Metabotropic actions of kainate receptors in the CNS

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Abstract
Kainate receptors (KARs), together with NMDA and α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPA), are typically described as ionotropic glutamate receptors. Although ionotropic functions for KARs are beginning to be characterized in multiple brain regions, both, in the pre- and post-synaptic compartments of the synapse, there is accumulating evidence that KARs mediate some of their effects without invoking ion-fluxes. Thus, since 1998, when the first metabotropic action of KARs was described in the modulation of GABA release in hippocampal interneurons, there have been increasing reports that some of the functions of KARs involve the participation of intracellular signalling cascades and depend on G protein activation. These surprising observations, attesting metabotropic actions of KARs, akin to those usually attributed to seven transmembrane region G protein-coupled receptors, make the physiological classification and description of glutamate receptors more complex. In the present review, we describe the metabotropic roles of KARs in the CNS and discuss the intriguing properties of this receptor which, structurally shows all the facets of a typical ionotropic receptor, but appears to express a metabotropic remit at some key synapses.

Keywords: G protein, GABA, glutamate, kainate receptors, metabotropic, protein kinase A, protein kinase C.


It is well established that glutamate is the major excitatory neurotransmitter in the mammalian CNS. Glutamate participates in normal synaptic transmission, as well as long-term potentiation (LTP) and long-term depression (LTD), believed to reflect the molecular and cellular bases for learning and memory processes (Jonas and Monyer 1999). Glutamatergic neurotransmission is also involved in some important developmental functions including neuronal maturation and synaptogenesis. Aberrations with the glutamate system may underlie some forms of epilepsy and contribute to neuronal pathologies arising from brain ischaemia and hypoglycaemia, as well as the aetiology of some disease states like Alzheimer’s, Parkinson’s, Huntington’s chorea and lateral amyotrophic sclerosis (Eubanks et al. 1993; Sander et al. 1995, 1997; Mulle et al. 1998; Jonas and Monyer 1999; McDonald et al. 1999).

Glutamate receptors are divided in two major families. The ionotropic receptors have three members, viz. NMDA-, α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)- and kainate (KA)-type, and the classically defined, seven transmembrane region, G protein-coupled metabotropic glutamate receptors (mGlur), consist of three subgroups, viz. types I, II and III (Hollmann and Heinemann 1994). In common with the other ionotropic glutamate receptors, kainate receptors (KARs) are homo- or heteromeric tetramers. The tetramers are composed of GluR5, GluR6, GluR7, KA1 or KA2 subunits, the former three having the ability to form low-affinity homomers, while the KA1 and KA2 impart higher agonist affinities in heteromeric assemblies.

While there is a considerable amount of information on NMDA and AMPA receptors and their function, KARs have been less tractable, primarily because, until recently, there was a lack of pharmacological tools to differentiate the activation of KA and AMPA receptors, which display...
cross-activation by their defining agonists. However, the availability of selective AMPA receptor antagonists in form of (1-(4-aminophenyl)-3-methylcarbamyl)-4-methyl-7,8-methylenedioxy-3,4-dihydro 5H-2,3 benzodiazepine (GYKI53655), since 1995, and (+)-4-(4-aminophenyl)-1,2-dihydro-1-methyl-2-propylcarbamoyl-6,7 methylenedioxy-phthalazine (SYM2206), more recently, has allowed KARs to be studied in isolation without the confounding aspects of AMPA receptor co-activation. This, together with the availability of transgenic mice with specific KAR subunits ablated, has greatly accelerated the exploration of the role of KARs in CNS physiology.

An important outcome of recent studies has been that there is now a definitive case for both the pre- and post-synaptic actions of KARs at several synapses. KARs are ubiquitously distributed in the CNS, where they were originally described as being post-synaptic and found in the principal cells and interneurons of the hippocampus, lateral amygdala, dorsal root ganglia, bipolar cells of the retina, cerebral cortex, globus pallidus and cerebellum (Huettnner 2003). Accumulating evidence now indicates that KARs are significantly localized in pre-synaptic terminals, where they modulate neurotransmitter release (Huettnner 2003; Lerma 2003).

Although classically, the actions of KARs have been attributed to the ionotropic activity, both pre- and post-synaptically some of the effects have been found to be temporally inconsistent with ionotropic mechanisms, and indeed display frank dependence on heterotrimeric G proteins. This therefore invokes overt metabotropic mechanisms of action for KARs, yet these are clearly not G protein-coupled receptors (GPCRs) as usually defined, i.e. single proteins with seven transmembrane regions and defined intracellular sites for G protein interaction (Pierce et al. 2002). Interestingly, precedence for these intriguing observations already exists from studies with AMPA receptors, which have been shown to mediate some of their effects by metabotropic routes (Wang and Durkin 1995; Wang et al. 1997; Hayashi et al. 1999; Kawai and Sterling 1999; Perkinton et al. 1999; Marin et al. 2001; Satake et al. 2004; Takago et al. 2005). KARs in their metabotropic guise have therefore now entered the neurochemist’s scientific arena.

In this review, we will consider the key evidence supporting the metabotropic actions of KARs, both pre- and post-synaptically. Specifically, in contrast to previous reviews providing a broad view of KAR physiology, we limit our discussion in this study to the most well-defined metabotropic modulatory influences of KARs. Thus, pre-synaptically, we review the role of KARs in both inhibiting and facilitating the release of GABA (as heteroreceptors) and

