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Kappa opioid receptor activation decreases inhibitory transmission and antagonizes alcohol effects in rat central amygdala

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ABSTRACT

Activation of the kappa opioid receptor (KOR) system mediates negative emotional states and considerable evidence suggests that KOR and their natural ligand, dynorphin, are involved in ethanol dependence and reward. The central amygdala (CeA) plays a major role in alcohol dependence and reinforcement. Dynorphin peptide and gene expression are activated in the amygdala during acute and chronic administration of alcohol, but the effects of activation or blockade of KOR on inhibitory transmission and ethanol effects have not been studied. We used the slice preparation to investigate the physiological role of KOR and interaction with ethanol on $GABA_A$ receptor-mediated synaptic transmission. Superfusion of dynorphin or U69593 onto CeA neurons decreased evoked inhibitory postsynaptic potentials (IPSPs) in a concentration-dependent manner, an effect prevented by the KOR antagonist norbinaltorphimine (norBNI). Applied alone, norBNI increased GABAergic transmission, revealing a tonic endogenous activity at KOR. Paired-pulse analysis suggested a presynaptic KOR mechanism. Superfusion of ethanol increased IPSPs and pretreatment with KOR agonists diminished the ethanol effect. Surprisingly, the ethanol-induced augmentation of IPSPs was completely obliterated by KOR blockade. Our results reveal an important role of the dynorphin/KOR system in the regulation of inhibitory transmission and mediation of ethanol effects in the CeA.

1. Introduction

Alcohol impacts many aspects of neuronal communication, including synaptic transmission, ion channels and intracellular signaling cascades. Synaptic transmission is a sensitive substrate for ethanol actions (Siggins et al., 2005). GABAergic transmission is affected by alcohol in several brain structures and contributes to the behavioral effects of alcohol as well as the development of alcohol dependence (Koob, 2004; Siggins et al., 2005; Weiner and Valenzuela, 2006). The central amygdala (CeA) plays a critical role in alcohol dependence (Davis et al., 1994; Koob, 2008) and behavioral studies have implicated CeA GABAergic transmission in the regulation of alcohol intake (Hyytia and Koob, 1995; Koob, 2003). In ex vivo recordings from CeA slices, ethanol increases GABAergic transmission by acting principally at a presynaptic site to augment GABA release (Nie et al., 2004; Roberto et al., 2003).

The endogenous peptide dynorphin activates kappa opioid receptors (KOR) in the brain (Chavkin et al., 1982), and the dynorphin/KOR system modulates affective-like states (Knoll and Carlezon, Jr., 2010). Several studies suggest that multiple physiological and behavioral effects of ethanol involve KOR (Dar, 1998; Matsuzawa et al., 1999; Pohorecky et al., 1989), and mice lacking KOR exhibit decreased alcohol consumption (Kovacs et al., 2005). Ethanol treatment upregulates dynorphin and its precursor prodynorphin as well as KOR mRNA in the CeA (D'Addario et al., 2011; Lam et al., 2008), and ethanol also increases prodynorphin levels in the nucleus accumbens and prefrontal cortex (D'Addario et al., 2011; Marinelli et al., 2006). Dynorphin peptide and gene expression are activated in the amygdala during acute and chronic administration of alcohol and the KOR antagonist nor-binaltorphimine (norBNI) reduces ethanol self-administration in alcohol-dependent animals (Walker and Koob, 2008). However, little is known about the role of the dynorphin/KOR system in regulating the cellular effects of ethanol.

Prodynorphin neurons are abundant in the CeA (Marchant et al., 2007), and KOR immunoreactivity is localized in the CeA and medial amygdala, with no specific staining in the lateral, basolateral

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and cortical amygdaloid nuclei (Mansour et al., 1996). Within the CeA, KOR-like immunoreactive fibers are localized predominantly in the medial division, identifying this area as a prime target for electrophysiological recordings. The synaptic network in CeA is mostly GABAergic and projection neurons also are GABAergic (Sah et al., 2003), thus we focused on inhibitory transmission in the medial division of the CeA in this study.

