Neuroscience 222 (2012) 181–190

OPIOIDS INHIBIT VISCERAL AFFERENT ACTIVATION OF CATECHOLAMINE NEURONS IN THE SOLITARY TRACT NUCLEUS

R. J. CUI,[a] B. L. ROBERTS,[a] H. ZHAO,[a] M. C. ANDRESEN[b] AND S. M. APPLEYARD[a]*

[a] *Department of Veterinary Comparative Anatomy, Physiology and Pharmacology, Washington State University, Pullman, WA 99164, USA*

[b] *Department of Physiology and Pharmacology, Oregon Health & Science University, Portland, OR 97239-3098, USA*

Abstract—Brainstem A2/C2 catecholamine (CA) neurons within the solitary tract nucleus (NTS) influence many homeostatic functions, including food intake, stress, respiratory and cardiovascular reflexes. They also play a role in both opioid reward and withdrawal. Injections of opioids into the NTS modulate many autonomic functions influenced by catecholamine neurons including food intake and cardiac function. We recently showed that NTS-CA neurons are directly activated by incoming visceral afferent inputs. Here we determined whether opioid agonists modulate afferent activation of NTS-CA neurons using transgenic mice with EGFP expressed under the control of the tyrosine hydroxylase promoter (TH-EGFP) to identify catecholamine neurons. The opioid agonist Met-enkephalin (Met-Enk) significantly attenuated solitary tract-evoked excitatory postsynaptic currents (ST-EPSCs) in NTS TH-EGFP neurons by 80%, an effect reversed by wash or the mu opioid receptor-specific antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP). Met-Enk had a significantly greater effect to inhibit afferent inputs onto TH-EGFP-positive neurons than EGFP-negative neurons, which were only inhibited by 50%. The mu agonist, DAMGO, also inhibited the ST-EPSC in TH-EGFP neurons in a dose-dependent manner. In contrast, neither the delta agonist DPDPE, nor the kappa agonist, U69,593, consistently inhibited the ST-EPSC amplitude. Met-Enk and DAMGO increased the paired pulse ratio, decreased the frequency, but not amplitude, of mini-EPSCs and had no effect on holding current, input resistance or current–voltage relationships in TH-EGFP neurons, suggesting a presynaptic mechanism of action on afferent terminals. Met-Enk significantly reduced both the basal firing rate of NTS TH-EGFP neurons and the ability of afferent stimulation to evoke an action potential. These results suggest that opioids inhibit NTS-CA neurons by reducing an excitatory afferent drive onto these neurons through presynaptic inhibition of glutamate release and elucidate one potential mechanism by which opioids could control autonomic functions and modulate reward and opioid withdrawal symptoms at the level of the NTS.

Key words: opioid, vagus, afferents, catecholamine, synaptic transmission, NTS.

INTRODUCTION

The solitary tract nucleus (NTS) broadly impacts homeostatic regulation as it is the primary site through which visceral afferent information concerning cardiovascular, respiratory and gastrointestinal systems enters the brain (Andresen and Kunze, 1994; Berthoud, 2008; Saper, 2002). The A_2/C_2 group of catecholamine (CA) neurons lie within the dorsal vagal complex (DVC) and are ideally situated to co-ordinate afferent signaling to multiple brain regions through their extensive projections, including the hypothalamus, amygdala, nucleus accumbens and other brainstem nuclei (Sawchenko and Swanson, 1981; Cunningham and Sawchenko, 1988; Sawchenko and Pfeiffer, 1988; Riche et al., 1990; Wang et al., 1992; Petrov et al., 1993; Jia et al., 1997; Suzuki et al., 1997; Ueta et al., 2000; Rogers et al., 2003; Reyes and Van Bockstaele, 2006; Travagli et al., 2006; Balcita-Pedicino and Rinaman, 2007; Rukhadze and Kubin, 2007). Release of norepinephrine and epinephrine at these target nuclei widely affects behaviors, including stress, anxiety, reward, food intake and cardiovascular function (Leibowitz et al., 1988; Cole and Sawchenko, 2002; Smith and Aston-Jones, 2008) and A_2/C_2 catecholamine neurons have been implicated in the regulation of these functions (Simon et al., 1985; Kubo et al., 1990; Itoh and Bunag, 1993; Rinaman, 2011).

