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Dynorphin activation of kappa opioid receptor reduces neuronal excitability in the paraventricular nucleus of mouse thalamus

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ABSTRACT

It has been reported that kappa opioid receptor (KOR) is expressed in the paraventricular nucleus of thalamus (PVT), a brain region associated with arousal, drug reward and stress. Although intra-PVT infusion of KOR agonist was found to inhibit drug-seeking behavior, it is still unclear whether endogenous KOR agonists directly regulate PVT neuron activity. Here, we investigated the effect of the endogenous KOR agonist dynorphin-A (Dyn-A) on the excitability of mouse PVT neurons at different developmental ages. We found Dyn-A strongly inhibited PVT neurons through a direct postsynaptic hyperpolarization. Under voltage-clamp configuration, Dyn-A evoked an obvious outward current in majority of neurons tested in anterior PVT (aPVT) but only in minority of neurons in posterior PVT (pPVT). The Dyn-A current was abolished by KOR antagonist nor-BNI, Ba^{2+} and non-hydrolyzable GDP analogue GDP-β-s, indicating that Dyn-A activates KOR and opens G-protein-coupled inwardly rectifying potassium channels in PVT neurons. More interestingly, by comparing Dyn-A currents in aPVT neurons of mice at various ages, we found Dyn-A evoked significant larger current in aPVT neurons from mice around prepuberty and early puberty stage. In addition, KOR activation by Dyn-A didn't produce obvious desensitization, while mu opioid receptor (MOR) activation induced obvious desensitization of mu receptor itself and also heterologous desensitization of KOR in PVT neurons. Together, our findings indicate that Dyn-A activates KOR and inhibits aPVT neurons in mice at various ages especially around puberty, suggesting a possible role of KOR in regulating aPVT-related brain function including stress response and drug-seeking behavior during adolescence.

1. Introduction

The paraventricular nucleus of the thalamus (PVT), a part of the midline and intralaminar thalamic complex, receives afferents from a number of brainstem areas involved in arousal and in the processing of somatosensory and visceral information (Cornwall and Phillipson, 1988; Krout et al., 2002; Krout and Loewy, 2000a,b; Otake et al., 1995; Phillipson and Bohn, 1994). PVT is an interface between sensory input and limbic and cortical structures of the extended amygdala. PVT receives afferents from the ventral pallidum (Haber et al., 1993; Zahm et al., 1996) and, in turn, projects to the infralimbic and prelimbic cortices and in the prefrontal cortex (PFC) (Berendse and Groenewegen, 1991; Bubser and Deutch, 1998; Conde et al., 1990; Freedman and Cassell, 1994; Moga et al., 1995), thus completing a functional corticofugal circuit and providing entry into parallel circuits (Zahm et al., 1996). The efferent projections of PVT differ from those of the mediodorsal thalamic nucleus by sending glutamatergic projections to the nucleus accumbens (NAC), amygdala, and subiculum (Berendse and Groenewegen, 1990; Bubser and Deutch, 1998; Moga et al., 1995; Su and Bentivoglio, 1990; Turner and Herkenham, 1991). In addition, PVT receives dopamine innervation (Groenewegen, 1988; Otake and Ruggiero, 1995; Takada et al., 1990) and a dense peptidergic innervation from hypothalamus (Freedman and Cassell, 1994; Kirouac et al., 2005, 2006; Otake, 2005; Parsons

Abbreviations: BNST, bed nucleus of the striaterminalis; CeA, central amygdala; Dyn-A, dynorphin-A; GIRK, G-protein-coupled inwardly rectifying potassium; GPCRs, G-protein coupled receptor; KOR, kappa opioid receptor; MDH, medial dorsal hypothalamus; MOR, mu opioid receptor; NAC, nucleus accumbens; PFC, prefrontal cortex; PVT, paraventricular nucleus of thalamus.

