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Kainate receptor physiology

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Glutamate receptors constitute a complex signalling system at most of the excitatory synapses in the brain. Of the known ionotropic glutamate receptors, kainate receptors are ubiquitous in the central nervous system, and a considerable amount of data indicates that this class of receptors is present at both sides of the synapse. Pre- and postsynaptic kainate receptors are able to regulate both transmission of information and excitability in a synapse-specific manner. Proteins interacting with kainate receptor subunits are being identified and functional studies have provided evidence of the existence of a dual signalling system. It has become clear that these receptors have a role in synaptic plasticity and that they might also have a fundamental role in epilepsy through the strategic control of network excitability. However, the role of kainate receptors in other brain pathologies remains obscure.

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Introduction

Glutamate receptors are integral membrane proteins which are responsible for mediating information transfer at most excitatory synapses in the brain. Ionotropic glutamate receptors belong to three receptor families (although a fourth class of ionotropic glutamate receptors of unknown function also exists: delta receptors), named after the agonists that activate them: *N*-methyl-D-aspartate (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPARs) and kainate receptors (KARs). These receptors are among the most studied molecules in the central nervous system. Similarly to the AMPARs and NMDARs, KARs are tetrameric combinations of five subunits: GluR5, GluR6, GluR7, KA1 and KA2. Of these, GluR5–7 can form functional homomeric or heteromeric receptors, whereas KA1 and KA2 only participate in heteromeric receptors, partnering any of the GluR5–7 subunits.

Many of the pharmacological agonists and antagonists active at KARs also interact with AMPARs [1]. This lack of pharmacological specificity has hindered our understanding of KAR function for several years. However, the discovery that the 2,3-benzodiazepines, particularly GYKI 53655 (LY300268 or the active isomer LY303070), antagonize AMPARs but not KARs [2,3], and the more recent development of compounds specific for KAR subunits and mice deficient for KAR subunits [4–6] have paved the way to study the synaptic physiology of KARs. As a result, KARs have been demonstrated to have different roles in synaptic transmission. At the postsynaptic level, KARs might carry part of the current charge of the synaptic response [7–10], similarly to the better characterized AMPARs and NMDARs. However, KARs are also targeted to a variety of presynaptic locations, where they might regulate transmitter release at both excitatory and inhibitory synapses [11–13]. An emerging characteristic of KARs is that they use two forms of signalling, a canonical pathway involving ion flux and another, noncanonical signalling pathway which links KAR activation to G protein activation [14,15,16•,17]. As such, KARs influence neuronal excitability and information transfer in the brain. Several questions concerning KARs are now starting to be (partially) resolved. For example, which proteins endow functional KARs with their idiosyncratic kinetics and regulate their localization? What impact does insertion and retrieval at the synapse have or how do they influence synaptic physiology and, ultimately, animal behaviour? How do the KARs contribute to overall synaptic activity? Although several reviews have addressed certain aspects of the molecular biology and physiology of KARs [1,18,19], here I examine the most recent findings regarding KARs with implications for their physiology.

KARs and the modulation of transmitter release

A considerable amount of effort has been devoted to determining the role of presynaptic KARs in the control of transmitter release, with the hippocampus being the most commonly used model structure [18]. Here, KARs are widely distributed in presynaptic buttons and they can bidirectionally regulate the release of glutamate at the mossy fibre to CA3 synapses [20]. In this way, a percentage of the extensive frequency-dependent facilitation, a hallmark of these synapses, is attributable to the activation of presynaptic KARs by synaptically released glutamate. This is reminiscent of applying low concentrations of exogenous kainate. However, rather than facilitating

mossy fibre synaptic transmission, higher concentrations of kainate depress it. Interestingly, this phenomenon is reproduced by synaptic activity, in that brief conditioning tetanus of associational fibres enhances the mossy fibre responses, whereas prolonged tetanus has a depressant effect [20]. Therefore, the characteristic short-term plasticity of mossy fibre–CA3 synapses is partly mediated by the long-lasting activation of a kainate autoreceptor. This mechanism has been shown to impose associative properties to mossy fibre long-term potentiation (LTP) because the activity in neighbouring mossy fibre synapses influences the threshold for inducing mossy fibre LTP [21]. The precise mechanism involved in the presynaptic facilitation of glutamate release is unclear. Facilitation of glutamate release is thought to occur because presynaptic KARs depolarize synaptic terminals to a level at which further release is enhanced. However, a mechanism involving the activation of a cAMP–protein kinase A signalling cascade has also been postulated [22]. Recent data have shown that inhibition, but not facilitation, of release by presynaptic KARs depends on a G-protein-dependent mechanism [23]. Therefore, it is possible that the threshold for activating one or other KAR signalling pathways would determine the physiological response.