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**Fig. 1** Metabotropic actions of kainate receptor (KARs) at synaptic connections to and from major hippocampal cell types. Topographic representation of (a) interneuron (cyan)-CA1 pyramidal cell (PC; blue), (b) CA3 Schaffer collateral (SC)-CA1 PC (blue) and (c) mossy fibre (MF)-CA3 PC (red) synapses. Boxes zooming (a–c) depict details of the respective synapse and also show schematic representations of the evoked post-synaptic current (inhibitory or excitatory) profiles under control conditions (CON) and in the presence of kainate (KA) or synaptic KAR activation. Insets show recording configurations: (i) as employed in (a) and (b) and (ii) as employed in (c). Synaptic connections are shown with respect to hippocampal strata (shown left): stratum oriens (st. ori.), stratum pyramidale (st. pyr.), stratum lucidum (st. luc.), stratum radiatum (st. rad.) and stratum lacunosum-moleculare (st. l.m.). (a) Pre-synaptic KARs at the hippocampal st. ori. or st. rad. interneuron-CA1 PC synapse are composed of GluR6-GluR6 and/or GluR5-KA2 subunits (although pharmacological evidence points to nerve terminal KARs having GluR6-containing but GluR5-lacking receptors). KARs present at these GABAergic pre-synaptic terminals produce an attenuation of GABA release onto CA1 PC dendrites. This modulation involves the pre-synaptic activation of a G<sub>MP</sub> protein, which stimulates phospholipase C (PLC) to produce diacylglycerol (DAG). DAG then activates protein kinase C (PKC), which phosphorylates, as yet unknown target, to decrease GABA release (a; PRE). (b) Pre-synaptic KARs at the SC-CA1 PC synapse are composed of GluR6 subunits at least. KARs at these GLUTAMERGIC pre-synaptic terminals produce a decrease in glutamate release onto CA1 neuron dendrites in the st. rad. This modulation involves pre-synaptic G protein activation and regulation of voltage-dependent Ca<sup>2+</sup> entry (b; PRE). Post-synaptic KARs at this synapse are probably extrasynaptic receptors (responding to ‘spillover’ glutamate) and contain GluR6 and KA2 subunits. Post-synaptic KAR activation produces a long-lasting decrease of a Ca<sup>2+</sup>-activated K<sup>+</sup> current (I<sub>CaK</sub>) that involves a G protein-mediated activation of PLC and downstream PKC. Protein kinase A (PKA) and mitogen-activated protein kinases may also be activated. KARs therefore increase post-synaptic excitability at this synapse (b; POST). (c) Dentate gyrus resident granule cells (green) project MFs which form GLUTAMERGIC synapses with the apical dendrites of CA3 PCs (red) in the st. luc. Pre-synaptic KARs at the MF terminals contain GluR6, KA2 and possibly KA1 and GluR7 subunits. Although there is pharmacological evidence for the presence GluR5 subunits, these subunits are not detectably expressed in granule cells. MF terminal KARs produce a bimodal effect on release depending on the agonist concentration. (c; PRE). At KA concentrations higher than 100 nmol/L, a decrease in glutamate release involves the activation of a G protein and the modulation of adenyl cyclase (AC) and PKA activity (c; PRE upper). At KA concentrations lower than 100 nmol/L, a facilitation of glutamate release also involves the activity of AC and PKA (c; PRE lower). Post-synaptic KARs at the MF-CA3 PC synapse contain GluR6 and KA2 subunits and produce a reversible decrease of the I<sub>CaK</sub> current that involves G protein, PLC and PKC activation. KARs therefore increase the post-synaptic excitability of this synapse (c; POST). At the same time, synthetically localized ionotropic KARs produce an excitatory post-synaptic potential. Activation of association/commissural terminals (A/C) on CA3 PC dendrites in the st. rad. can produce ‘spillover’ glutamate to heterosynthetically activate MF-CA3 PC synapses in the st. luc. A/C terminals themselves express GluR6 (and possibly GluR5) containing KARs that suppress glutamate release (autoreceptor mechanism undefined). In contrast, perforant pathway (PP) terminals synapsing onto CA3 PC dendrites in the st. l.m. express GluR5/6 containing KARs that facilitate glutamate release (autoreceptor mechanism undefined).
glutamate (as autoreceptors). Post-synaptically, we discuss the intriguing role of KARs in modulating neuronal (ionotropic) excitability through a metabotropic influence.

**Pre-synaptic metabotropic actions of kainate receptors**

Many of the studies looking at KAR function have used spontaneous or evoked post-synaptic synaptic currents to assess the effect of applied agonist. With this type of model (Fig. 1, Inset I and II), in addressing a KAR-mediated regulation of neurotransmitter release intimated by a post-synaptic response, key questions to consider are: (i) Are the KARs truly pre-synaptic on nerve terminals, or is there an indirect effect on release as a result of post-synaptic somatodendritic (or even axonal) receptors in the proximal neuron? and (ii) Is the pre-synaptic receptor activity ionotropic or metabotropic?

The first question can be addressed by delineating effects on parameters such as paired-pulse facilitation (PPF), coefficient of variation (a change in $1/CV^2$ correlated with the change in post-synaptic current amplitude) and synaptic failures, which reflect nerve terminal resident modulation (Bekkers and Stevens 1990; Clements 1990; Malinow and Tsien 1990; Korn and Faber 1991; Thomson and Deuchars 1995; Rodriguez-Moreno et al. 1997), from effects on the afferent/pre-synaptic fibre volley, which reflect modulation at somatodendritic receptors in the proximal neuron (Andersen et al. 1978; Henze et al. 1997; Kamiya and Ozawa 1998, 2000; Schmitz et al. 2001). Additionally, the demonstration of KAR-dependent modulation by synthetically released glutamate (in the presence of AMPA and NMDA receptor blockade) provides further credence to the presence of pre-synaptic KARs (reviewed by Pinheiro and Mulle 2006). A useful neurochemical model for confirming nerve terminal KAR-mediated modulation has been the...
synaptosomal preparation which is devoid of cell-body and axonal elements. As such, this preparation directly assays the release of neurotransmitter and only reports the modulation thereof by pre-synaptic receptors present at the terminal itself (Sánchez-Prieto et al. 1996; Perkinton and Sihra 1998, 1999; Wang et al. 2002, 2006; Wang and Sihra 2003, 2004).

Addressing the second question: defining whether a KAR-mediated response is ionotropic or metabotropic, rather depends on determining electrophysiological parameters such as holding current and input resistance changes (Freking et al. 1999; Ruiz et al. 2005; Negrete-Díaz et al. 2006). This is usually only technically possible for post-synaptic receptors, because the small size of most nerve terminals precludes such measurement therein. Notwithstanding, an assessment of whether effects of KA on synaptic responses are rapid or long-lasting can allude to the type of mechanism. Thus, while an ionotropic response should typically deactivate in a subsecond time-scale, a long-lived response is often indicative of a potential metabotropic action of KARs (Chittajallu et al. 1996; Negrete-Díaz et al. 2006). However, in corroboration of the latter, a key diagnostic is whether the response is sensitive to inhibitors of the putative metabotropic signalling pathways thought to be involved. We review hereafter, examples of pre-synaptic KAR-mediated responses that have been assessed with the application of the aforementioned criteria, and thereby allude to metabotropic mechanisms in the pre-synaptic heteroreceptor control of neurotransmitter GABA and autoreceptor modulation of glutamate release itself.

**Metabotropic actions of kainate receptors involved in modulation of GABA release – heteroreceptors**

The initial indications of a metabotropic function of KARs in the brain came from studies showing that this glutamate receptor subtype could produce a depression of the evoked inhibitory post-synaptic current (eIPSC) at synapses established between stratum oriens (Fig. 1a, st. ori.) or stratum radiatum (Fig. 1a, st. rad.) interneurons and CA1 pyramidal cells (CA1 PCs) in the hippocampus (Rodríguez-Moreno et al. 1997; Clarke et al. 1997; Rodríguez-Moreno and Lerma 1998; Fig. 1a). Using the sort of criteria discussed above, these authors delineated this modulation to be as a result of pre-synaptic effects of KARs. Furthermore, and surprisingly at the time, the mechanism of KAR coupling to a decrease in GABA release at these synapses was postulated as being metabotropic. The basis of this hypothesis was the demonstration that the synaptic depression is: (i) dependent on the activation of a pertussis toxin (PTX)-sensitive G protein; (ii) suppressed by inhibitors of phospholipase C (PLC) and protein kinase C (PKC) and (iii) independent of pre-synaptic ion channel activity (Rodríguez-Moreno and Lerma 1998). This was the first clear exemplification of a metabotropic action of KARs described in the CNS. The model proposed, invokes that pre-synaptic KARs located on pre-synaptic inhibitory terminals activate $G_{i/o}$ coupled to PLC to produce the second messenger diacylglycerol. Subsequent activation of a pool of PKC by diacylglycerol then results in a decrease of GABA release (Rodríguez-Moreno and Lerma 1998; Fig. 1a).

