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Research Report

Subunit-dependent modulation of kainate receptors by muscarinic acetylcholine receptors

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ABSTRACT

Interactions between cholinergic and glutamatergic neurotransmitter systems influence synaptic transmission and plasticity. While previous studies have examined cross-talk between acetylcholine (ACh) and NMDA or AMPA receptors, little is known about the effect of ACh on kainate receptors (KARs). We show that stimulation of m1 or m3 muscarinic ACh receptors (mAChRs) for 2 min potentiates recombinant KAR currents in a long lasting fashion. Muscarinic AChR activation potentiates heteromeric GluK2/GluK4 and GluK2/GluK5 receptors, but not homomeric GluK2 receptors. In hippocampal slices kainate potentiates mossy fiber axon excitability. Transient mAChR activation enhances this action of kainate, suggesting a novel mechanism through which acetylcholine could modulate synaptic transmission in the hippocampus. KAR over-activation has been implicated in excitotoxic cell death. To establish the functional significance of the interaction between mAChRs and KARs we examined the effect of mAChR activation on KAR-mediated excitotoxicity. We find that during pharmacological blockade of NMDA and AMPA receptors, KAR activation with AMPA produces significant cell death in primary cortical culture. Concanavalin A (Con A), which selectively blocks KAR desensitization, markedly increases this KAR-mediated neurotoxicity. Brief activation of mAChRs with pilocarpine significantly enhances KAR-mediated excitotoxicity both in the presence and absence of Con A. We conclude that KARs are modulated in a subunit dependent manner by mAChRs. We suggest that ACh may induce long lasting alterations in neuronal excitability and enhance excitotoxicity in part by potentiating KAR function.

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Abbreviations: ACh, acetylcholine; AChR, acetylcholine receptor; ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; AMPAR, AMPA receptor; ANOVA, analysis of variance; D-APV, D-(–)-2-Amino-5-phosphonopentanoic acid; CCh, carbachol; CNQX, 6-Cyano-7-nitroquinoxaline-2,3-dione; CP-465,022, 3-(2-Chlorophenyl)-2-[2-[6-[(diethylamino)methyl]-2-pyridinyl]ethenyl]-6-fluoro-4(3*H*)-quinazolinone hydrochloride; Con A, concanavalin A; EPSC, excitatory postsynaptic current; GluK1-3, kainate GluR5-7 subunits; GluK4–5, kainate KA1–2 subunits; GluN1, NMDA NR1 subunit; GluN2B, NMDA NR2B subunits; GYKI 52466, 4-(8-Methyl-9*H*-1,3-dioxolo[4,5-*h*][2,3]benzodiazepin-5-y l)-benzenamine hydrochloride; KAR, kainate receptor; LDH, lactate dehydrogenase; mAChR, muscarinic acetylcholine receptor; MF, mossy fiber; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; PKC, protein kinase C; TTX, tetrodotoxin

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1. Introduction

Interactions between cholinergic and glutamatergic neurotransmitter systems modulate synaptic transmission and neuronal excitability. These interactions also regulate forms of synaptic plasticity thought to be important in learning and memory (Blokland, 1995). Dysfunctional interactions between these transmitter systems have been proposed to contribute to a number of neurodegenerative and psychiatric disorders. Brain acetylcholine levels fluctuate greatly with changes in behavioral state (i.e. stress, arousal, fear) and during the sleep–waking cycle (Marrosu et al., 1995; Acquas et al., 1996). These behaviorally induced changes in acetylcholine levels have the potential to regulate glutamatergic function in a state dependent manner. Because of this, a great deal of effort has been focused on understanding the interaction between cholinergic and glutamatergic neurotransmission.

It is well established that acetylcholine, acting on muscarinic receptors (mAChRs) potentiates currents through the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor (NMDAR) (Markram and Segal, 1992; Marino et al., 1998). This potentiation of NMDAR-mediated currents is present only during mAChR stimulation. In contrast, mAChRs can produce lasting changes in synaptic efficacy at glutamatergic synapses by influencing AMPA receptor currents. Both mAChR-induced long term potentiation (Auerbach and Segal, 1994; Fernandez de Sevilla et al., 2008) and long term depression (Kirkwood et al., 1999) have been reported. Muscarinic receptors appear to influence AMPA receptor currents through indirect mechanisms, including alterations in AMPA receptor trafficking (Fernandez de Sevilla et al., 2008). An interaction between mAChRs and KARs has not been reported. Therefore, one goal of this study was to determine whether mAChRs influence KAR currents.