It is now widely accepted that presynaptic KARs also control the release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the hippocampus. Although KAR stimulation might enhance the release of GABA at connections between interneurons [24,25], they inhibit GABA release at interneuron–pyramidal cell connections [11,12,14,26]. It seems that the spillover of glutamate from adjacent terminals could activate KARs at presynaptic GABA terminals [27]. The specific role of receptors controlling GABA release in hippocampal synaptic networks remains unclear. However, in experiments carried out *in vivo*, infusion of kainate reduces the efficiency of recurrent inhibition, producing a state of hyperexcitability that leads to the generation of recurrent epileptic spikes [11]. Accordingly, KARs antagonists are able to prevent and/or abolish epileptic activity induced by pilocarpine in rats [28]. Therefore, the excitability of the network seems to be under the fine control of the extracellular concentrations of glutamate through the transient and/or tonic activation of KARs.

An important issue that has been a matter of debate over the past few years is the mechanism by which KARs affect release at these inhibitory synapses. Although evidence has been presented that GABA release is inhibited by a G protein and protein kinase C (PKC)-dependent mechanism [14], it was postulated that this was the result of an indirect effect, given the large increase in interneuron firing induced by KAR agonists. Although it is possible that the large barrage of inhibitory postsynaptic currents received by pyramidal cells might cause some short-circuiting [29], there is now a general consensus that this is not sufficient to account for such inhibition. KARs might strongly regulate CA1 GABAergic circuitry through two distinct and opposing mechanisms. Whereas activation of somatodendritic KARs can increase the activity of GABA interneurons, presynaptic KARs might diminish inhibition. These two effects are mediated by receptor populations that are functionally, molecularly and pharmacologically distinct, with one situated in somatodenritic and/or axonal compartments and the other at presynaptic terminals directly inhibiting GABA release [26,30,31•].