Opioids modulate many functions influenced by afferent inputs and CA neurons in the NTS. Injections of opioid agonists into the NTS increase food intake (Kotz et al., 1997) and decrease baroreceptor reflex (Hassen and Feuerstein, 1987; Gordon, 1990), while antagonists have the converse effects (Van Giersbergen et al., 1989; Xu et al., 1992; Giraudo et al., 1998; Kotz et al., 2000; Glass et al., 2002; Kim et al., 2009). NTS-CA neurons are also proposed to be important for the rewarding aspects of opioids as re-expression of dopamine beta-hydroxylase, an enzyme critical for catecholamine synthesis in NTS neurons restores morphine-induced conditioned place preference (Olson et al., 2006). These neurons also undergo adaptive responses following chronic exposure to opioids (Van Bockstaele et al., 2001) and gene

*Corresponding author. Address: Department of Veterinary Comparative Anatomy, Physiology and Pharmacology, Program in Neuroscience, Washington State University, 100 Dairy Road, Pullman, WA 99164, USA. Tel: +1-509-335-7784; fax: +1-509-335-4650.
E-mail address: appleyas@vetmed.wsu.edu (S. M. Appleyard).

Abbreviations: AP, action potential; CA, catecholamine; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LC, Locus Coeruleus; Met-Enk, Met-enkephalin; MOP-Rs, mu opioid receptors; NTS, solitary tract nucleus; ST-EPSCs, solitary tract-evoked excitatory postsynaptic currents.

0306-4522/12 $36.00 © 2012 Published by Elsevier Ltd. on behalf of IBRO.
http://dx.doi.org/10.1016/j.neuroscience.2012.07.010

transcription is activated following opioid withdrawal (Laorden et al., 2002; Benavides et al., 2005) concomitant with an activation of both aversive (Delfs et al., 2000) and stress responses (Fuertes et al., 2000a, 2000b; Laorden et al., 2000; Nunez et al., 2008, 2010; Navarro-Zaragoza et al., 2010, 2011). Furthermore, brainstem CA neuron projections, including the A_2/C_2 group, making up the ventral noradrenergic bundle appear to be critical for both the aversive effects following acute morphine withdrawal and the stress-induced reactivation of morphine-conditioned place preference following abstinence (Wang et al., 2001). Norepinephrine release in the extended amygdala is hypothesized to mediate many of the aversive aspects of opioid withdrawal as well as stress-induced relapse of drug-seeking for opioids and other drugs of abuse including cocaine, ethanol and nicotine (Smith and Aston-Jones, 2008).

NTS-CA neurons are strongly and directly activated by incoming visceral afferents through the release of the excitatory transmitter glutamate (Appleyard et al., 2007; Cui et al., 2011). However, the propagation of afferent information to downstream targets by NTS-CA neurons depends on the translation of afferent glutamate release into postsynaptic action potential (AP) activity. This can be influenced by multiple factors, including presynaptic modulation of glutamate release (Bailey et al., 2006; Appleyard et al., 2007; Peters et al., 2008; Cui et al., 2011) and expression of different types of ion channels (Appleyard et al., 2007; Bailey et al., 2007). Opioids inhibit the release of norepinephrine acutely in NTS slices (Arakawa et al., 1991; Al-Khrasani et al., 2003). However, the cellular mechanisms by which opioids regulate glutamate release and influence the activity of this critical group of CA neurons are not known. The goals of this study were to determine whether opioids modulate afferent activation of NTS-CA neurons and the cellular mechanisms involved.

