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Kainate Receptors Play a Role in Modulating Synaptic Transmission in the Olfactory Bulb

Laura J. Blakemore, [a,b] **John T. Corthell** [a,b] **and Paul Q. Trombley** [a,b]*

[a] *Program in Neuroscience, Florida State University, Tallahassee, FL, United States*

[b] *Department of Biological Science, Florida State University, Tallahassee, FL, United States*

Abstract—Glutamate is the neurotransmitter used at most excitatory synapses in the mammalian brain, including those in the olfactory bulb (OB). There, ionotropic glutamate receptors including N-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) play a role in processes such as reciprocal inhibition and glomerular synchronization. Kainate receptors (KARs) represent another type of ionotropic glutamate receptor, which are composed of five (GluK1–GluK5) subunits. Whereas KARs appear to be heterogeneously expressed in the OB, evidence as to whether these KARs are functional, found at synapses, or modify synaptic transmission is limited. In the present study, coapplication of KAR agonists (kainate, SYM 2081) and AMPAR antagonists (GYKI 52466, SYM 2206) demonstrated that functional KARs are expressed by OB neurons, with a subset of receptors located at synapses. Application of kainate and the GluK1-selective agonist ATPA had modulatory effects on excitatory postsynaptic currents (EPSCs) evoked by stimulation of the olfactory nerve layer. Application of kainate and ATPA also had modulatory effects on reciprocal inhibitory postsynaptic currents (IPSCs) evoked using a protocol that evokes dendrodendritic inhibition. The latter finding suggests that KARs, with relatively slow kinetics, may play a role in circuits in which the relatively brief duration of AMPAR-mediated currents limits the role of AMPARs in synaptic transmission (e.g., reciprocal inhibition at dendrodendritic synapses). Collectively, our findings suggest that KARs, including those containing the GluK1 subunit, modulate excitatory and inhibitory transmission in the OB. These data further suggest that KARs participate in the regulation of synaptic circuits that encode odor information. Published by Elsevier Ltd on behalf of IBRO.

Key words: glutamate receptors, olfaction, glutamate, GABA, ATPA, SYM 2081.

INTRODUCTION

Glutamate is the neurotransmitter used at most excitatory synapses in the mammalian brain, including those in the olfactory bulb (OB). Both ionotropic and metabotropic glutamate receptors play a role in synaptic transmission and neuromodulation (Zhuo, 2017). Ionotropic glutamate receptors comprise three families, which are named based on their selective synthetic agonist: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (Dingledine et al., 1999; Lodge, 2009; Alexander et al., 2017). In the central nervous system (CNS), rapid synaptic excitation is largely mediated by postsynaptic AMPA receptors (AMPARs) and NMDA receptors (NMDARs) (Koles et al., 2016), while kainate receptors (KARs) act

*Correspondence to: P. Q. Trombley, Florida State University, Department of Biological Science, 3011 King Life Sciences Building, 319 Stadium Drive, Tallahassee, FL 32306-4295, United States.
E-mail address: ptrombley@fsu.edu (P. Q. Trombley).