We report here that KOR activation reduced GABAergic synaptic responses whereas KOR blockade increased it, revealing a tonic endogenous KOR activity that suppresses inhibition in the CeA. Dynorphin diminished but did not prevent the ethanol-elicited increase of GABAergic transmission, whereas KOR antagonism blocked the effect of ethanol. We conclude that dynorphin reduces inhibitory transmission and KOR activation tonically controls neuronal activity in the CeA. Dynorphin also antagonizes the effect of ethanol in the CeA of the alcohol-naïve rat brain, suggesting an important role of the dynorphin/KOR system to regulate CeA tone during challenge with ethanol.

2. Materials and methods

2.1. Slice preparation

All experimental protocols were consistent with guidelines issued by the National Institutes of Health and were approved by The Scripps Research Institute's Institutional Animal Care and Use Committee. We prepared CeA slices as previously described (Roberto et al., 2003) from male Sprague-Dawley rats (120–200 g) that were anesthetized with halothane (3%) and decapitated. In brief, the brains were rapidly removed and placed into ice-cold artificial cerebrospinal fluid (ACSF) gassed with 95% O_2 and 5% CO_2. Transverse slices 400 μm thick were cut on a Vibratome Series 3000 (Technical Products International, St. Louis, MO), incubated in an interface configuration for about 30 min, and then completely submerged and superfused at a constant flow rate of 2–4 ml/min with warm (31 °C), gassed ACSF of the following composition in mM: NaCl, 130; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4 \cdot 7H_2O$, 1.5; $CaCl_2$, 2.0; $NaHCO_3$, 24; glucose, 10. We added drugs to the ACSF from stock solutions to obtain known concentrations in the superfusate. The inner recording chamber had a total volume of 0.8 ml, so drug concentrations reach 90% of the nominal concentration within 2 min.

2.2. Recordings

We recorded neurons in the medial subdivision of the CeA with sharp micropipettes filled with 3M KCl (impedance range of 60–90 MΩ) using current-clamp mode. We held most neurons near their resting membrane potential (RMP), and the RMP observed in control conditions was maintained throughout the experiment. Data were acquired with an Axoclamp-2 preamplifier (Axon Instruments, Foster City, CA) and stored for later analysis using pClamp software (Axon Instruments). Pharmacologically-isolated GABA receptor-mediated inhibitory postsynaptic currents (IPSPs) were evoked by stimulating locally within the CeA through a bipolar stimulating electrode while superfusing the slices with the glutamate receptor blockers 6-cyano-7-nitroquinoxaline-2,3-dione (DNQX, 20 μM) and DL-2-amino-5-phosphonovalerate (APV, 30 μM). We also applied the $GABA_B$ receptor antagonist CGP 55845 (1 μM) to isolate the $GABA_A$ receptor-mediated component of the IPSP.

2.3. Electrophysiological protocols

To determine the experimental response parameters for each cell, we performed an input–output protocol consisting of a range of current stimulations (typically between 50 and 250 mA; 0.125 Hz), starting at the threshold current required to elicit an IPSP up to the strength required to elicit the maximum amplitude. The stimulus strength eliciting 50% of the maximum response was chosen to conduct experiments and maintained throughout the entire duration of the experiment. Stability of IPSPs was established by stimulating for at least 15 min prior to beginning experiments. The synaptic responses were quantified by averaging two consecutive responses (30 s apart, i.e. 1 data point/min) and calculating the IPSP amplitude with Clampfit software (Axon Instruments). We examined paired-pulse facilitation (PPF), a phenomenon whereby a secondary evoked synaptic response is increased by a preceding primary stimulation of equal intensity (Siggins et al., 2005; Thomson, 2000), in each neuron using 100 msec inter-stimulus intervals; we calculated the paired-pulse ratio as the second IPSP amplitude over that of the first IPSP.

