, it is still not clear what the 'flipped' state represents, though Dr Sivilotti speculates that it may correspond to a capping of the agonist molecule within its binding domain (Sivilotti, 2010); this is an exciting viewpoint which is bound to stimulate further discussion and experimentation.

In his talk, David Weiss from the University of Texas Health Science Center at San Antonio went a step further towards addressing how receptor structure relates to function. Over the last few years, the Weiss lab has championed the use of voltage-clamp fluorometry (VCF) to study GABA receptor function. The VCF technique's great advantage is that it

Figure 1. The symposium participants
From left to right, back row, J-M. Fritschy, S. Oliet, C. Mulle, D. Madden, J. Howe, G. Swanson, D. Bowie and D. S. Weiss; front row, P. Paoletti, L. Sivilotti, R. A. McKinney and R. Cossart.

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provides information on receptor activation via voltage-clamp recordings as well as changes in protein conformation. The latter measurement is inferred from the shift in intensity of environmentally sensitive fluorophores strategically engineered into the receptor complex. The talk centred on the ongoing controversy of how the Cys loop receptor family couples agonist binding to channel activation. Recent work suggests that the agonist-binding pocket is composed of six loops (A–F) with Loop F playing a direct role in linking events in the agonist binding pocket to the channel gate, at least for nAChRs. However, the Weiss lab's more recent findings suggest instead an indirect involvement for GABARs (Khatri *et al.* 2010), indicating that Loop F may be crucial for locking the agonist molecule into the binding site (Khatri & Weiss, 2010). It will be interesting in future experiments to understand how Loop F manages to fulfil distinct roles in nAChRs and GABARs.

Pierre Paoletti from the Ecole Normale Supérieure in Paris discussed the importance his lab has recently attributed to the amino-terminal domain (NTD) of NMDA-type ionotropic glutamate receptors (iGluRs) (Gielen *et al.* 2009). They valiantly took on the challenge of trying to understand why NMDARs composed of different NR2 subunits differ substantially in their ability to respond to agonist binding (i.e. open-channel probability). It had been assumed that subunit-specific gating resided in the basic gating core module, that is, the agonist-binding domains together with the pore region. However to much surprise, the Paoletti group in collaboration with Jon Johnson's team at the University of Pittsburg revealed that it could all be explained by differences in the NTD (Gielen *et al.* 2009). The NTD is already known to bind allosteric modulators such as Zn^{2+}. Their findings add a new and exciting perspective on this region of the protein that is often overlooked by glutamate receptor aficionados. And how does the NTD determine channel open probability? The authors have an answer for that too. It seems that NTD-driven gating hinges on the equilibrium set up between spontaneous open-cleft and closed-cleft conformations, a model that nicely explains why open-channel probability and allosteric modulators are NR2 subunit dependent.

The next talk by James Howe from Yale University School of Medicine in Connecticut shifted the audience's attention to the role of transmembrane AMPA receptor regulatory proteins (or TARPs) and how they affect glutamatergic transmission. In several recent landmark papers, the Howe lab, working closely with the lab of fellow Yale colleague Susumu Tomita, has teased apart how different TARP family members, including stargazin, endow native AMPARs with distinct signalling properties (Cho *et al.* 2007; Morimoto-Tomita *et al.* 2009). For the last 10–15 years, it had been generally accepted that synapse-specific differences in the kinetic behaviour of AMPARs can be explained entirely on the basis of receptor subunit composition. However, the impact that TARPs have on AMPARs has compelled the entire field to abandon that idea in favour of the AMPA receptor–TARP complex. To date, their magnum opus has been to identify the molecular basis for the long standing observation that native AMPARs exhibit a biphasic dose–response relationship (Raman & Trussell, 1992), which Drs Howe and Tomita have termed autoinactivation (Morimoto-Tomita *et al.* 2009). Using AMPAR constructs with covalently tethered TARPs, they convincingly show that autoinactivation is completely lost, thus proving that AMPARs autoinactivate by TARP disassociation. At central synapses, it appears that this mechanism can account for paired-pulse depression as well as protecting neurons from excitotoxic damage. With the recent identification of another family of AMPAR auxiliary proteins (Schwenk *et al.* 2009), it is evident that this field of research will remain centre-stage well into the future.