Direct support for KARs present on interneurons, physiologically effecting inhibitory modulation of GABA release at interneuron-CA1 principal cell synapses, came from studies by Min et al. (1999). These authors demonstrated that synaptically released, endogenous glutamate (evoked by conditioning stimulation of glutamatergic afferents in CA1 region of the hippocampus), caused synaptic disinhibition. In the absence of any evidence for axo–axonic synapses of glutamate terminals onto interneurons, the results with the experimental paradigm used suggest that a ‘spillover’ of glutamate could heterosynaptically activate KARs to modulate GABA release (Fig. 1a, Inset I). Although, this intimates an immediate effect of released glutamate on synaptically located KARs, the fact remains that axonal KARs are also activated under same stimulation paradigm (Semyanov and Kullmann 2001). This leaves the question begging: are KARs effecting a decrease in eIPSC at interneurons synapses, somatodendritic (extrasynaptic) or nerve terminal-resident? Interestingly, with respect to this issue, pertaining to the subcellular mechanism(s) of KAR action, Min et al. (1999) did note that the depression of the inhibitory post-synaptic currents outlasted the conditioning stimulation. Given that ionotropic KAR-mediated currents typically display rapid deactivation kinetics, this is therefore indicative of a metabotropic action of KARs on interneurons.

Support for nerve terminal-resident KARs producing G protein-dependent inhibition of GABA release, as postulated by Rodríguez-Moreno and Lerma (1998), came from biochemical studies with isolated nerve terminals (synaptosomes). Synaptosomes, by definition, are devoid of a somatodendritic compartment and can thus only reflect nerve terminal resident receptor activity. Using this preparation (Cunha et al. 1997; Perkinton and Sihra 1999) or interneuron micrcultures where the pre-synaptic cell could be monitored (Rodríguez-Moreno et al. 1997), KA application produces a clear decrease of endogenous GABA release in the absence of any somatodendritic KAR involvement. Indicating a metabotropic mechanism of action, Cunha et al. (1999) demonstrated, first, a coupling of KARs to $G_{i/o}$ activity in hippocampal membranes. Secondly, the group (Cunha et al. 1997, 2000) showed that the KAR-mediated depression of GABA release from hippocampal synaptosomes is sensitive to PTX and PLC inhibitors. Collectively, these observations confirm that the inhibition of GABA release at interneuron-CA1 PC synapses is likely mediated by a novel metabotropic action of KARs present in nerve endings per se.
Altogether, the foregoing data strongly support the hypothesis that the inhibition of GABA release, observed at st. ori. or st. rad. interneuron-CA1 PC synapses, is mediated by pre-synaptic KARs activating G_{i/o} coupled to PLC and downstream PKC (Fig. 1a, PRE). Notwithstanding, there remains considerable polemic as to even the existence of metabotropic actions of KARs, with several studies citing alternative ionotropism-based explanations for the reported observations.

Thus, based on the fact that ionotropic KARs occur in the somatodendritic compartment of interneurons, Frerking et al. (1999) proposed that the inhibitory effect of KARs on the eIPSC recorded in CA1 PCs is mediated by the activation of pre-synaptic, metabotropic GABA_B receptors, inhibiting interneuron GABA release by well-documented GPCR mechanisms. The GABA_B receptors are purportedly activated by an initial increase in GABA release evoked by the stimulation of the ionotropic somatodendritic KARs (activation of these KARs produces an increase in the spontaneous inhibitory post-synaptic current (sIPSC) frequency, while decreasing the eIPSC amplitude). In addition to the indirect effect of KARs, through an initial increase in GABA release stimulating pre-synaptic GABA_B receptors, Frerking et al. (1999) also suggest that the decrease in series resistance recorded in the post-synaptic neuron could reflect the activation of post-synaptic GABA_A receptors by the released GABA. Furthermore, the presence of post-synaptic ionotropic KARs may also purportedly contribute to shunting of the evoked inhibitory post-synaptic potential (Bureau et al. 1999). All of the aforementioned effects could mitigate the eIPSC amplitudes at GABAergic interneuron-CA1 PC synapses and therefore account for the decrease produced by KA application. However, balancing the previously mentioned points of contention, several lines of evidence provide argument against the foregoing hypotheses stipulating an ionotropic remit for KARs.

First, standing against the proposal that the inhibitory effect of KARs are mediated by the activation of GABA_B autoreceptors, a number studies have shown that the KAR-mediated inhibition of GABA release persists in the presence of a diverse range of GABA_B receptors blockers (Clarke et al. 1997; Rodriguez-Moreno et al. 1997, 2000; Frerking et al. 1998; Min et al. 1999). Thus, while a scenario involving GABA_B receptor-mediated feedback inhibition of GABA release (leading to a decrease in eIPSC amplitudes) has been confirmed with the effects of KA on spinal cord inhibitory neurons (Kerchner et al. 2001a), this ionotropically initiated mechanism does not explain the KA-induced reduction of the eIPSC amplitude at hippocampal interneuron-CA1 PC synapses.

Secondly, Rodriguez-Moreno et al. (2000) demonstrated that the two KAR-mediated effects in st. ori. interneurons, viz. a KA-elicited increase of the sIPSC frequency and a decrease of the eIPSC amplitude in the same neurons, were, in fact, independent phenomena and could be dissociated by the differential use of agonists. Thus, application of 3–10 μmol/L of the endogenous agonist glutamate causes a clear decrease of eIPSC amplitude, but had no effect on the sIPSC frequency. On the other hand, 0.3 μmol/L (S)-3-amino-3(3-hydroxy-5-tert-butylisoxazol-4-yi) propanoic acid (APTA; GluR5 selective agonist) produces an unambiguous increase in the sIPSC frequency, but without affecting the eIPSC amplitude. Importantly, application of KA still increases the sIPSC, even when its modulatory (metabotropic) effect on the eIPSC has been abolished by PTX treatment of slices. This verifies that the two effects of KAR activation on the sIPSC (somatodendritic) and eIPSC (nerve terminal) are independent and separable events. The conclusion from this is that, while somatodendritic KARs clearly operate to increase interneuron excitability, inhibition of GABA release is achieved by a distinct population of KARs located at the interneuron terminals.

The differential activation of two KAR populations located in separate subcellular compartments of the same interneurons potentially depends on the properties imparted by different subunit compositions of the receptors, or intracellular interactions that affect receptor function. Additionally, one key issue with regard to functional compartmentalization of KARs pertains to the physiological concentrations of glutamate perceived by the receptors as determined by the extent of the synaptic glutamate release and subsequent ‘spillover’ of neurotransmitter to nonsynaptic locations.