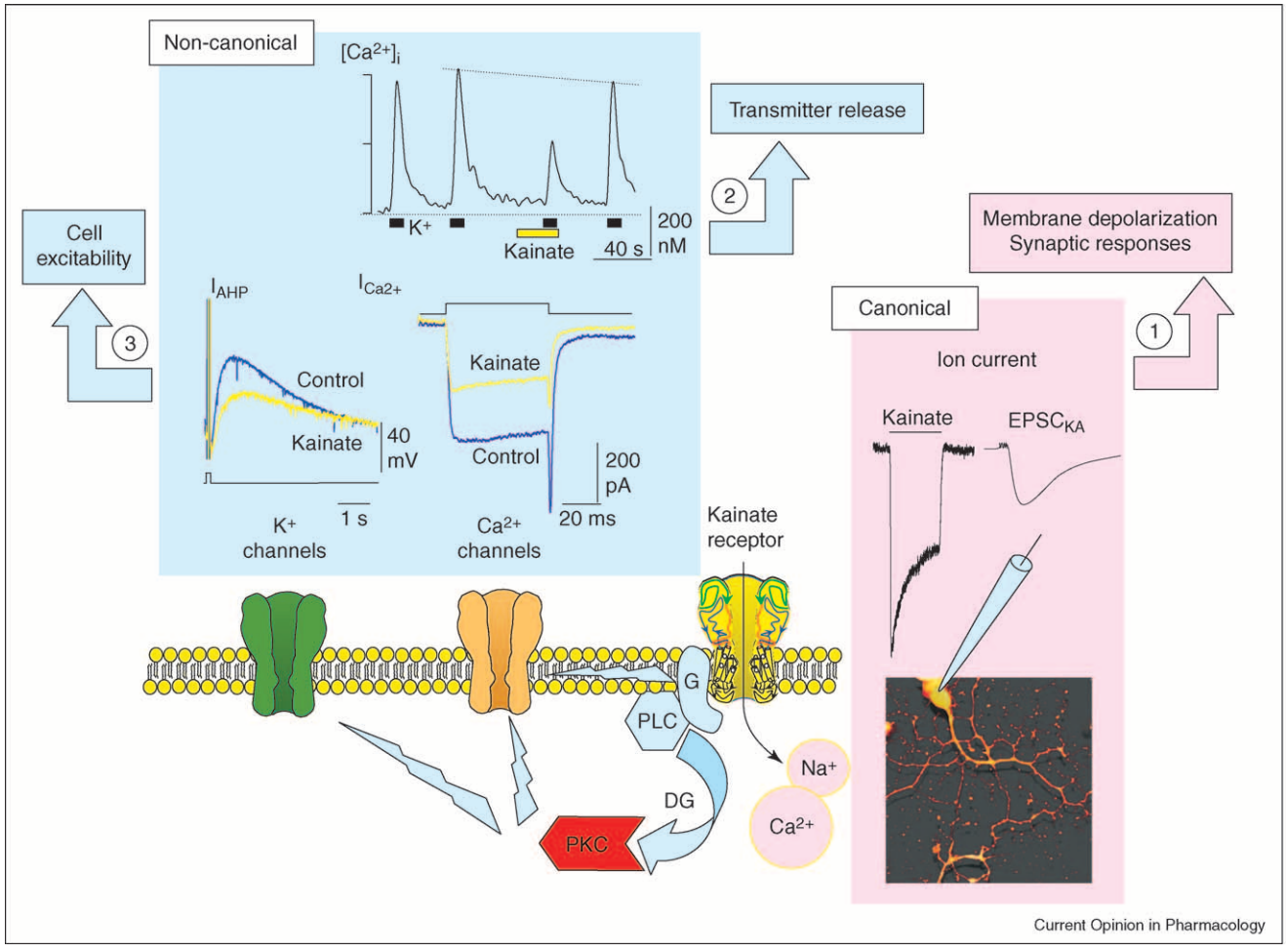
Dual signalling of KARs

An emerging characteristic of KARs and other ionotropic glutamate receptors is that signalling might occur through ion flux and by receptor coupling to G proteins, which in turn might regulate voltage-dependent Ca^{2+} channels (Figure 1). This was first observed in the hippocampus [14,32] and later confirmed in other preparations [23,32–34]. Indeed, in cultured dorsal root ganglion cells that almost exclusively express GluR5 and KA2 KAR subunits, activation of KARs produced the release of Ca^{2+} from intracellular stores in a G-protein-dependent manner and the concomitant activation of PKC inhibited voltage-dependent N-type Ca^{2+} channels [15]. This noncanonical signalling is independent of ion channel activity and provides insights into the dual signalling of KARs because both pathways depend on a common ionotropic subunit, GluR5. It is still unknown how an ion channel becomes coupled to a G protein. However, it seems likely that an intermediate or linker protein participates. Unfortunately, none of the KAR-interacting proteins so far identified carry out this role and, thus, this intermediary element remains to be identified. More work is necessary, probably involving proteomic analysis [35••] to identify this intermediary element. In addition, this property does not seem to be restricted to a given KAR subunit. Postsynaptic KARs can also inhibit afterhyperpolarizing currents through this signalling system, and the GluR6 and/or KA2 subunit is involved rather than GluR5 [16•,17]. What seems to be a constant rule is that the inhibitory activity of KARs is linked to noncanonical signalling. The variety of signals activated by metabotropic KARs indicates certain unpredictability in the coupling of KARs to G proteins, and much work is needed to clarify when KARs might work through classical or through noncanonical signalling.

KAR trafficking

In recent years, several scaffolding proteins have been identified that are crucial for efficient AMPAR and NMDAR targeting and function [36–38]. Alternative splicing and RNA editing of ionotropic glutamate receptors has also been shown to have important roles in receptor assembly and trafficking, and several recent studies have identified important motifs that regulate KAR delivery to the plasma membrane [39]. Similarly