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPAR, AMPA receptor; AP5, 2-amino-5-phosphonopentanoic acid; ATPA, (RS)-2-Amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl) propanoic acid; AUC, area under the curve; CNS, central nervous system; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione disodium; DDI, dendrodendritic inhibition; CTZ, cyclothiazide; DRG, dorsal root ganglion; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; EPL, external plexiform layer; ET cells, external tufted cells; GABA, gamma-Aminobutyric acid; GAD, glutamic acid decarboxylase; GL, glomerular layer; GYKI 52466, 1-(4-Aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride; GYKI 53655, 1-(4-Aminophenyl)-3-methylcarbamyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride; ICC, immunocytochemical; IPSC, inhibitory postsynaptic current; IPSP, inhibitory postsynaptic potential; JG cells, juxtaglomerular cells; KAR, kainate receptor; mIPSC, miniature IPSC; MF, mossy fiber; M/T cell, mitral/tufted cell; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide; Neto1, Neuropilin Tolloid-like 1; Neto2, Neuropilin Tolloid-like 2; NMDA, N-methyl-D-aspartate receptor; NMDAR, NMDA receptor; OB, olfactory bulb; ON, olfactory nerve; ONL, olfactory nerve layer; OSN, olfactory sensory neuron; PG cell, periglomerular cell; QX314, lidocaine *N*-ethyl bromide; RT-PCR, reverse transcription polymerase chain reaction; SYM 2081, (2S,4R) 4-methylglutamic acid; SYM 2206, 4-(4-Aminophenyl)-1,2-dihydro-1-methyl-2-propylcarbamoyl-6,7-methylenedioxyphthalazine; UBP302, (S)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxybenzyl) pyrimidine-2,4-dione; TTX, tetrodotoxin; VGLUT1, vesicular glutamate transporter 1.

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principally to modulate neuronal excitability and synaptic transmission at both presynaptic and postsynaptic sites (Contractor et al., 2011; Lerma and Marques, 2013; Sihra and Rodriguez-Moreno, 2013).

In the OB, both AMPARs and NMDARs play a role in a number of processes including correlated spiking, reciprocal inhibition, and glomerular synchronization (Schoppa et al., 1998; Isaacson and Strowbridge, 1998; Schoppa and Westbrook, 2002; Halabisky and Strowbridge, 2003; Schoppa, 2006a). However, the potential role of KARs in such processes remains unclear. Studies that used a variety of techniques, including in situ hybridization (Gall et al., 1990), autoradiography (Nadi et al., 1980; Bailey et al., 2001), activity-dependent labeling (Edwards and Michel, 2003), and immunohistochemistry (Petralia et al., 1994; Montague and Greer, 1999; Davila et al., 2007), suggest that KARs are heterogeneously expressed in the OB. However, evidence as to whether KARs in the OB are functional, found at synapses, or modify synaptic transmission is limited.

KARs are tetrameric receptors comprised of the glutamate receptor subunits originally named GluR5-7, KA1, and KA2. New nomenclature for ligand-gated ion channels was introduced in 2009 (Collingridge et al., 2009), which re-named GluR5, GluR6, GluR7, KA1, and KA2 as GluK1-GluK5. GluK1–GluK3 form functional homomeric receptors when expressed in heterologous systems (Egebjerg et al., 1991; Sommer et al., 1992; Schiffer et al., 1997; Pinheiro and Mulle, 2006), although whether native KARs can exist as homomers remains unclear (Carta et al., 2014). GluK4 and GluK5 only form functional receptors when combined with one of the GluK1–GluK3 subunits (Lerma, 2006; Pinheiro and Mulle, 2006; Lerma and Marques, 2013; Carta et al., 2014), which generates KARs with varying kinetics and agonist affinities (Perrais et al., 2010; Carta et al., 2014).

KARs are widely dispersed in the CNS. Functional presynaptic KARs are found in brain regions including the hippocampus (Chittajallu et al., 1996; Rodriguez-Moreno et al., 1997; Clarke et al., 1997; Vignes et al., 1998; Negrete-Diaz et al., 2006; Andrade-Talavera et al., 2012), thalamus (Kidd et al., 2002; Andrade-Talavera et al., 2013), hypothalamus (Liu et al., 1999), cortex (Perkinton and Sihra, 1999; Kidd et al., 2002; Rodriguez-Moreno and Sihra, 2013), amygdala (Negrete-Diaz et al., 2012), and cerebellum (Falcon-Moya et al., 2018). Functional postsynaptic KARs are found in areas including the hippocampus (Castillo et al., 1997; Vignes and Collingridge, 1997; Cossart et al., 1998; Frerking et al., 1998), retina (DeVries and Schwartz, 1999), amygdala (Li and Rogawski, 1998), cortex (Wu et al., 2005; Campbell et al., 2007), auditory brainstem (Vitten et al., 2004), cerebellum (Bureau et al., 2000), and spinal cord (Li et al., 1999). Immunocytochemical (ICC) data, including our own, suggest that KARs in the OB are found on mitral/tufted (M/T) cells, the bulb's principal output neurons, as well as interneurons including periglomerular (PG) cells and granule cells (Petralia et al., 1994; Montague and Greer, 1999; Davila et al., 2007). Our previous ICC data further suggest that GluK1-containing KARs are more prone to be located at or near synapses than GluK2/3-containing KARs (Davila et al., 2007). One goal of the present study was to examine the characteristics and distribution of functional KARs on M/T cells and interneurons in the OB, including the presence of KARs at synapses.