From transgenic mice studies, it is apparent that both GluR5- and GluR6-subunits are required for interneuron-CA1 synaptic sensitivity to KA. Coassembly with one or other of these subunits with KA2 would be predicted to produce higher affinity KARs, typical of those in the nerve terminals that mediate a decrease of GABA release. However, in transgenic mice in which GluR5 or GluR6 KAR subunits have been ablated, compensatory changes have been described (Christensen et al. 2004). This confounds the interpretation of data towards elucidating the actual native subunits that form endogenous KARs. Consequently, despite some novel insights into KAR function obtained using knockout studies, predicting a contingent link between the distinct agonist affinities of the somatodendritic versus terminal KARs, and KAR subunit compositions, is not possible from the current knowledge of KAR localization and trafficking (Christensen et al. 2004; Jaskolski et al. 2005). Notwithstanding the now compelling evidence for metabotropic disinhibition produced by pre-synaptic KARs, there is also evidence of the receptors increasing GABA release from interneurons. Thus, KAR activation produces a consistent increase in spontaneous GABA release evoked by low KA concentrations at CA1 interneuron–interneuron synapses (Cossart et al. 2001). Paired recordings of inter-
neuron-CA1 PC synapses indicate that, while low concentrations of KA mediate facilitation of release in pairs reporting low initial release probability, those with high release probability, together with being susceptible to inhibition by higher [KA], are actually inhibited by KAR antagonism (Jiang et al. 2001). The latter ‘defacilitation’ argues for somatodendritic KARs in interneurons with high release probability being tonically active, perhaps as a result of activation by glutamate spillover from nearby synaptic terminals or from non-synaptic sources such as astrocytes (Liu et al. 2004). Although the facilitatory effects of KA are generally consistent with ionotropic effects of extrasynaptic somatodendritic KARs, there are two interesting points of note from these studies. First, in the study looking at interneuron–interneuron synapses, the facilitation by KAR activation is not sensitive to Ca\(^{2+}\) channel block by Cd\(^{2+}\) (Cossart et al. 2001). This is somewhat unexpected if an ionotropic function of KARs is operational in the observed facilitation of GABA release. Second, in the study looking at pairs of interneurons and CA1 PCs, the facilitatory effect of KA is relatively long-lasting. Thus, the enhancement GABA release by KARs displays features of metabotropic function. It remains to be seen in future studies, whether the case for this develops as compellingly as it has for the metabotropic KAR-mediated inhibition of GABA release.

The foregoing discussion has focused on examples of actual or suspected metabotropic regulation of GABA release by KARs. There are numerous other instances of KAR-mediated regulation of GABA release e.g. in the cortex (Ali et al. 2001; inhibitory effect), amygdala (Braga et al. 2003; bidirectional effect), striatum (Chergui et al. 2000; indirect inhibitory effect), spinal cord (Kerchner et al. 2001a; indirect inhibitory effect) and hypothalamus (Liu et al. 1999; facilitatory effect), where ionotropic mechanisms are thought to be operational. In some cases, KARs appear to effect indirect metabotropic influences, e.g. adenosine receptors in striatal cells (Chergui et al. 2000) and GABA\(_{A}\)-receptors in spinal interneurons (Kerchner et al. 2001a). In still others, the mechanistic details have yet to be established. What is clear from the accumulated evidence is that, KARs act by ionotropic and metabotropic mechanisms, likely operating in parallel, to tune synaptic inhibition (GABA release) in an activity dependent manner.

### Metabotropic actions of kainate receptors involved in modulation of glutamate release – homosynaptic autoreceptors

Together with the heterosynaptic regulation of GABA release by KARs, there is compelling evidence that these glutamate receptors are involved in the modulation of glutamate release and that this action is mediated through metabotropic mechanisms at some synapses.

It is well established that KAR activation at hippocampal synapses produces a modulation of glutamate release. Thus, at the CA3 Schaffer Collateral (SC)-CA1 PC synapse, KAR activation produces a decrease in glutamate release (Chittajallu et al. 1996; Kamiya and Ozawa 1998; Vignes et al. 1998; Frerking et al. 2001; Clarke and Collingridge 2002; Lauri et al. 2006; Fig. 1h, PRE). In contrast to this monotonic regulation by KARs at glutamatergic terminals projecting to CA1 PCs, at the mossy fibre (MF)-CA3 PC synapse (Fig. 1, Inset II), the activation of KARs produces a biphasic effect depending on the KA concentration applied. Thus, at low (< 50 nmol/L) KA concentrations, glutamate release is increased (Contractor et al. 2000; Lauri et al. 2001a,b, 2003; Schmitz et al. 2001; Breustedt and Schmitz 2004; Rodriguez-Moreno and Sihra 2004). However, at higher (> 100 nmol/L) KA concentrations, glutamate release is decreased (Kamiya and Ozawa 2000; Schmitz et al. 2000; Contractor et al. 2001, 2003; Negrete-Diaz et al. 2006, 2007; Fig. 1c, PRE).

### Schaffer collateral-CA1 pyramidal cell synapses

The mechanism underlying the KAR-mediated decrease of glutamate release at the CA3 SC-CA1 PC synapse remains contentious. While some authors attribute the modulation to an ionotropic action of pre-synaptic KARs, whereby depolarization leads to the inactivation of Ca\(^{2+}\) channels (Chittajallu et al. 1996; Kamiya and Ozawa 1998), others (Frerking et al. 2001) argue that pre-synaptic KAR activation produces a decrease of glutamate release dependent on a metabotropic mechanism of action. In support of the latter view, Frerking et al. (2001) first, confirmed that the pharmacology of the synaptic modulation at CA3 SC-CA1 PC synapses is coherent with the involvement of KARs. Second, a pre-synaptic origin for the regulation was established based on observations that KA effects an increase in PPF and causes a decrease in 1/CV\(^2\) that correlates with a decrease in the mean excitatory post-synaptic current (EPSC) amplitude. Finally and crucially, a metabotropic mode of KAR action was assigning based on observations that the glutamate release inhibition produced by KA is abrogated by the G protein inhibitors, N-ethylmaleimide (NEM) and PTX. Intriguingly, despite the implied dependence of the regulation on \(G_{i,\alpha}\), treatment of slices with H-7, a broad spectrum protein kinase inhibitor, has no effect on the modulation by KA. This suggests that 2nd messenger-mediated stimulation of protein kinases is not involved. How might this metabotropic regulation therefore occur? One suggestion is that the regulation is intrinsic to the membrane, whereby membrane-delimited \(\beta\gamma\) subunits of G protein are proposed to directly bind to, and inhibit pre-synaptic Ca\(^{2+}\) channels and thereby depress release (Frerking et al. 2001), in manner akin to regulation of release by several inhibitory pre-synaptic GPCRs (Brown and Sihra 2007). Alternatively, instead of phosphorylation-dependent modulation, direct second
mechanism-mediated regulation is plausible given the increasing revelations of kinase-independent control of exocytosis (Silinsky and Searl 2003; Springett et al. 2004; Seino and Shibasaki 2005).