The structure–function session was concluded by a talk on kainate-type glutamate receptors (KARs) by one of us, Derek Bowie from McGill University in Montréal. A few years ago, the Bowie lab made the unexpected finding that KARs fail to respond to agonist stimulation unless external cations and anions, such as Na^+ and Cl^-, are present (Wong *et al.* 2006). It turns out that the observation is not due to a failure of agonist binding but rather to a novel ion-dependent gating mechanism that, so far, is unique to KARs. It is even absent from closely related AMPA- and NMDA-type iGluR family members which makes this feature of KARs an ideal target for the development of selective ligands (Bowie, 2010). This idea is all the more appealing given the growing appreciation of KAR involvement in several CNS disorders (Bowie, 2008). Despite these advances, it is still not clear how ion-dependent gating of KARs is involved in neuronal signalling, suggesting there is still much more to uncover about this interesting but often inconspicuous iGluR in the future.

The second half of the symposium began in earnest on the Neurophysiology of amino acid neurotransmitter receptors. The first speaker was Rosa Cossart from the Université de la Méditerranée in Marseille, France who discussed patterned activity in developing cortical structures (Allene & Cossart, 2010). She argued that understanding network activity at both the cellular and systems level is necessary if we are to understand any defects that may arise during CNS development. Her talk concentrated on two types of spontaneous synapse-driven network patterns: cortical early network oscillations (cENOs) and giant depolarizing potentials (GDPs). cENOs are large scale oscillatory calcium waves driven by glutamatergic transmission, which, to date, seem to be specific to the neocortex (Allene *et al.* 2008). As cENOs are observed under specific conditions, such as anoxia, Dr Cossart questioned their physiological relevance. To do this, she reviewed the mechanisms and developmental profiles and their dynamics to give insight into their physiological role during brain maturation. In contrast to cENOs, GDPs are driven by GABAergic transmission, and are further distinguished by their distinct spatiotemporal dynamics based on electrophysiological and optical recordings. It is still not clear what the exact physiological role of NMDAR driven ENOs is *in vivo*. However, as outlined by Dr Cossart, the most compelling way forward is to use a combination of multi-cell imaging with electrophysiology to address these issues.

The second speaker was Christophe Mulle from the Bordeaux Neuroscience Institute in France. Dr Mulle's group has made seminal contributions to our understanding of kainate-type iGluRs, but the topic of his talk in Vienna was on activity-dependent NMDAR synaptic plasticity (Rebola *et al.* 2010). Activity-dependent bidirectional control of synaptic efficacy in long-term potentiation (LTP) and depression (LTD) is thought to represent, in part, the molecular basis of learning and memory. In this context, most work has centred on AMPAR trafficking into and out of synapses, with NMDARs acting as coincidence detectors of postsynaptic and presynaptic excitability. However, Dr Mulle presented a convincing argument that synaptic NMDA-type

glutamate receptors are also up- and/or down-regulated in an activity-dependent manner (Rebola *et al.* 2008). Moreover, much like AMPARs, NMDARs also undergo long-term potentiation (LTP) and depression (LTD), suggesting that there is still much to study about the emerging role for NMDARs at central synapses.

Stéphane Oliet from the Neurocentre Magendie, which is also located in Bordeaux, France, presented a summary of his exciting work on the tripartite synapse. Like Dr Mulle, his talk focused on NMDARs, but looked at the way the gliotransmitter, D-serine, can act as a co-agonist (Bains & Oliet, 2007). Earlier work had established that ambient levels of glycine (Gly) were an absolute requirement for NMDAR activation without giving due consideration to the possibility that other endogenous amino acids may behave similarly. For example, it has been shown in recombinant receptors that D-serine is as potent an activator of NMDARs as Gly. Dr Oliet put this idea into a more physiological context by showing that D-serine is in fact the preferred endogenous ligand of NMDARs in many areas of the brain (Panatier *et al.* 2006). Intriguingly, D-serine is synthesized in glial cells and subsequently released when they are activated by L-glutamate in the synaptic cleft. Importantly, the concentration of D-serine that accumulates in the synaptic cleft determines the number of NMDARs that are available for activation. As well as highlighting the importance of glial cells to NMDAR synaptic plasticity, Dr Oliet's work underlines the critical contribution of glial cells to the tripartite synapse, which has implications for future work on synaptic plasticity mechanisms as well as CNS disorders such as epilepsy.