Kainate receptors (KARs) are widely expressed throughout the CNS where they contribute to synaptic transmission and plasticity (Lerma, 2003). KARs are tetramers composed of combinations of GluK1–3 subunits that have a low affinity for glutamate and GluK4–5 subunits that have a high affinity for glutamate. Only the GluK1–3 subunits can produce functional homomeric receptors. KARs are located at both presynaptic and postsynaptic sites (Lerma, 2003), where they modulate neurotransmitter release or mediate excitatory neurotransmission, respectively.

Over-activation or dysfunction of KARs has been implicated in a number of neurological disorders, including schizophrenia (Bah et al., 2004) and epilepsy (Vincent and Mulle, 2009). In the hippocampus, activation of axonal KARs with a low concentration of kainate can enhance the excitability of mossy fibers (Schmitz et al., 2000; Kamiya and Ozawa, 2000). KARs have also been implicated in excitotoxic cell death. *In vitro*, KAR over-activation is neurotoxic in primary neuronal culture (Jensen et al., 1999; Giardina and Beart, 2001). *In vivo*, KAR antagonists are neuroprotective in both global and focal ischemia models (O'Neill et al., 2000). Muscarinic modulation of KAR function could therefore regulate neuronal excitability and alter excitotoxicity in pathological states.

In this study, we investigated the functional interaction between mAChRs and KARs. Our goal was to determine whether mAChR activation regulates KAR function and whether this regulation influences excitotoxicity mediated by KARs. Using recombinant KARs expressed in *Xenopus* oocytes, we found that stimulation of m1 or m3 mAChRs for 2 min produces a long lasting and subunit-dependent potentiation of KAR currents. In hippocampal slices, we found that kainate-induced potentiation of mossy fiber axon excitability was enhanced by prior activation of mAChRs. The ability of mAChR activation to potentiate KAR currents suggested that mAChR activation would also potentiate KAR-mediated excitotoxicity. Indeed, we found that KAR-mediated excitotoxicity in primary cortical culture was markedly increased by prior mAChR stimulation. These results indicate a significant interaction between mAChR and KAR transmitter systems and suggest that ACh may induce long lasting alterations in neuronal excitability and enhance excitotoxicity in part by potentiating KAR function. This neurotransmitter interaction may have important implications in a variety of neurological disorders, including epilepsy.

2. Results

2.1. *Muscarinic receptor activation potentiates kainate receptor-mediated current*

Because of the widespread expression of GluK2 in brain and its reported physiological importance (Lerma, 2003), this study focused on muscarinic regulation of GluK2-containing KARs. Kainate currents were evoked in *Xenopus* oocytes expressing GluK2, GluK2/GluK4 or GluK2/GluK5 receptors alone or in combination with m1 or m3 mAChRs. Steady state kainate currents at GluK2 receptors were evoked with domoate (10 μM), while steady state currents at GluK2/GluK4 and GluK2/GluK5 receptors were evoked with AMPA (30 μM). At 30 μM, AMPA selectively activates GluK4 and GluK5-containing KARs. Muscarinic receptors were stimulated with a 2 min application of pilocarpine (100 μM), a selective mAChR agonist. In oocytes in which m1 or m3 mAChRs had not been injected, pilocarpine produced no current, indicating that oocytes in this study did not express native mAChRs. However, in oocytes expressing m1 or m3 mAChRs, pilocarpine produced a large inward current attributable to Cl^- efflux through Ca^{2+}-activated Cl^- channels endogenous to the oocytes. The presence of this pilocarpine-induced current indicated effective surface expression of mAChRs.

Potentiation of KAR currents immediately followed pilocarpine application (Fig. 1A and B), but was dependent on the KAR subunits expressed (Fig. 1C). Stimulation of m1 or m3 mAChRs with pilocarpine significantly potentiated current at GluK2/GluK4 (m1, 133±3%, $n=8$, $P<0.01$; m3, 119±4%, $n=6$, $P<0.01$) and GluK2/GluK5 (m1, 172±10%, $n=8$, $P<0.01$; m3, 168±16%, $n=8$, $P<0.01$) receptors 30 min after pilocarpine washout (Fig. 1C). In contrast, activation of m1 mAChRs did not potentiate current at GluK2 homomeric receptors (106±5%, $n=6$), suggesting that muscarinic potentiation of KARs requires the GluK4 or GluK5 subunit. Current elicited by GluK2/GluK5 receptors was significantly more potentiated than that at GluK2/GluK4 receptors ($P<0.05$). However, potentiation of KARs produced by m1 and m3 mAChRs was similar. To determine whether pilocarpine