In support of the metabotropic action of KARs on CA3-CA1 PC terminals, Lauri et al. 2006 have also described a G protein-mediated action of these KARs during development. In addition, Kamiya and Ozawa (1998) have shown that the activation of KARs produces a decrease in intracellular Ca\(^{2+}\) concentration that correlates with the decrease in glutamate release. The observation that there was no change in the pre-synaptic (afferent) fibre volley upon application of KA, obviates the involvement of any ionotropic, depolarizing influences of KAR activation in the modulation observed. In earlier studies, Chittajallu et al. (1996) observed that KA application produces an inward current while decreasing glutamate release in slices. However, although the observed inward current recovers rapidly and completely, the effect of KA on the evoked EPSC is long-lasting. The long-lived EPSC in this study, points to KARs on SC nerve terminals depressing glutamate release through a metabotropic mechanism, perhaps involving a reduction in voltage-dependent Ca\(^{2+}\) channel activity. A similar metabotropic regulation of glutamate release by KARs, contingent on the control of intracellular Ca\(^{2+}\) concentration, occurs in dorsal root ganglion cells by (Rozas et al. 2003). Here, KARs, exclusively composed of GluR5 subunits, are functionally coupled to a PTX-sensitive G protein, the activation of which produces a reduction in Ca\(^{2+}\) channels activity as well as increasing Ca\(^{2+}\) release from intracellular stores. Notably, this regulation occurs without the prior initiation of depolarization-dependent ion-fluxes, again, implicating a metabotropic mechanism.

In addition to the CA3 SC input onto CA1 PCs, CA3 PC also project association/commissural (A/C) pathway terminals onto the apical dendrites of CA3 PC in the st. rad. Here, these terminals heterosynaptically regulate the major MF-CA3 PC synapse in the st. luc. (Fig. 1). The A/C terminals express KARs that inhibit glutamate release. Given the common origin of SC and A/C terminals projecting from CA3 PCs, it is plausible that these terminals might share a common metabotropic mechanism of KAR-mediated inhibition of glutamate release. However this remains to be established experimentally.

**Mossy fibre-CA3 pyramidal cell synapses**

One of the most intriguing aspects of KAR function is the bidirectional control of glutamate release they offer at MF-CA3 PC synapses; nanomolar concentrations of KA facilitating glutamate release, but higher concentrations agonist inhibiting release (Fig. 1c, PRE). The facilitatory effect underlies the frequency facilitation seen at this synapse, confirming that synaptically released glutamate can activate KARs homo- and heterosynaptically and that KARs are located at, or close to, the nerve terminals. The question remains: how does KAR activity enhance or reduce glutamate release form the same synapse, depending on agonist concentration?

Some groups have argued that the effects of KA at the MF-CA3 PC synapse could be attributed to classical ionotropic or depolarizing effects of KAR activation. Thus the inactivation of K\(^{-}\)channels might underlie the enhancement of release, while the inactivation of Na\(^{+}\) and/or Ca\(^{2+}\) channels and/or electrical shunting, might produce the depression of release (Kamiya and Ozawa 2000; Schmitz et al. 2001; Kamiya et al. 2002). Low concentrations of KA, by causing an after-depolarization of MF terminals, are also purported to increase nerve terminal Ca\(^{2+}\) levels, whereby an enhancement of glutamate release leads to frequency facilitation. Although, these data are indeed consistent with an ionotropic mechanism, it is notable that the after-depolarization in MF terminals is long-lasting. This is suggestive of facilitatory KARs at MF-CA3 PC synapses being metabotropic too.

In line with this notion, the facilitation of glutamate release produced by low concentrations of KA is seen to involve an adenylyl cyclase/cAMP/protein kinase A (AC/cAMP/PKA) signalling cascade, such that prior activation of the signalling pathway occludes the facilitatory effects of KARs at MF-CA3 synapses (Rodriguez-Moreno and Sihra 2004). Additionally, glutamate release from hippocampal synaptosomes is facilitated by the application of KA (Rodriguez-Moreno and Sihra 2004). These facilitatory pre-synaptic effects of KA on MF-CA3 synapses or synaptosomes are not however prevented by the action of G protein inhibitors. In the absence of G protein involvement, how might this putative metabotropic regulation occur? One possibility is that a Ca\(^{2+}\)-dependent activation of the AC/cAMP/PKA pathway, as first postulated in relation to the synaptic plasticity observed at the MF-CA3 synapse (Weisskopf et al. 1994), might occur because of small local increases in Ca\(^{2+}\) effected by KARs stimulating Ca\(^{2+}\)/calmodulin-dependent ACs. Interestingly in this regard, recent work has indicated that KAR-gated Ca\(^{2+}\)-influx produces a Ca\(^{2+}\)-induced intracellular Ca\(^{2+}\) release to support facilitation and LTP at MF-CA3 PC synapses (Lauri et al. 2003). Whether such intracellular Ca\(^{2+}\) release subserves an AC/cAMP/PKA pathway in the synaptic potentiation would be interesting to see. Strictly speaking though, this type of metabotropic mechanism is obviously an indirect consequence of prior KAR ionotropic activity.

The mechanism of KA-mediated facilitation of glutamate release in general remains contentious. In a study by Perkinton and Sihra (1999) using cerebrocortical synaptosomes, although KA enhanced 4-aminopyridine-induced glutamate release, there was no parallel facilitation of ionotropic parameters such as membrane potential or intracellular [Ca\(^{2+}\)], over and above that produced by the secretagogue itself. Although it is possible that the averaging

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effect of measurements from a synaptosomal population might mask a small local effect on a subgroup of nerve terminals expressing KARs, the data do not generally support an ionotropic mechanism for the facilitation of glutamate observed. Indeed, from the established criteria for the effects of ionotropic stimulation by KARs (Kullmann 2001; Semyanov and Kullmann 2001), if anything, at the high concentrations of KA used, an inhibition of the 4-aminopyridine(4AP)-mediated stimulus-release coupling might have been predicted as a result of Na\(^+\)- and/or Ca\(^{2+}\)-channel inactivation. This has never been observed for glutamate release in this preparation, though at the same time, GABA release is seen to be inhibited by KA treatment (Perkinton and Sihra 1999).