Only a few studies have provided evidence of functional KARs in the OB. A 2003 study that used 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide (NBQX), an AMPAR/KAR antagonist, as well as 4-(4-Aminophenyl)-1,2-dihydro-1-methyl-2-propylcarbamoyl-6,7-methylenedioxyphthalazine (SYM 2206), a noncompetitive AMPAR antagonist, examined the role of glutamate receptors in the OB (Lowe, 2003). In that study, mitral cell somatodendritic excitation was attributed to fast AMPAR- and KAR-mediated currents, as well as slow high-affinity NMDAR-mediated currents. However, this study was limited to mitral cells. Furthermore, these methods (flash photolysis of caged glutamate) do not distinguish between pre- and post-synaptic receptors, nor between synaptic and extrasynaptic receptors. In 2006, Schoppa reported a potential role for KARs in mediating synaptic events in granule cells evoked by patterned olfactory nerve ("dynamic") stimulation (Schoppa, 2006a). In our 2007 study, we found that KAR activation increases excitatory spontaneous activity but attenuates evoked glutamatergic transmission between OB neurons, likely via a presynaptic depolarizing mechanism (Davila et al., 2007). Another goal of the present study was to explore the potential roles of both presynaptic and postsynaptic KARs in mediating synaptic transmission in the OB.

Studies in various brain regions suggest that KARs modulate synaptic transmission via a variety of mechanisms (Contractor et al., 2011; Rodrigues and Lerma, 2012; Lerma and Marques, 2013; Negrete-Diaz et al., 2018). These include postsynaptic depolarization and mediation of a small component of the synaptic current at some excitatory synapses (e.g., mossy fiber-CA3 pyramidal cell synapse) (Castillo et al., 1997; Vignes and Collingridge, 1997; Lerma and Marques, 2013) and presynaptic modulation of the release of neurotransmitters such as glutamate (Chittajallu et al., 1996; Schmitz et al., 2000; Frerking et al., 2001; Rodriguez-Moreno and Sihra, 2004; Andrade-Talavera et al., 2012; Negrete-Diaz et al., 2012; Sihra and Rodriguez-Moreno, 2013; Andrade-Talavera et al., 2013; Rodriguez-Moreno and Sihra, 2013; Falcon-Moya et al., 2018) and gamma-Aminobutyric acid (GABA) (Rodriguez-Moreno et al., 1997; Rodriguez-Moreno and Lerma, 1998; Liu et al., 1999; Cossart et al., 2001; Mathew et al., 2008). In contrast to AMPARs and NMDARs, some KAR-mediated modulation of synaptic transmission involves metabotropic (G protein-mediated)/non-canonical signaling in addition to traditional ionotropic receptor activity (Rodriguez-Moreno and Sihra, 2007; Rodrigues and Lerma, 2012; Lerma and Marques, 2013; Negrete-Diaz et al., 2018). The ionotropic pathway is responsible for membrane depolarization and the synaptic current as well as the facilitation of transmitter release at some synapses (Schmitz et al., 2001b; Cossart et al., 2001; Lerma and Marques, 2013). The metabotropic/non-canonical