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Swim stress differentially blocks CRF receptor mediated responses in dorsal raphe nucleus

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Summary Modulation of the serotonergic (5-HT) neurotransmitter system arising from the dorsal raphe nucleus (DR) is thought to support the behavioral effects of swim stress, i.e., immobility. *In vivo* pharmacological and anatomical studies suggest that corticotropin-releasing factor (CRF) and γ-aminobutyric acid (GABA) synaptic transmission closely interact to set the response of the DR to swim stress. To investigate the cellular basis of these physiological mechanisms the effects of ovine CRF (oCRF) on $GABA_A$-dependent miniature inhibitory postsynaptic currents (mIPSCs) in 5-HT and non-5-HT DR neurons in acute mesencephalic slices obtained from rats either naïve or 24 h after a 15 min swim stress session were tested. In this study, the effect of swim stress alone was to decrease the holding current, i.e., hyperpolarize the neuron, and to increase the amplitude and charge of mIPSCs recorded from non-5-HT neurons. Ovine CRF (10 nM) induced an increase in mIPSC frequency in 5-HT neurons recorded from naïve rats, an effect that was suppressed by swim stress. The inward current elicited by oCRF in both 5-HT and non-5-HT neurons was also blocked by swim stress. Ovine CRF increased mIPSCs amplitude and charge in both 5-HT and non-5-HT neurons, but this effect was not modified by swim stress. In concert with our previous findings that swim stress decreased input resistance, action potential threshold and action potential duration and increased glutamatergic synaptic activity the overall primary effect of swim stress is to increase the excitability of 5-HT neurons. These data provide a mechanism at the cellular level for the immobility induced by swim stress and identifies critical components of the raphe circuitry responsible for the altered output of 5-HT neurons induced by swim stress.

1. Introduction

The dorsal raphe nucleus (DR) is a major source of serotonergic (5-hydroxytryptamine, 5-HT) innervation of the mammalian forebrain (Azmitia and Segal, 1978; Jacobs and Azmitia, 1992; Mamounas et al., 1991). Dorsal raphe 5-HT projections are implicated in the physiological and behavioral responses to stressors and in the development of stress-related

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clinical conditions of mood disorders (Lowry, 2002; Lowry et al., 2005; Maier et al., 1993; Petrov et al., 1992).

The neuropeptide corticotrophin-releasing factor (CRF) is a key mediator of different aspects of stress responses including the activation of the HPA axis (Bale and Vale, 2004; Herman et al., 2003; Owens and Nemeroff, 1993). In addition CRF is thought to be involved in the response of the DR to certain stressors (Cooper and Huhman, 2007; Leonard, 2005; McEuen et al., 2008; Roche et al., 2003; Staub et al., 2005, 2006). Extensive evidence exists demonstrating the expression of CRF and its receptors CRF1 and CRF2 in the DR (Austin et al., 1997; Chalmers et al., 1995; Day et al., 2004; Van Pett et al., 2000). However, CRF expression is heterogeneous among the different DR subregions. CRF fibers in the caudal part of the DR are mainly located to the dorsal and lateral aspects of the nucleus while at a more rostral level these fibers are concentrated in the ventromedial DR (Austin et al., 2003; Kirby et al., 2000; Valentino et al., 2001). Detailed immunohistochemical labeling reveals that the rich CRF innervation of DR is primarily in contact with GABA-containing dendrites than with 5-HT dendrites in both the ventromedial and dorsolateral DR (Lowry et al., 2000; Waselus et al., 2005). Ultrastructural studies provide further evidence of synaptic specializations involving CRF-immunoreactive terminals and DR dendrites in the lateral wings of the DR (Valentino et al., 2001; Waselus et al., 2005).