The decrease of glutamate release produced by high KA concentrations at MF-CA3 PC synapses has, on the one hand, been attributed to the ionotropic consequences of KAR activation, but on the other hand, has metabolotropic characteristics. The parsimonious explanation for the inhibition by KAR activation is that strong depolarization with high concentrations of agonist leads to Na\(^+\)- and/or Ca\(^{2+}\)-channel inactivation and/or electrical shunting, and this then results in reduced terminal excitability leading to depression of glutamate release. However, on closer examination, the depression of glutamate release at MF-CA3 synapses was also shown to be underpinned by a metabolotropic mechanism in recent study by Negrete-Díaz et al. (2006). In this report, the site of KAR action is demonstrated to be pre-synaptic by virtue of observations that KA application alters PPF, increases the number of failures of evoked EPSCs and effects a change in the 1/CV\(^2\) that correlates with the change in synaptic response (Negrete-Díaz et al. 2006). Most notably, the effect of KA is found to be long-lasting, with the slow recovery suggesting a metabolotropic influence of KAR activation. This is in variance with the previously proposed ionotropic mechanism of KAR-mediated modulation at MF-CA3 synapses, where the change and recovery of the KA-evoked holding current was fast (Kamiya and Ozawa 2000). Notwithstanding, a crucial point supporting a metabolotropic inhibition of release by KARs at MF-CA3 synapses, is the observation that depression is prevented by the treatment of the slices with the G\(_{i/o}\) inhibitor, PTX. Finally, the depression also appeared to be contingent on the activity of an AC/cAMP/PKA signalling cascade (Negrete-Díaz et al. 2006; Fig. 1c, PRE).

Intriguingly, the AC/cAMP/PKA pathway described as being obligatory for the decrease of glutamate at MF-CA3 PC synapses also appears to be essential in a shared induction of KAR-mediated synaptic depression and low-frequency stimulation-mediated LTD (Negrete-Díaz et al. 2007). LTD at MF-CA3 synapses has also been shown to be mediated by type II mGluR coupled to a decrease in PKA activity (Kobayashi et al. 1996; Tzounopoulos et al.1998). Thus, two types of glutamate receptors, viz. KARs (in a metabolotropic guise) and type II mGluRs, appear to collude in this form of plasticity. This mirrors the observations with LTP at MF-CA3 PC synapses, where KAR-mediated facilitation collaborates with excitatory, type I, mGluR activation (Contractor et al. 2001).

Overall, KARs appear to participate intimately in the key forms of synaptic plasticity displayed at the MF-CA3 synapse (Bortolotto et al. 2005; Negrete-Díaz et al. 2007). Interestingly, in the developing hippocampus, KARs also have metabolotropic actions that mediate the regulation of glutamate release and network activity in response to synaptic activation (Lauri et al. 2005). During the first post-natal week of hippocampal development, endogenous glutamate appears to regulate release in an action potential-independent manner, by tonically activating KARs at CA3 glutamatergic synapses. In sum, at terminals synapsing onto PCs in the hippocampus, KARs inhibit glutamate release via a metabolotropic, G protein and PKC-dependent mechanism, while, at the same time, the receptors facilitate glutamate release onto CA3 interneurons, by a presumed ionotropic mechanism, albeit with metabolotropic attributes according to some accounts.

The metabolotropic influences of KARs are not restricted to examples in the hippocampus and spinal cord (Rozas et al. 2003). In a very recent paper, Jin et al. (2006) showed that KARs are present in the rat globus pallidus. Here, KARs are localized both, pre- and post-synaptically, with the presynaptic KARs acting as autoreceptors to produce a decrease in glutamate release, through a PTX-sensitive and PKC-dependent metabolotropic mechanism.

As with KARs modulating GABA, we have concentrated on discussing examples of metabolotropic regulation of glutamate release by KARs which are currently matter of active debate. There are numerous instances of KAR-mediated regulation of glutamate release where the modulation is patently ionotropic, or mechanistic details are still under consideration. These include examples in the developing cortex (Kidd et al. 2002; inhibitory effect), amygdala (Li et al. 2001; facilitatory effect), nucleus accumbens (Crowder and Weiner 2002; Casassus and Mulle 2002; inhibitory effect), dorsal root ganglion cells (Kerchner et al. 2001b; inhibitory effect) and cerebellum (Delaney and Jahr 2002, facilitatory and inhibitory effect). Again, as with GABA release, pre-synaptic KARs evidently operates through a combination of ionotropic and metabolotropic mechanisms to modulate excitatory glutamatergic transmission.

Post-synaptic metabolotropic actions of kainate receptors

In the last 5 years or so, increasing importance has been assigned to the metabolotropic actions of KARs in neuronal excitability. Melyan et al. (2002) described a KAR-mediated inhibition of the slow afterhyperpolarization (sAHP) in CA1 PCs (Fig. 1b, POST). The sAHP is generated by a voltage-
independent Ca-dependent K⁺ current (slow afterhyperpolarization current; \( I_{\text{sAHP}} \)), activates proportionally to the number and frequency of short bursts of action potentials and lasts up to several seconds in duration (Lancaster and Adams 1986). The inhibitory effect of KA application on the sAHP has been pharmacologically established as being due to activation of KARs, but the regulation is not contingent on any ionotropic actions of KARs or changes in network activity. Most notably, this KAR-mediated modulation is blocked by the PTX-sensitive G protein inhibitor, NEM, and the PKC inhibitor, calphostin C, confirming that KARs impinge on the sAHP through a metabotropic activity (Melyan et al. 2002).

Further work has confirmed that PKC-mediated inhibition of the sAHP by KARs in CA1 pyramidal neurons involves GluR6-containing receptors. Most importantly, the effect is recapitulated by synaptically released glutamate, again, in a G protein and PKC-dependent manner (Melyan et al. 2004). Most recently, studies, on the protein kinase requirements for metabotropic action of KARs in CA1 pyramidal neurons, indicate that, together with PKC, PKA and mitogen-activated protein kinases (MAPKs) are also instrumental in the long-lasting KA-mediated modulation of the sAHP. In these studies, given that MAPK stimulation occurs downstream of PKC activation at least (Grabauskas et al. 2007), it may be that the long-term effects of MAPK-dependent phosphorylation underlie the near irreversible effect of KAR stimulation on the sAHP in CA1 neurons. The presence of the KAR-mediated regulation of the sAHP represents a novel physiological function for the receptor at glutamatergic synapses. Thus operation of metabotropic KARs, directly controls the excitability of pyramidal neurons. This contribution of KARs to the prolongation of the excitability of pyramidal neurons may physiologically play a part in synaptic plasticity, but gone awry, could well be involved in epileptogenic activity (Melyan et al. 2002, 2004; Grabauskas et al. 2007).

The regulation of post-synaptic excitability by metabotropically acting KARs are not restricted to CA1 PCs, but also occurs in CA3 neurons. Thus, Fisahn et al. (2005) described KARs that mediate a metabotropic regulation of the \( I_{\text{aAHP}} \) and medium afterhyperpolarization current (\( I_{\text{mAHP}} \)) in mouse hippocampal CA3 PCs. One intriguing effect of this KAR activation is a long-term change in intrinsic neuronal excitability and in neuronal firing patterns, such as single-spike and spike-burst firing. The KA-induced decrease of \( I_{\text{aAHP}} \) and \( I_{\text{mAHP}} \) amplitude is PKC-dependent and absent in pyramidal neurons from GluR6\(-/-\), but not in GluR5\(-/-\), transgenic mice. The upshot from these observations is that activation of GluR6-containing KARs reduces the AHP amplitude through regulation of both the \( I_{\text{aAHP}} \) and \( I_{\text{mAHP}} \) in CA3 pyramidal neuron (cf. CA1 pyramidal neurons), and thereby influences the firing frequency of these neurons. Interestingly, in contrast to the effect of KAR activation on the sAHP in CA1 PCs, the AHP reduction is reversible in CA3 neurons (Ruiz et al. 2005). This suggests that the effects of KARs underlying post-synaptic excitability may occur by distinct metabotropic mechanisms at CA3 and CA1 synapses.