2.4. Drugs

Drugs were added to the superfusate in known concentrations. U69593 and norbinaltorphimine (norBNI) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and dissolved in dimethylsulfoxide. Dimethylsulfoxide final concentration was 0.05–0.1%, which did not affect the studied synaptic responses in control experiments. Dynorphin A [1-17], CTAP and bicuculline were purchased from Tocris (Ellisville, MO), and ethanol from Remet (La Mirada, CA). We purchased all other chemicals from Sigma-Aldrich (St Louis, MO).

2.5. Statistics

We took measures every minute before drug superfusion (control) and during drug superfusion. All values are expressed as mean ± s.e.m. We performed statistical analysis using GraphPad Prism version 5.00 (GraphPad Software, San Diego California USA). In the case of a single drug application, we used Student's paired *t*-test. One-way repeated measures ANOVA with the Tukey post hoc test were performed in a multiple drug application. Statistical significance was set at $p < 0.05$.

3. Results

We recorded intracellularly from medial CeA neurons using sharp micropipettes and we evoked pharmacologically isolated $GABA_A$–mediated synaptic responses via local stimulations through an electrode placed near the recording site (see Roberto et al., 2010). The average resting membrane potential (RMP) of our neuronal sample was −77.0 ± 0.6 mV ($n = 60$).

3.1. Kappa opioid receptor ligands decrease inhibitory transmission at a presynaptic site

We first assessed the effect of dynorphin (dynorphin A [1-17]), an endogenous kappa receptor agonist, on GABAergic transmission. Superfusion of 1 μM dynorphin consistently decreased Inhibitory PostSynaptic Potentials (IPSPs) in 80% of the neurons exposed to the peptide (12 of 15.) The depressant effect of dynorphin developed immediately after the start of application and reached a maximum effect after 9 min of superfusion (Fig. 1A,B). Upon washout of the peptide, IPSP amplitude recovered to control level within 20 min (4 of 4 experiments). On average (mean of 4 points after steady level reached and just before change of condition), IPSP amplitudes were significantly decreased to 79 ± 4% of control ($n = 12$, $t = 6.568$). The dynorphin decrease of IPSP amplitude persisted upon extended application of the peptide for 25 min ($n = 2$ at 1 μM; $n = 2$ at 2 μM), demonstrating a lack of short term desensitization of KOR in our preparation. Another neuron did not respond and another 2 showed a slight IPSP increase (10–15%) upon exposure to dynorphin. We used the selective KOR antagonist nor-binaltorphimine (norBNI) (Portoghese et al., 1987) to confirm that dynorphin decreased IPSPs by activating KOR. In the presence of 0.2 μM norBNI, dynorphin did not affect IPSPs which remained at 98 ± 4% of pre-dynorphin level ($n = 4$; Fig 1C). The dynorphin-elicited decrease of inhibitory transmission was concentration-dependent. We observed no effect of 0.1 μM dynorphin (101 ± 4% of control, $n = 3$), whereas IPSPs were decreased to 96 ± 5% of control with 0.2 μM dynorphin ($n = 3$), to 91 ± 5% with 0.5 μM ($n = 4$) and 77 ± 5% with 2 μM ($n = 6$). We used a sigmoidal (logistic) fit to analyze the concentration–response relationship (Fig. 2A). The apparent EC50 for dynorphin to decrease IPSPs was 0.5 μM. We conclude that the endogenous peptide dynorphin concentration-dependently decreases inhibitory transmission in CeA by activating KOR.

We also determined the effect of the highly selective synthetic KOR agonist U69593 (U69) (Raynor et al., 1994) on $GABA_A$–mediated transmission. These experiments were designed to confirm KORs as the locus of action for dynorphin effects and as such, we utilized only two concentrations typical of recently published electrophysiological studies in brain slices (Lemos et al., 2012; Li et al., 2012). Upon superfusion of 0.5 μM U69, IPSP amplitude decreased to 78 ± 6% of control ($n = 4$; Fig. 1D). A concentration of 1 μM U69 did not elicit a larger effect, decreasing IPSPs to 76 ± 6% of control ($n = 4$; Figs. 1D and 2A). Combining those two concentrations, U69 significantly decreased IPSP amplitude to 77 ± 5% of control ($n = 8$, $t = 4.478$). Another neuron did not respond to U69.