EXPERIMENTAL PROCEDURES

NTS slices

Hindbrains of male TH-EGFP mice (6–16 weeks old) were prepared as previously described (Appleyard et al., 2007). All animal procedures were conducted with the approval of the Animal Care and Use Committees at either OHSU or WSU and in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Guide). The hindbrain was removed and placed for 1 min in cold (0–4 °C) artificial cerebral spinal fluid composed of (mM): 125 NaCl, 3 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 dextrose, 2 $CaCl_2$, and bubbled with 95% O_2/5% CO_2. The medulla was trimmed to a 2-cm block (rostral–caudal) centered on the obex. A wedge of tissue was removed from the ventral surface to align the ST with the cutting plane when mounted in a vibrating microtome (Leica VT-1000S). Slices (250 μm thick) cut with a sapphire knife (Delaware Diamond Knives, Wilmington, DE) contained the ST in the same plane as the NTS but more dorsal than the dorsal motor nucleus of the vagus (DMNV). Slices were submerged in a perfusion chamber and all recordings performed at 31–35 °C and pH 7.4. The osmolarity was adjusted to 301–305 mOsm using dextrose. Neurons were visualized using an upright microscope (Olympus BX51 or Zeiss Axioskop, Center Valley, PA). Recording electrodes were filled with a solution (mM): 10 NaCl, 130 K gluconate, 11 EGTA, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 2 NaATP, 0.2 NaGTP; pH 7.3; 297–301 mOsm. Neurons were recorded from NTS within 200 μm rostral or caudal from obex and medial to the ST-medial NTS (mNTS). Patch electrodes, 3–5 MΩ, were guided to neurons using differential interference contrast (DIC) optics illuminated with infrared light (Olympus B51 or Zeiss Axioskop). Voltage clamp recordings were made with an Axopatch 700B and pClamp 9 or 10 software (Molecular Devices, Sunnyvale, CA). Only neurons with holding currents not exceeding 100 pA at $V_H = -60$ mV for the 15-min control period (input resistance > 150 MΩ) were studied further. Synaptic currents were evoked with an ultrafine concentric bipolar stimulating electrode (50 μm ID, F. Haer) placed on the ST 1–3 mm from the recording electrode. Electrical stimuli were delivered from an isolated programmable stimulator (Master-8, AMPI, Jerusalem, Israel) triggered to deliver a burst of five stimuli (20–50 Hz). Highly consistent ST latencies (SD latency or jitter < 200 μs) identified neurons that are second order to ST were studied (Doyle and Andresen, 2001). Most analyses examined the first excitatory postsynaptic current (EPSC) of each train and averaged responses across multiple trials (>20) within each neuron unless otherwise noted. All drugs were obtained from Tocris Cookson (Ballwin, MO, USA) or Sigma (St. Louis, MO, USA). As previously characterized, TH-EGFP neurons often receive multiple monosynaptic afferent inputs, as well as polysynaptic inputs, resulting in a compound EPSC (Appleyard et al., 2007) and all drug effects are reported on the compound EPSC amplitudes. Failure to detect a synaptic event at the expected latency for a given solitary tract-evoked EPSC (ST-EPSC) was considered a synaptic failure and failure rate was calculated as the number of missed events divided by the number (>20) of successive ST shock trials and expressed as the percent failing. The failure rate reported is for the compound ST-EPSC, acknowledging that this may underestimate each individual input's failure rate. However, all neurons studied had at least one direct input that must fail for there to be an overall increase in failure rate.

Statistical analysis

All data are presented as average ± SEM. Statistical comparisons were made using unpaired Student's *t*-test or one-way ANOVA, and Fisher's PLSD post hoc analysis where appropriate (see individual results; Statview 4.57, Abacus Concepts). The Kolmogorov–Smirnov test (K–S test) determined the significance of the drug effect for individual neurons when analyzing the mEPSC data (Mini analysis, Synaptosoft). $p < 0.05$ was considered a statistically significant difference.

RESULTS

Opioid inhibition of ST-stimulated EPSCs

Horizontal brainstem slices were cut to preserve a lengthy segment of the ST in the same plane as the cell bodies of NTS (Fig. 1A). This configuration allows the stimulating electrode to be placed on the visible ST at a sufficient distance from the recording area to minimize focal activation of local interneurons and interconnecting fibers (Bailey et al., 2008). Brief shocks (100 μs duration) to the ST evoked excitatory postsynaptic currents (ST-EPSCs) in the TH-EGFP neurons. As we have described previously, ST-EPSCs in TH-EGFP neurons were mediated by non-NMDA ionotropic glutamate receptors and had nearly invariant latencies, few failures, and substantial frequency-dependent amplitude depression