The function of post-synaptic KARs in CA3 PC function has been further elucidated in a very interesting paper by Ruiz et al. (2005). In this study, the authors suggest that heteromeric KARs with distinct subunit compositions, respectively mediate the ionotropic and metabotropic effects of KA at hippocampal MF synapses. Thus at low KA concentrations (5 and 30 nmol/L), or by synaptically released endogenous glutamate, KAR activation reversibly inhibits the slow Ca²⁺-activated K⁺ current (\( I_{\text{sAHP}} \)) and thus invokes an increase of neuronal excitability. This effect is sensitive to block by NEM and calphostin C, and therefore intimates a G protein-coupled metabotropic mechanism involving PKC. This metabotropic effect occurs concurrently with the ionotropic KAR-mediated EPSC in the CA3 pyramidal neurons, showing, for first time, that post-synaptic KARs can operate simultaneously through both ionotropic and metabotropic signalling at the same synapse.

The novelty of the approach taken by Ruiz et al. (2005) in dissecting the different modes of KAR function was the use of KAR-subunit knockouts. These expedited the delineation of the specific subunits underpinning the ionotropic and the G coupled mechanisms. Thus, using GluR6\(-/-\) and KA2\(-/-\) mice, GluR6 and KA2 containing KAR heteromers are shown be essential for the inhibition of the \( I_{\text{aAHP}} \) in CA3 PCs by low concentrations of KA. In GluR6\(-/-\) mice, both ionotropic synaptic transmission and the inhibition of the \( I_{\text{aAHP}} \) mediated by endogenously released glutamate from MFs, are lost. In KA2\(-/-\) mice, however, inhibition of the \( I_{\text{aAHP}} \) is ablated, but the KAR-mediated EPSC is preserved. Interestingly, biochemical analysis indicates that the KA2 containing KARs, required for \( I_{\text{aAHP}} \) modulation, physically interact with the Gz_{q11}-protein (Ruiz et al. 2005). In sum, at MF synapses, the proposed model for KAR-mediated modulation involves two concurrent modes of receptor action: (i) direct ionotropic activity of GluR6 containing KARs and (ii) indirect, G protein-coupled, activity requiring the binding of glutamate to KA2 containing KARs. Significantly, the supporting data and this model affirm that metabotropic action of KARs need not rely on the activation of KAR-mediated inward current.

From studies in CA1 and CA3 pyramidal neurons, it is evident that KAR signalling through a metabotropic mode enhances hippocampal excitability (Melyan et al. 2002, 2004; Fisahn et al. 2005; Ruiz et al. 2005). While it is not clear which KAR-subunits, if any, are responsible for a physical interaction of Gz_{i/o} with the KARs affecting the \( I_{\text{aAHP}} \) in CA1 PCs, the identification of the KA2 subunit as the link between KARs and Gz_{q} leading to \( I_{\text{aAHP}} \) modulation in CA3 neurons, may provide key insights into how KARs couple with G proteins to have their metabotropic influences. It will therefore be interesting to see if KA1 subunits show a similar
ability to KA2 subunits in interacting with G proteins, and thus perhaps bridging GluR5/6/7 subunits for metabotropic signalling.

**Potential mechanism underlying the metabotropic actions of KARs**

The foremost question in considering non-ionotropic signalling by KARs is: how exactly does KARs couple with G proteins to mediate the various metabotropic effects postulated above? KAR subunits have a structure and membrane topology that is, in principle, difficult to reconcile with the structure of the prototypic metabotropic receptor superfamily, viz., seven transmembrane region GPCRs, which are known to have specific sites that interact with heterotrimeric G proteins (Pin and Duvoisin 1995; Pierce et al. 2002). Indeed, as heterodimers, any potential G protein binding site on KARs might be cryptic because of the involvement of multiple cytoplasmic domains from more than one subunit in the oligomer, constituting binding sites based on secondary/tertiary structure. Notwithstanding, studies with goldfish KA-binding proteins have reported PTX-sensitive agonist binding and an agonist-dependent ADP ribosylation of a 40 kDa protein, data arguing in favour of the direct interaction of a KARs with a G protein (Willard et al. 1991, 1992; Ziegler et al. 1992). Willard and Oswald (1992) also proposed that heterologously expressed frog KARs could bind to GTP-binding proteins through a direct interaction.

Despite these latter studies, given the lack of obvious G protein interacting motifs in KAR subunits, until recently, putative indirect docking of G proteins to KARs has remained the most tenable proposition, perhaps involving the binding of adaptor proteins as intermediaries (Rodríguez-Moreno and Sihra 2007). Most importantly, the KA2-dependent coimmunoprecipitation of Gq by an anti-GluR6 antibody that coprecipitates both GluR6 and KA2 subunits. That the Gq interaction is mediated through the KA2 subunit is surmised from the lack of Gq in GluR6 immunoprecipitates from in KA2−/− mice. The identity of the G protein involved in the KAR-mediated inhibition of the I\(_{\text{AHP}}\) in CA3 pyramidal neurons therefore differs from that in CA1 neurons, where KARs affect the I\(_{\text{AHP}}\) through a PTX-sensitive G\(_{\text{lo}}\)-protein. The modulation of the I\(_{\text{AHP}}\) by CA3 PC KARs is however congruent to Gq-coupled modulation of the I\(_{\text{AHP}}\) by de facto GPCRs, including the muscarinic M(3)-cholinergic receptor and metabotropic (mGluR5) receptor (Krause et al. 2002). Most importantly, the KA2-dependent communoprecipitation of Gq represents the first convincing demonstration of functional KARs interacting with G proteins. The molecular determinants of the interactions of ionotropic receptor subunits with Gq may indeed be more universal given that Chen et al. (2006) have found a very similar interaction between Gq and a potassium channel. Nonetheless, it will be interesting to see if coimmunoprecipitation experiments transpire a similar scenario with respect to the interaction of KARs with G\(_{\text{lo}}\); this functionally defined partnership appearing to underpin many of the observed metabotropic actions of the receptor reviewed herein.

Following the delineation of KA2 interaction with Gq, KARs definitively enter the neurochemists domain. Assuming a direct interaction, the motif(s) within the KA2 that mediate G protein binding warrant detailed mapping. This knowledge and use of subunit-specific antibodies in coimmunoprecipitation experiments promise exciting possibilities for elucidating the precise function of metabotropic KARs.