The next speaker, Jean-Marc Fritschy from the University of Zurich in Switzerland, switched the focus from excitation to the role of inhibitory $GABA_A$ receptors. Dr Fritschy is recognized internationally for his work in this field of research. He speculated on the molecular events that might lead to up- and down-regulation of inhibitory GABAergic synapses during homeostatic synaptic plasticity (Tyagarajan & Fritschy, 2010). As synaptic scaling has been extensively studied at glutamatergic synapses, Dr Fritschy argued that coordinated changes in both excitatory and inhibitory synapses occurs by a synchronized mechanism involving scaffolding proteins. In this regard, he identified PSD-95 as the candidate scaffolding protein at glutamatergic synapses and, by analogy, proposed that gephyrin fulfills a similar role at GABAergic synapses (Tyagarajan & Fritschy, 2010). To support this argument, Dr Fritschy presented a concise but highly informative review on the structure and regulation of $GABA_A$ receptors by gephyrin and how he thinks tethered complexes may bring about global changes in inhibitory synapses. The talk was concluded by speculating that phosphorylation was the key regulatory event that triggers homeostatic synaptic changes.

The final talk after a long but exciting afternoon was by the other one of us, Anne McKinney from McGill University in Montréal. Dr McKinney provided a summary of the current status of her work on the role of dendritic spines in synaptic transmission (McKinney, 2010). Her special emphasis over the last decade has been to examine changes in spine morphology in the context of hippocampal plasticity mechanisms (Richards *et al.* 2005). Specifically, Dr McKinney highlighted the various roles played by two subfamilies of ionotropic glutamate receptor (iGluRs), namely NMDARs and AMPARs, in determining the formation and disappearance of spines. Interestingly, NMDARs and AMPARs have distinct roles in this regard which is dependent on the time point of neuronal circuit development. Since spine morphology is dynamically regulated and abnormal in several important neurological disorders, Dr McKinney argued that the study of dendritic spines may provide necessary clues to unlock the nature of mental retardation, epilepsy and disorders of ageing.

Two other notable talks, by Geoff Swanson from Northwestern University in Chicago and Dean Madden from Dartmouth Medical School in New Hampshire, are worth mentioning in closing. Though not included in the symposium, their findings were very much in keeping with the theme of amino acid neurotransmitter receptors. Dean Madden outlined his lab's use of electron microscopy to study the quaternary structure of calcium-permeable and -impermeable AMPA receptors (Midgett & Madden, 2008). Up until recently, all of our structural information has relied on analysis of partial structures of the extracellular regions of iGluRs. However, the Madden lab has provided a much-needed step forward by considering the assembled structure in its entirety, showing its elongated appearance, overall twofold symmetry and large central vestibule. And as this editorial went to press, the need to look at the entire iGluR structure was given a substantial and dramatic boost by the publication from Eric Gouaux's lab at the Vollum Institute of the complete AMPAR X-ray crystal structure (Sobolevsky *et al.* 2010). This finding alone will inject further vigour in the structure–function analyses of iGluRs over the next few years. And finally, Geoff Swanson presented experiments that re-examined the long-held view that agonist-induced closure in the ligand-binding pocket of KARs determines agonist efficacy. In a collaborative study with Jette Kastrup's lab at the University of Copenhagen, he showed compelling electrophysiology and X-ray crystallography evidence that even partial agonists can elicit full closure in the ligand-binding pocket (Frydenvang *et al.* 2009). Their findings come at a time when other groups report similar observations (Zhang *et al.* 2006; Fay *et al.* 2009) which together cast significant doubt on previous attempts to relate AMPAR or KAR structure to function. The challenge for the future will be to work towards structural models of these receptors that satisfies the complexities of their functional properties.

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