The effects of CRF on DR are well documented from *in vivo* and *in vitro* pharmacological investigations in rodents. An intracerebroventricular (icv) injection of CRF decreases the firing rate of DR neurons. It also changes 5-HT release in projection areas with a region in a dose-dependent pattern (Kirby et al., 2000; Price et al., 1998; Price and Lucki, 2001). At low doses (1—10 ng) CRF decreases neuronal activity but at higher doses (30 ng) it has the opposite effect. The latter dose also results in a decrease of 5-HT release in both lateral septum and striatum (Kirby et al., 2000; Price et al., 1998; Price and Lucki, 2001). Corticotropin-releasing factor1 antagonists can reverse these effects indicating a role for this specific receptor (Kirby et al., 2000; Price and Lucki, 2001). Other studies provide evidence for a role for CRF2 neuromodulation as well. Urocortin 2 (Ucn2), a CRF2 agonist, activates c-fos expression in 5-HT neurons in the dorsal mid to caudal DR when injected icv (Staub et al., 2005). A microinjection of Ucn2 in DR at low doses results in a decrease of 5-HT neuron activity. At higher doses, the firing rate of 5-HT neurons increases whereas the firing rate of non-5-HT neurons decreases, leading to the hypothesis that there is disinhibition of 5-HT neurons (Pernar et al., 2004). Recently, our laboratory demonstrated that GABAergic synaptic activity is modified in the ventromedial (vmDR) DR by bath application of CRF. Activation of CRF1 receptors increases the frequency of GABA release and the amplitude of $GABA_A$ receptor mediated mIPSCs in 5-HT neurons. Activation of CRF2 also increases mIPSC amplitude and increases inward current in 5-HT containing neurons. In non-5-HT neurons, CRF1 receptors mediate an increase in inward current only (Kirby et al., 2008).

Corticotropin-releasing factor has an important role in the adaptation of DR to swim stress. Indeed, Price et al. (2002) show that swim stress reduces the ability of both subsequent swim stress and CRF icv to alter 5-HT release in lateral septum. Moreover, the administration of a CRF antagonist in the 24 h interval between the two swim stress sessions prevents the occurrence of adaptation to subsequent swim stress. At a cellular level, we previously demonstrated that swim stress has significant effects on active and passive cellular characteristics, glutamatergic EPSC synaptic activity and $5\text{-}HT_{1B}$ receptor mediated inhibition of EPSC activity in the DR that was neurochemically specific (Kirby et al., 2007). In 5-HT neurons, input resistance, action potential threshold and action potential duration decreases, glutamatergic EPSC frequency increases, EPSC amplitude decreases and the $5\text{-}HT_{1B}$ mediated inhibition of EPSC activity increases. In non-5-HT neurons swim stress only decreases EPSC amplitude (Kirby et al., 2007). In this paper we further our investigation of swim stress induced alterations in DR physiology. We test the hypothesis that swim stress decreases both GABAergic synaptic activity on 5-HT neurons and its modulation by CRF.

2. Materials and methods

2.1. Animals and stress experiments

Male Sprague—Dawley rats (75—150 g; Taconic Farms, Germantown, NY) were housed 3 per cage with a 12 h/12 h light/dark cycle, lights on at 7:00 am, with free access to food and water. Animals were randomized for treatment group. Swim stress was performed using procedures similar to those previously described elsewhere (Porsolt et al., 1977a,b; Roche et al., 2003). Swim stress was conducted in the morning so that 24 h later the rats could be sacrificed for the electrophysiology experiments. This procedure that has been used previously by many laboratories, produces extensive immobility in the rats when tested 24 h later (Porsolt et al., 1977a,b; Kirby and Lucki, 1998; Price et al., 2002). Rats were placed in a large glass cylinder filled with water at room temperature ($25 \pm 1\ ^\circ C$) and left to swim for 15 min. They were then towel dried, allowed to recover for 15 min in a warming cage containing a heating pad (37 °C) and returned to their home cages. Naïve controls remained unhandled in their home cages. All experiments on animals were conducted in accordance to the NIH Guide for the Care and Use of Laboratory Animals and following protocols approved by the Institutional Animal Care and Use Committee.

2.2. Slice preparation

Twenty-four hours after the swim stress session, brain slices were prepared as previously described (Beck et al., 2004; Lemos et al., 2006). Animals were rapidly decapitated and the brain placed in ice-cold artificial cerebrospinal fluid (ACSF) in which sucrose (248 mM) was substituted for NaCl. Coronal slices 200 μm thick were cut from the mesencephalic region with a Leica VT1000S vibroslicer (Leica, Allendale, NJ) and placed in a holding vial containing ACSF bubbled with 95% O_2/5% CO_2. They were allowed to rest at 34 °C for 1 h and then kept at room temperature until used for recording. The composition of the ACSF was (mM): NaCl 124, KCl 2.5, NaH_2PO_4 1.25, $MgSO_4$ 2.0, $CaCl_2$ 2.5, dextrose 10 and $NaHCO_3$ 26, pH 7.25.

2.3. Electrophysiological recordings

Slices were immersed in a recording chamber continuously superfused with ACSF at 32 °C and bubbled with 95% O_2/5%