Apart from G protein interaction, what else could determine whether a KAR behaves ionotropically or metabotropically? Certainly, subunit composition analysis does not provide a hard and fast rule, notwithstanding the aforementioned interaction of KA2 subunits with Gq that has now been established. Glu5/6/7 KAR subunits are alternatively spliced to give proteins with different C-termini (Pinheiro and Mulle 2006). A priori, these might impart differential functional properties on the incumbent KARs, but because of the endoplasmic reticulum retention of many of the heterologously expressed KAR subunits, it is only at present possible to ascribe trafficking or compartmentalization functions to these domains of KAR subunits. Other than the C-terminus, the roles, if any, of the short intracellular loops of KAR subunits remain unexplored in any context of KAR function.

One interesting possibility is that the mode of KAR action depends on interactions with the variety of glutamate receptor interacting proteins, as well as the cytoskeleton, both pre-synaptically and at post-synaptic densities. This is certainly of issue in the trafficking of KAR receptors (see Isaac et al. 2004; Jaskolski et al. 2005; Lerma 2006; Pinheiro and Mulle 2006 for reviews), but it remains to be seen whether intracellular interactions can also determine the mode of action KAR. Interestingly, there is some evidence that interaction of post-synaptic density protein-95/synapse-associated protein-90 (PSD-95/SAP-90) with KARs can affect receptor function albeit in an ionotropic context (desensitization) and in relation to receptor clustering (Garcia et al. 1998). Together with PSD-95/SAP-90, SAP-102 also interacts with GluR6 and KA2 (evidently key subunits in metabotropic KAR actions) and SAP-97 found pre-synaptically (Garcia et al. 1998; Mehta et al. 2001), interacts with GluR6 exclusively (Garcia et al. 1998). It will be interesting to see whether these interactions have functional consequences on the KAR function together with their canonical roles in dictating subcellular localization of receptors (Jaskolski et al. 2005; Lerma 2006; Pinheiro and Mulle 2006).
Other interactions, including those mediating trans-synaptic interactions may also affect the functional readout of KARs (Coussen et al. 2002, 2005).

A Common theme: ionotrophic glutamate receptors leading a double life

Some enlightenment on the issue of mechanism of metabotropic KAR action may come from parallel studies with other ionotropic receptors that have also been shown to have a metabotropic propensity. Thus in cortical neurons, AMPA receptor activation leads to the stimulation of MAPK through G protein \( \beta \gamma \) subunits (Wang and Durkin 1995), as well as inhibition of AC by \( \mathrm{G}_{\alpha_i} \), (Wang et al. 1997). The former activation may be mediated, in part, by an interaction of AMPA receptors with the protein tyrosine kinase Lyn and activation of MAPK in this way may lead to the observed increase in brain-derived neurotrophic factor mRNA (Hayashi et al. 1999). AMPA receptors also activate a G protein that suppresses cGMP-gated currents (Kawai and Sterling 1999). More recently, AMPA receptors with metabotropic actions have been found to play a role in the cerebellum. Thus, while climbing fibre excitatory inputs to the Purkinje cells are ionotropic, inhibition of cerebellar GABAergic interneurons depends on AMPA-mediated activation of GTP-binding proteins coupled to the down-regulation of pre-synaptic \( \mathrm{Ca}^{2+} \) channels (Satake et al. 2004). Finally, AMPA receptors with metabotropic actions are similarly involved at the calyx of Held, where a G protein-dependent pre-synaptic inhibition of \( \mathrm{Ca}^{2+} \) currents leads to the attenuation of glutamatergic synaptic transmission (Takago et al. 2005).

The observation that KARs and AMPA receptors have simultaneous ionotropic and metabotropic modes of action begs the question as to whether the same may be relevant to NMDA receptors also. While there ample evidence now for both post-synaptic and pre-synaptic functions of NMDA receptors (McMahon et al. 1989; Krebs et al. 1991; Wang 1991; Desce et al. 1992; Pittaluga and Raiteri 1992; Wang et al. 1992; Nicholls 1998; Casado et al. 2002), there is paucity of data pointing to NMDA receptors being metabotropic. Notwithstanding this, Itano et al. (1992) have ascribed a ‘metabotropic’ role to NMDA receptors that inhibit AC activity in dispersed rat hippocampal neurons. While it remains to be seen whether or not this a true metabotropic effects, what is conspicuous is a common theme with KA, AMPA and NMDA receptors in that they all modulate AC in addition to their ionotropic activity. Whether the AC coupling is an independent metabotropic activity or contingent upon prior ionotropic activity remains to be established for each individual synapatic case.

Finally, it would appear that the coupling of ionotropic channels to G proteins is not restricted to ligand-gated channels. Hence, association of \( G_{\beta \gamma} \) subunits with the alpha subunit of the voltage-dependent \( \mathrm{Na}^+ \) channel may control neuronal excitability (Marin et al. 2001). Evidently therefore, ligand- and voltage-gated ion channel proteins have the capacity to behave as signal initiators or transducers, quite apart from their ion-conducting remit for which they were first defined.

Conclusions and perspectives

In summary, KARs function with dual signalling mechanisms. As ionic channels they participate post-synaptically to provide a component of the EPSC, depolarize neurons and produce a pre-synaptic increase or decrease of neurotransmitter release. What is abundantly clear now is that KARs can alternatively actuate as metabotropic receptors and have functions that are independent of any ionotropic activity. This bimodal aspect is in common with AMPA receptors, but, so far, distinct from ionotropic NMDA receptors. In their alternative guise, KARs display a variety of metabotropic actions including: (i) activation of \( \mathrm{G}_{\alpha_i} \) or \( \mathrm{G}_{\alpha_p} \) proteins leading to PKC stimulation; (ii) activation of a G protein leading to stimulation of AC/cAMP/PKA signalling; (iii) activation of a G protein leading to kinase-independent, possibly membrane-delimited, G\( \beta \gamma \)-mediated regulation; and (iv) G protein-independent activation of PKA. While the role of PKC in the metabotropic activity of KARs has been established for some time now, more recent studies have invoked an essential role for PKA in the facilitatory and inhibitory actions KARs at some central synapses. The question now is: what are the substrates for these kinases that expedite the regulation (Sihra and Nichols 1993). While post-synaptically, the channel supporting the I\(_{\text{AHP}}\) represents an a intriguing target, pre-synaptically it will be important to determine whether phosphorylation targets of KARs are those cited for classical GPCR-mediated regulation of exocytosis (Brown and Sihra 2007).

Despite the numerous examples of metabotropic effects being activated by KARs working through G proteins, the exact mechanism of coupling between KARs and G protein (directly or indirectly) remains elusive. Crucially, the metabotropic actions of KARs effect the important pre-synaptic modulation of glutamate or GABA release and, at the same time, control neuronal excitability in general by pre-synaptic or post-synaptic mechanisms. The burning question remains as to physiological significance for the existence KARs which, not only have both ionotropic and metabotropic actions, but such diverse ones at that, and sometimes in the same neurons. KARs will no doubt continue to surprise us with newer and more elaborate issues to question and debate.

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