Figure 1



Dual signalling by KARs. KARs could signal as cationic ion channels (the canonical pathway), through which they depolarize the membrane (1). However, they can also set in motion a noncanonical signalling pathway involving the activation of a pertussis toxin-sensitive G protein (G). As a consequence, phospholipase C (PLC) is also activated, leading to the activation of PKC, probably by the increase in diacylglycerol (DG). This second-messenger system could inhibit Ca^{2+} channels, as illustrated by the direct recording of Ca^{2+} currents ($I_{Ca}{}^{2+}$, lower recording) and intracellular Ca^{2+} signal ($[Ca^{2+}]_i$) (top trace) [15]. This action might lead to the modulation of transmitter release (2). Although Ca^{2+} channels could also be inhibited by a membrane-delimited process, the activation of PKC could produce the inhibition of the Ca^{2+}-dependent K^+ current responsible for the repolarization of the membrane after repetitive firing (afterhyperpolarizing current [I_{AHP}]) [16]. The inhibition of such a current by KARs drastically increases membrane excitability (3). EPSC, excitatory postsynaptic current.

to AMPARs, assembly of KARs in the endoplasmic reticulum (ER) probably involves the formation of subunit dimers which further assemble into a dimer of dimers [40]. ER retention signals, consisting of a stretch of basic residues, have been identified at the carboxy-terminal domain of GluR5–2b, a splice variant of GluR5 [41]. Indeed, disrupting R896 promotes ER exit and surface delivery of GluR5–2b receptors in both heterologous cells and neurons, and this residue is conserved in GluR6 and GluR7. However, in GluR6 and GluR7, the conserved R896 does not appear to have the same function [41]. ER export of the receptors is also regulated by a pair of positively charged residues in the carboxy-terminal region, R900 and K901. Surrounding residues seem to shield these amino acids, preventing them from acting as a retention signal [41]. In GluR6, a stretch of residues, rather than retention motifs, has been identified as a forward-trafficking signal [39], and is a cluster of positively charged amino acids which has a similar function in other receptors. Meanwhile, surface expression of KA2 subunits is controlled both by the arginine-rich ER retention signal and a dileucine endocytotic motif [41]. RXR-based retention signals prevent surface expression of homomeric receptors containing only KA2 (and probably KA1), whereas GluR52b or GluR52c subunits enable KAR subunits to exit the ER as heteromeric receptors only [42]. Indeed, these RXR sequences can be masked by oligomerization with other proteins. Although GluR6

and GluR7 might reach the plasma membrane as homomers, the efficiency is increased by heteroligomerization with other subunits, which might shield these motifs. Therefore, the surface delivery of KARs is largely determined by their subunit composition.

Regulation of KAR surface expression

Although ER retention has a rate-limiting role in KAR trafficking, the mechanism controlling surface expression remains unclear. Important residues for ER retention or exit are proximal to PDZ-binding motifs which, as in most glutamate receptors, are also present at the carboxy-terminal domain of KARs. Several proteins containing PDZ motifs interact with KARs, although most of these are promiscuous and also bind to AMPAR and possibly NMDAR. Those that also interact with AMPAR include PSD95/SAP90, CASK (calcium calmodulin-associated serine/threonine kinase), GRIP (glutamate receptor-interacting protein), PICK1 (protein-interacting with C kinase 1) and syntenin [35••,42–44]. However, PDZ proteins such as PICK1, GRIP and PSD95 do not have any significant role in GluR5 or GluR6 ER exit [39,41]. It has recently been shown that the surface expression of KARs can rapidly be altered through protein–protein interactions, and these proteins have also been implicated in the trafficking of AMPARs and KARs to the synapse [45]. These proteins appear to regulate KAR and AMPAR trafficking differently because disrupting the interaction with GRIP decreases the synaptic responses through KARs, while concomitantly increasing the AMPAR component. Similarly, interfering with the interaction of PICK1 with KARs and AMPARs was found to depress the KAR-mediated excitatory postsynaptic current, leaving the current mediated by AMPARs intact [45]. It is likely that synaptic expression of KARs is a tightly regulated process which depends on interactions with still unknown scaffolding proteins and other proteins that influence the trafficking and targeting of KARs. For example, the rules governing polarized targeting of KARs to axons and dendrites remain unknown. The study of knockout mice for different subunits has provided no links between subunit composition and the compartment to which KARs are targeted. Identification of these (chaperone) proteins will enable us to understand the mechanisms that regulate KAR signalling and function. Indeed, a recent study [35••] using a proteomic approach has identified that GluR6a and GluR6b each interact with a different set of cytosolic proteins (e.g. calmodulin, calcineurin, profiling II) and that some of these proteins contribute to the fine regulation of trafficking and function of GluR6-containing KARs.

