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Research report

Activation of 5-hyrdoxytryptamine 7 receptors within the rat nucleus tractus solitarii modulates synaptic properties

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ABSTRACT

Serotonin (5-HT) is a potent neuromodulator with multiple receptor types within the cardiorespiratory system, including the nucleus tractus solitarii (nTS) - the central termination site of visceral afferent fibers. The 5-HT_7 receptor facilitates cardiorespiratory reflexes through its action in the brainstem and likely in the nTS. However, the mechanism and site of action for these effects is not clear. In this study, we examined the expression and function of 5-HT_7 receptors in the nTS of Sprague-Dawley rats. 5-HT_7 receptor mRNA and protein were identified across the rostrocaudal extent of the nTS. To determine 5-HT_7 receptor function, we examined nTS synaptic properties following 5-HT_7 receptor activation in monosynaptic nTS neurons in the in vitro brainstem slice preparation. Application of 5-HT_7 receptor agonists altered tractus solitarii evoked and spontaneous excitatory postsynaptic currents which were attenuated with a selective 5-HT_7 receptor antagonist. 5-HT_7 receptor-mediated changes in excitatory postsynaptic currents were also altered by block of 5-HT_{1A} and $GABA_A$ receptors. Interestingly, 5-HT_7 receptor activation also reduced the amplitude but not frequency of $GABA_A$-mediated inhibitory currents. Together these results indicate a complex role for 5-HT_7 receptors in the nTS that mediate its diverse effects on cardiorespiratory parameters.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a potent neuromodulator in the cardiorespiratory system, with varying and sometimes contradictory effects. Currently there are 14 known 5-HT receptors (5-HTRs) in 7 sub-families. With the exception of 5-HT_3R, all of these receptors are G-protein coupled receptors that activate downstream pathways to elicit their physiological effects. The diverse effects of 5-HT are likely due, in part, to the many different 5-HTRs and their

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activated second messengers within the autonomic nervous and respiratory pathways.

The nucleus tractus solitarii (nTS) contains the first central synapses to receive visceral afferents from baroreceptors and peripheral chemoreceptors, is intrinsically O_2 and CO_2/pH sensitive, and has reciprocal connections to many central cardiorespiratory nuclei within the brainstem and forebrain (Accorsi-Mendonça and Machado, 2013; Dean, 2010; Kline, 2008; Matott et al., 2014). The nTS is densely innervated by 5-HT fibers originating from the ventral raphe (Thor and Helke, 1988), peripheral ganglia (Nosjean et al., 1990; Thor et al., 1988), and perhaps the nTS itself (Calzà et al., 1985). Several 5-HTRs have been anatomically and functionally identified in the nTS, including the 5-HT1, 2, 3, and 4 receptor subtypes (Raul, 2003). For instance, 5-HT_{1A}Rs are located postsynaptically on nTS neurons where they inhibit evoked and spontaneous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) and decrease respiration (Ostrowski et al., 2014). Activation of 5-HT_2R in the nTS lowers heart rate, blood pressure, and delays the hypoxic ventilatory response (Comet et al., 2007; Kanamaru and Homma, 2009), and activation of 5-HT_{2A}R and 5-HT_{2C}R augment nTS EPSCs (Austgen and Kline, 2013; Austgen et al., 2012). Presynaptic 5-HT_3Rs augment nTS neurotransmission by increasing spontaneous glutamate release (Cui et al., 2012) as well as inhibit cardiac reflex responses (Sévoz et al., 1997; Weissheimer and Machado, 2007). 5-HT_4Rs in the nTS attenuate cardiopulmonary reflexes (Edwards and Paton, 1999).