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et al., 2006). Increasing evidences indicate that PVT is involved in drug-seeking behavior (Martin-Fardon and Boutrel, 2012) and stress response (Heydendael et al., 2014, 2011). Glutamatergic efferents from PVT are closely apposed to dopamine fibers in the NAC shell (Pinto et al., 2003) and stimulation of PVT produces an efflux of dopamine in this brain region (Jones et al., 1989; Parsons et al., 2007). Early studies indicate that acute psychostimulant administration activates PVT (Deutch et al., 1998) and lesions of PVT block the conditioned locomotor response to a cocaine-paired environment (Young and Deutch, 1998). In addition, the lesion or chemical inactivation of PVT suppresses drug-seeking behaviors (Hamlin et al., 2009; Marchant et al., 2010). These studies indicate the importance of PVT neurons in the regulation of drug reward, stress and motivation.

The endogenous opioid system is a modulator of drug reward and stress responses (Hebb et al., 2005; Laurent et al., 2015). In contrast to mu opioid receptor (MOR) agonists, kappa opioid receptor (KOR) agonists function as negative reinforcers. Neurochemical and electrophysiological data have shown that selective KOR activation decreases dopamine release in NAC (Chefer et al., 2005; Di Chiara and Imperato, 1988; Shippenberg et al., 2007). Dynorphin-A (Dyn-A) is the selective endogenous ligand for KOR which is distributed throughout the brain including thalamus (Hollt et al., 1980). Neurons expressing dynorphin in medial dorsal hypothalamus (MDH) send innervations onto PVT neurons (Marchant et al., 2010). In addition, the high expression of KOR has been found in midline thalamic nuclei particularly the PVT of rat brain (George et al., 1994; Mansour et al., 1986). Intra-PVT infusion of KOR agonist inhibits drug-seeking behavior (Marchant et al., 2010). However, to date, it is still unclear whether dynorphin affects the activity of PVT neurons. Therefore, in this study, we used patch-clamp technique to test the hypothesis that Dyn-A activates KOR and inhibits the excitability of PVT neurons in mouse slices.

2. Material and methods

2.1. Brain slice preparation

C57BL/6 mice at different age from 9 to 150 days were used for the experiments. Acute brain slices were cut for patch-clamp recording. Animals were deeply anesthetized with isoflurane. Following decapitation, the brains were rapidly excised and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose (gassed with 95% O_2/5% CO_2); 310 mOsm. Coronal brain slices (300 μM) containing PVT were prepared using a Leica VT1000S vibratome (Leica instruments Ltd., Wetzlar, Germany). Brain slices were then transferred to an incubation chamber filled with ACSF and incubated at room temperature (22 °C). After a recovery period of at least 1 h, slices were transferred to a recording chamber and continuously perfused with ACSF. All procedures in this study were approved by NIH Guide and the Animal Care and Use Committee of Central South University.

2.2. Patch-clamp recording

Whole-cell patch recordings were performed on PVT neurons that were visualized using an infrared-differential interference contrast (DIC) optical system combined with a monochrome CCD camera and monitor. Pipettes used for whole-cell recording were pulled from thin-walled borosilicate glass capillary tubes (outer diameter 1.5 mm, inner diameter 1.1 mm, World Precision Instruments) using a P-97 micropipette puller (Sutter Instruments, Novato, CA) and had resistances ranging from 3 to 6 MΩ when filled with pipette solution containing (in mM) 130 K-gluconate, 10 KCl, 1 $MgCl_2$, 10 HEPES, 1.1 EGTA, 2 Mg-ATP, 0.5 Na_2-GTP, and 5 Na_2-phosphocreatine (pH 7.3 with KOH; 290–295 mOsm). EPC-10 amplifier (HEKA, Lambrecht, Germany) was used to collect the data. Pulse 8.74 software (HEKA, Lambrecht, Germany) was used to acquire and analyze the data. Igor pro 6.0 was used to make figures. Recording was performed under room temperature (22 °C).

2.3. Statistical analysis

Data are expressed as mean ± SEM. Group statistical significance was assessed using Student's *t* test for comparison of two groups, one-way and two-way ANOVA followed by a Bonferroni *post hoc* test for three or more groups. $P < 0.05$ was considered statistically significant.