The slower kinetics and higher apparent affinity shown by native KARs when compared with recombinant KARs remains a mystery. It was initially thought that KARs might lie outside of the synapse, resulting in a reduced sensitivity to the synaptic release of glutamate [46]. However, this is unlikely because it has been shown unequivocally that synaptic KARs can be activated by quantal release, retaining their typical slow kinetics [47,48]. Although the modification of recombinant KARs by interacting proteins has not been studied systematically (with the exception of the study by Garcia *et al.* [43]), no change in kinetics or in the affinity of these receptors have been reported when they assemble with scaffolding proteins. It is possible that such an association would modify their intrinsic properties, similarly to the behaviour of AMPARs in association with transmembrane AMPA receptor regulatory proteins (TARPs) (as recently reported [36]). Association with TARPs not only modifies trafficking but also the affinity and gating of AMPAR channels [49••,50•]. It is still unclear why there are so many extrasynaptic KARs compared with synaptic KARs [51]. Interestingly, glutamate released from astrocytic processes could activate KARs diffusely, as seen in the CA1 field [52]. Therefore, KARs might still be one of the key mediators of communication between glia and neurons, providing an additional function for these receptors.

KAR activity, synapse formation and physiology

In juvenile stages in rodents, it appears that KARs are involved in axonal navigation. During maturation, the filopodia of axons move rapidly towards their targets, although this becomes slower as development proceeds. Long-term blockage of KARs in developing hippocampal slices prevents this normal developmental decrease in the speed of axonal growth [33]. Thus, axonal motility is bidirectionally regulated by neuronal activity through KARs. Whereas low concentrations of kainate stimulate motility, higher concentrations block it. Interestingly, it seems that different downstream mechanisms are involved in these two actions. The inhibition of motility at higher kainate concentrations is dependent on the activation of a G protein, suggesting a role for noncanonical KAR signalling in synapse formation. In summary, glutamate could regulate filopodial motility as a function of the extent of presynaptic KAR activation.

LTP at thalamocortical synapses is related to a rapid switch from KAR- to AMPAR-mediated synaptic transmission [53], suggesting that KARs and AMPARs are subject to differential trafficking. The reduction in the number of KAR-expressing synapses coincides with periods that are crucial for activity-dependent plastic changes in thalamocortical synapses. Recent data indicated that these changes might involve the internalization of GluR6-containing receptors, which initially traffic to early endosomes and subsequently enter recycling or degradation pathways [54•]. Interestingly, NMDAR activation leads to the rapid endocytosis of GluR6 KARs, which might or might not recycle back into the plasma membrane. This differential sorting of internalized KARs and AMPARs would provide a rational mechanism for the

rapid and longer-term activity-dependent regulation of KARs in neurons.

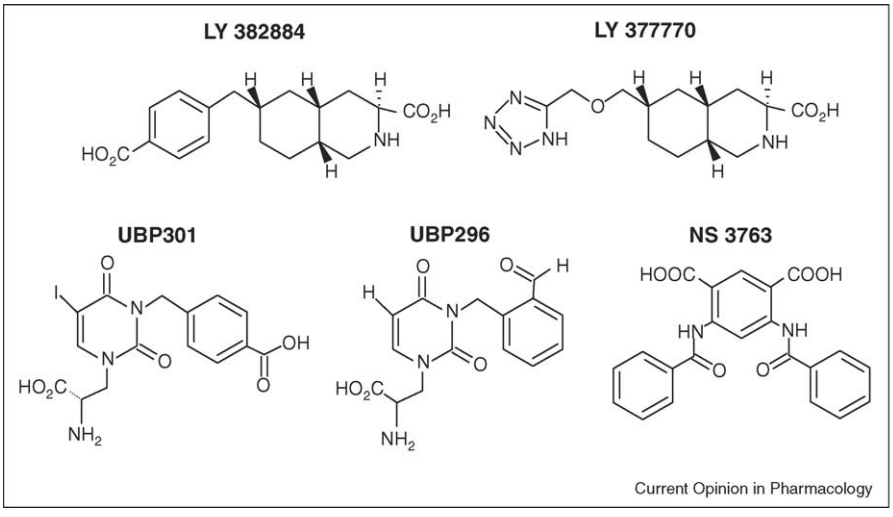
KAR downregulation during development [53] suggests a limited contribution of postsynaptic KARs to information transfer after the crucial period of experience-evoked plasticity. This raises the question as to the role of postsynaptic KARs in the later stages. However, the role of KARs in synaptic integration might be underestimated because the longer-lasting, albeit smaller, current provoked by KAR activation in CA1 interneurons endows these synapses with new, unpredicted properties [55]. Moreover, after synaptogenesis, these same presynaptic receptors might also have a significant role in information transfer. Indeed, as found in the hippocampal mossy fibre to CA3 pyramidal cell synapse, presynaptic KARs might set the gain of the synapse for action potential-dependent and -independent glutamate release [23].