5-HT_7R, the most recently discovered 5-HT receptor (Bard et al., 1993; Lovenberg et al., 1993; Ruat et al., 1993; Shen et al., 1993), has been demonstrated via in situ hybridization throughout the CNS, including nTS (Gustafson et al., 1996). 5-HT_7R has been implicated to have functional or modulatory roles in respiratory (Hoffman and Mitchell, 2013, 2011; Nichols et al., 2012) and cardiovascular reflexes (Damaso et al., 2007; Jordan, 2005; Kellett et al., 2005a; Oskutyte et al., 2009). For example, block of 5-HT_7R within the brainstem of anesthetized rats attenuated the vagal-mediated bradycardia evoked by the cardiopulmonary, baro- and chemoreflexes (Kellett et al., 2005a). This was confirmed in conscious rats where brainstem 5-HT_7R blockade attenuated the bradycardia and pressor response to cardiopulmonary and peripheral chemoreceptor reflex activation (Damaso et al., 2007). The 5-$HT_{1/7}$ agonist (5-carboxamidotryptamine, 5-CT) ionophoresed directly onto nTS neurons resulted in a mix of excitatory and inhibitory responses, as well as non-responding neurons (Oskutyte et al., 2009). 5-CT induced neuronal excitation, as well as vagal-afferent induced activity, were reduced or eliminated by 5-HT_7R block. Taken together, these studies suggest 5-HT_7R within the brainstem enhance several cardiorespiratory reflexes by augmenting neuronal discharge. Given the prominent role of the nTS in integrating visceral reflexes and the identification of 5-HT_7R mRNA in this nucleus, the nTS is therefore a likely target for this effect. However, the synaptic mechanisms by which these 5-HT_7R mediated effects in the nTS may occur are largely unknown. The goal of the present study was to determine the functional role of 5-HT_7Rs on synaptic transmission in monosynaptic second-order nTS neurons.

2. Results

2.1. 5-HT_7R is present in the nucleus tractus solitarii

With the exception of one in situ hybridization study (Gustafson et al., 1996), the distribution or presence of 5-HT_7R within the nTS has not been described. Therefore, we used RT-PCR and immunoblot to examine the presence and distribution of 5-HT_7R mRNA and protein within the caudal nTS. 5-HT_7R mRNA was localized in the nTS and was significantly greater compared to the nodose-petrosal ganglia (NPG), the location of visceral afferent somas (Fig. 1A, $p=0.023$ paired t-test). There was not a significant difference in nTS mRNA expression relative to the cerebellum, where abundant 5-HT_7R has been shown (Geurts

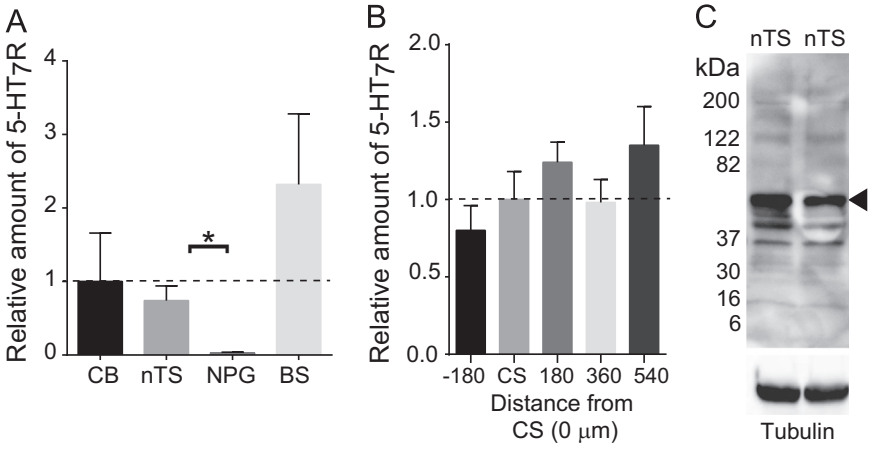

Fig. 1 – 5-HT_7R mRNA and protein are present in the nTS. (A) Relative 5-HT_7R mRNA levels normalized to cerebellum (CB) determined from RT-PCR. The amount of mRNA in the nTS is significantly higher than that found in the nodose-petrosal ganglia (NPG). General brainstem tissue (BS), excluding the nTS, was not different than cerebellum or nTS. ΔΔCT method, $n=5$ each. (B) Relative 5-HT_7R mRNA across the rostrocaudal nTS, normalized to calamus scriptorius (CS, 0 μm). ΔΔCT method, $n=3$ individual sections/level. (C) Immunoblot from nTS samples (100 μg) showing band for 5-HT_7R protein at expected 54 kDa (arrow). Tubulin loading control is shown below $n=2$.