2.4. Drugs and drug application

CTAP and Dynorphin-A (1–13, human, rat, porcine) were purchased from Phoenix Pharmaceuticals (Beijing, China). [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin (DAMGO), GDP-β-s, nor-binaltorphmine (nor-BNI), tetrodotoxin (TTX), bicuculline (Bic), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonopentanoic acid (D-AP5) were obtained from Sigma–Aldrich (St. Louis, MO). Unless specifically mentioned in the result, drug solutions were administered using gravity by flow pipe (about 500 μm tip diameter) directed toward the recorded cell. During the whole recording, normal ACSF was continuously supplied to the slice through bath application.

3. Results

3.1. Differential effect of Dyn-A on PVT neurons from anterior to posterior area

Both the anatomy and function PVT have been reported to be different in anterior and posterior subregions (Barson et al., 2015; Hsu et al., 2014). To test the effect of Dyn-A on PVT neuron, we measured Dyn-A current at voltage of −60 mV on neurons in PVT from anterior to posterior subregions including anterior PVT (aPVT), middle PVT (mPVT) and posterior PVT (pPVT) (Fig. 1A). Dyn-A (3 μM) evoked obvious outward current (peak amplitude larger than 3 pA) on 13 out of 16 tested aPVT neurons (Fig. 1C). In contrast, Dyn-A (3 μM) evoked obvious outward current on 9 out of 16 tested mPVT neurons and 5 out of 16 tested pPVT neurons (Fig. 1C). The average peak amplitude of Dyn-A current was 26.9 ± 5.2 pA, 9.1 ± 2.1 pA and 2.4 ± 0.7 pA from 16 tested aPVT, mPVT and pPVT neurons, respectively (Fig. 1D). We then assessed Dyn-A current of amplitude larger than 3 pA from various PVT subregions. The average of peak Dyn-A current was 32.9 ± 5.1 pA ($n = 13$), 15.5 ± 2.4 pA ($n = 9$) and 6.3 ± 0.7 pA ($n = 5$) from aPVT, mPVT and pPVT neurons, respectively (Fig. 1B and E). These results together indicate that more neurons in aPVT than in pPVT responded to Dyn-A and the outward current evoked by Dyn-A was significantly larger in aPVT neurons.

3.2. Dyn-A effect on aPVT neurons from mice at various ages

In the thalamus and amygdala, KOR expression level was reported to be different in young and old rats (Maggi et al., 1989). To determine whether Dyn-A effect of mouse aPVT neurons is age-dependent, we observed the outward current induced by Dyn-A on aPVT neurons in mice at aged 2–20 weeks. At holding potential of −60 mV, Dyn-A (3 μM) evoked obvious outward current on aPVT neurons from mice at various ages (Fig. 2A–F). In young mice at aged 2 weeks, Dyn-A (3 μM) evoked an obvious outward current with peak amplitude of 15.2 ± 1.9 pA ($n = 10$, Fig. 2A and G). In contrast, at prepuberty stage, Dyn-A current was significantly increased to 31.0 ± 5.5 pA ($p < 0.05$, one-way ANOVA, $n = 9$, Fig. 2B and G) on aPVT neurons from 3 weeks old mice and 34.5 ± 6.3 pA ($p < 0.05$, one-way ANOVA, $n = 9$, Fig. 2C and G) on neurons from mice of 4 weeks old. However, at adult stage, Dyn-A current was decreased to 18.2 ± 3.2 pA ($n = 10$, Fig. 2D and G) and to 16.0 ± 3.8 pA ($n = 9$, Fig. 2E and G) on aPVT neurons from 8 and 12 weeks old mice, respectively. These results indicated that Dyn-A currents from aPVT neurons of adult mice were similar to that of young mice of 2 weeks old. To further determine whether Dyn-A current is stable on neurons from old mice, we studied the effect of Dyn-A on aPVT neurons from 20 weeks old mice. Compared to aPVT neurons from 8 weeks old adult mice, Dyn-A (3 μM) evoked an outward current of 16.6 ± 4.2 pA ($n = 10$, Fig. 2F and G) on neurons from 20 weeks old mice. No significant difference was found in peak amplitude of Dyn-A current between mature adult and old mice ($p > 0.05$).