KARs and pathology

As in physiology, the progression in understanding the role of KARs in pathology has been hampered by the lack of tools allowing easy blockade of responses caused by KAR activation. However, useful compounds have lately been developed by Elli Lilly (Figure 2; see also [56]). In particular, LY382884 [(3S,4aR,6S,8aR)-6-((4-carboxyphenyl)methyl-1,2,3,4,4a,5,6,7,8,8a-decahydro isoquinoline-3-carboxylic acid] seems to antagonize KARs at concentrations that do not affect AMPARs or NMDARs. Furthermore, LY382884, like another molecule of this series (LY293558), is claimed to be a selective antagonist of neuronal GluR5-containing receptors [56]. Similarly, LY377770, an active enantiomer of LY294486, appears to be selective for receptors containing GluR5 subunits, and antagonizes kainate responses in dorsal root ganglion cells (IC_{50} of 0.51 μM) [28]. Novel derivatives have been synthesized that show varying levels of selectivity and potency for AMPARs or KARs depending upon the substituents added [57,58]. One of these antagonists, (S)-3-(4-carboxybenzyl)-5-iodowillardiine (UBP301), is selective for native receptors expressed by dorsal root ganglion cells (i.e. GluR5-containing KARs) over AMPARs. Structural changes made to this parent structure provided a number of derivatives with some specificity. Amongst them, UBP296 behaved as a potent antagonist as assessed by its ability to block KAR-mediated, but not AMPAR-, NMDAR- and group I mGlu receptor-mediated, responses on neonatal rat motoneurones. The S enantiomer of this compound, UBP302, is even more potent than UBP296 at antagonising kainate responses in dorsal roots. Finally, the only non-competitive antagonist known of KARs, NS3763, is selective for GluR5 homomeric receptors [31•].

Curiously enough, although GluR5 subunits are essential for KAR-mediated responses in dorsal root ganglion neurons and for presynaptic regulation of transmitter release in the spinal dorsal horn [15,59], nociceptive thresholds are unaffected in GluR5- and GluR6-deficient mice [60]. However, there is evidence that KARs are involved in

Figure 2



Selective antagonists of KARs. Most of the compounds so far described, such as LY382884 and LY377770, are selective for GluR5-containing receptor complexes and are inactive at AMPARs, GluR6 homomeric receptors and GluR6/KA-2 heteromers. A willardiine derivative, UBP301, is a new GluR5 antagonist, showing a nearly 30-fold selectivity for kainate over AMPA receptors. A derivative of this compound, UBP296, was found to be the most potent antagonist, as it presents K_b values of 0.6, 0.8 and 1.0 μM for preventing glutamate-induced calcium influx in cells expressing GluR5, GluR5/GluR6 and GluR5/KA2 receptors, respectively [58]. The decahydroisoquinoline LY377770 has demonstrated effectiveness for preventing epilepsy [28] and for neuroprotection in two models of cerebral ischaemia [78]. However, UBP compounds seem to have advantages over LY377770 and LY382884: they have greater selectivity for GluR5 versus AMPARs and improved water solubility. NS3763 is a non-competitive antagonist with selectivity for GluR5 homomeric receptors [31•].

persistent pain and fear memory [61]. The possible contribution of KARs to pain is also supported by pharmacological data. The GluR5 selective antagonist, LY293558, has demonstrated preclinical and clinical efficacy in models of pain [62–65]. Such a profile definitively raises the possibility that KARs antagonists could be effective for the treatment of certain forms of allodynia and hyperalgesia, as well as acute migraine.

Several lines of evidence suggest that KARs might be involved in certain brain disorders, the best established involving excitatory imbalances linked to epilepsy. Indeed, for several years, kainate injection has provided an animal model to study human temporal lobe epilepsy. The inhibition of GABA release leading to recurrent epileptic activity might account for the acute epileptogenic effect of kainate [11]. However, this mechanism does not explain the chronic epilepsy generated months after kainate treatment. It is well known that both in this model of temporal lobe epilepsy and in human patients, sprouting of glutamatergic fibres occurs, establishing a large number of aberrant synapses. It has recently been shown that the synaptic response mediated by KARs provides a substantial component of the excitatory transmission at these functional aberrant synapses [66••]. Indeed, granule cells of the dentate gyrus (DG) target KARs to the synapses made by sprouting mossy fibres, and although the DG normally only displays AMPAR-mediated responses, mossy fibre to DG synapses have an additional KAR-mediated component which could amount to as much as half of the total synaptic current. Thus, aberrant KAR-operated synapses form under pathological conditions, and KARs definitively participate in the pathogenesis of temporal lobe epilepsy, raising the possibility of designing antiepileptic therapies based on KAR antagonism.

Because obsessive compulsive disorder might be the consequence of glutamatergic dysfunction, the expression of KARs has been examined in patients with this condition, in an attempt to find associations with single nucleotide polymorphisms (SNPs) in two KARs, GluR6 and GluR7 (GRIK2 and GRIK3 in humans) [67]. Although no strong associations were found, GRIK2 SNP I867 was transmitted less readily than expected, supporting a functional role for this variant, which has recently been associated with autism. Although it has not been possible to define a compelling link between KARs and other diseases, an allele of the GluR6 gene is associated with early-onset Huntington's disease [68]. Thus, in this disease, linkage disequilibrium with a GluR6 variant or with another gene in this region could exist. Indeed, GluR6-mediated excitotoxicity has been implicated in the pathogenesis of Huntington's disease [69]. Allelic variants of GRIK1 (GluR5) but not GRIK2 (GluR6) appear to contribute a major genetic determinant of the pathogenesis of juvenile absence epilepsy and related phenotypes [70,71]. Interestingly, the GluR5 gene is located on chromosome 21q22.1, and physical mapping situates it near to the amyloid β precursor protein and superoxide dismutase 1 regions [72], making it a possible candidate for familial amyotrophic lateral sclerosis and other diseases [73]. In addition, GluR5 might influence the phenotypes associated with partial trisomy or monosomy of chromosome 21. Although we await more compelling evidence, recent results have indicated that there is a disequilibrium of GRIK2 (GluR6) transmission in autism [74] and schizophrenia [75•]. However, no linkage has been found with GluR5 (GRIK1) SNPs and their haplotypes in schizophrenia [76]. Thus, until a definitive role for KARs in these disorders is demonstrated, this remains a matter for speculation.

Conclusions

KARs remained elusive to physiologists until the selective AMPAR antagonist GYKI53655 was discovered and the role of KARs in several processes began to be understood. Further knowledge is accumulating with the aid of mice deficient for KAR subunits or engineered with modified KARs. However, possible functional compensations must be taken into account in these mice [53]. It has emerged that these receptors could be located postsynaptically and mediate part of the synaptic current at certain synapses. Perhaps more importantly, KARs are also presynaptic, where they can modulate the release of both glutamate and GABA in a bidirectional fashion. Therefore, KAR activity can exert tight control of the excitability of brain networks. It has also emerged that KARs display dual signalling capabilities because they are canonical ion channels and can also couple to G proteins, triggering a second-messenger cascade (noncanonical signalling). The speculation as to how an ion channel can activate a G protein has encouraged the study of proteins that interact with KARs, which could influence their targeting as well as their signalling capacities. Furthermore, high-resolution structural analyses are resolving the atomic structure of KAR subunits [77••], providing insights into molecular gating and agonist selectivity. Although it is clear that KARs participate in short- and long-term synaptic plasticity, their role in brain pathologies remains unclear, with the exception of epilepsy, where KARs seem to have a fundamental role, probably through the strategic control of network excitability.

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