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5-HT_{1B} AUTORECEPTORS DIFFERENTIALLY MODULATE THE EXPRESSION OF CONDITIONED FEAR IN A CIRCUIT-SPECIFIC MANNER

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Abstract—Located in the nerve terminals of serotonergic neurons, 5-HT_{1B} autoreceptors are poised to modulate synaptic 5-HT levels with precise temporal and spatial control, and play an important role in various emotional behaviors. This study characterized two novel, complementary viral vector strategies to investigate the contribution of 5-HT_{1B} autoreceptors to fear expression, displayed as freezing, during contextual fear conditioning. Increased expression of 5-HT_{1B} autoreceptors throughout the brain significantly decreased fear expression in both wild-type (WT) and 5-HT_{1B} knockout (1BKO) mice when receptor levels were increased with a cell-type-specific herpes simplex virus (HSV) vector injected into the dorsal raphe nucleus (DRN). Additional studies used an intersectional viral vector strategy, in which an adeno-associated virus containing a double-floxed inverted sequence for the 5-HT_{1B} receptor (AAV-DIO-1B) was combined with the retrogradely transported canine adenovirus-2 expressing Cre (CAV-Cre) in order to increase 5-HT_{1B} autoreceptor expression only in neurons projecting from the DRN to the amygdala. Surprisingly, selective expression of 5-HT_{1B} autoreceptors in just this circuit led to an increase in fear expression in WT, but not 1BKO, mice. These results suggest that activation of 5-HT_{1B} autoreceptors throughout the brain may have an overall effect of attenuating fear expression, but activation of subsets of 5-HT_{1B} autoreceptors in particular brain regions, reflecting distinct projections of serotonergic neurons from the DRN, may have disparate contributions to the ultimate response.

Key words: serotonin, dorsal raphe, amygdala, viral vector, adeno-associated virus, canine adenovirus-2.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter that is involved in a wide variety of emotional and cognitive behaviors; dysfunction of the serotonergic system is implicated in psychiatric illnesses such as depression, post-traumatic stress disorder, and anxiety. Serotonergic neurons in the brain have cell bodies that reside in the raphe nuclei of the midbrain and brainstem but project extensively to nearly every brain region (Jacobs and Azmitia, 1992). The dorsal raphe nucleus (DRN) provides most of the serotonin fibers to the forebrain and is somatotopically organized; subpopulations of serotonergic neurons that have unique anatomical connections may also impact different elements of complex emotional behavior (Lowry et al., 2008; Gaspar and Lillesaar, 2012).

5-HT_{1B} receptors are metabotropic $G\alpha_{i/o}$ receptors (Bouhelal et al., 1988; Schoeffter and Hoyer, 1989) that are primarily localized to nerve terminals (Boschert et al., 1994; Ghavami et al., 1999; Riad et al., 2000) and exist both as autoreceptors on serotonergic neurons and as heteroreceptors on nonserotonergic cells (Hen, 1992). Since 5-HT_{1B} autoreceptors are more strongly activated during bouts of intense serotonergic activity at the sites of release, they provide localized autoregulation of serotonin neurotransmission (Sari, 2004). Several lines of evidence suggest that the level of 5-HT_{1B} autoreceptor expression is a key determinant of stress reactivity (Neumaier et al., 2002a; Kaiyala et al., 2003), and several studies suggest that drugs targeting the 5-HT_{1B} receptor have promise as adjunctive therapy with selective serotonin reuptake inhibitors (SSRIs) (Roberts et al., 1999; Gardier, 2009; Ruf and Bhagwagar, 2009).

Differentiating between 5-HT_{1B} autoreceptors and 5-HT_{1B} heteroreceptors is crucial in revealing their individual roles in brain circuits and behavior but poses a technical challenge. 5-HT_{1B} autoreceptors and heteroreceptors are identical in sequence and structure but are expressed in different neuron types. 5-HT_{1B} autoreceptors are diffusely distributed at nerve terminals throughout the entire brain, but are intermixed with 5-HT_{1B} heteroreceptors in most brain regions. Therefore, it is difficult to target 5-HT_{1B} autoreceptors exclusively using conventional methods like drug administration, as 5-HT_{1B} heteroreceptors will also be affected. The 5-HT_{1B} knockout mice currently available for research have a constitutive deletion of the gene, and while they have a remarkable phenotype with decreased anxiety and increased aggression (Gingrich and Hen, 2001;

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Abbreviations: 1BKO, 5-HT_{1B} receptor constitutive knockout mouse; AAV, adeno-associated virus; CAV, canine adenovirus; DIO, double-floxed inverted open reading frame; DRN, dorsal raphe nucleus; HA, hemagglutinin; HSV, herpes simplex virus; NGS, normal goat serum; ORF, open reading frame; SERT, serotonin transporter; Tph, tryptophan hydroxylase; WT, wild type.

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Groenink et al., 2003; Guilloux et al., 2011), it is unclear whether these behavioral effects relate to the loss of 5-HT_{1B} autoreceptors or heteroreceptors during early development or adulthood. One approach for manipulating 5-HT_{1B} autoreceptor function is via systemic injections of the brain-penetrant agonist CP-94,253 at low doses, which preferentially activates autoreceptors over heteroreceptors (Sarhan et al., 2000; McDevitt et al., 2011); however, it is possible that heteroreceptors may also be affected even at low doses, tending to confound any conclusions based solely on pharmacological experiments. An alternative technique utilizes viral-mediated gene transfer (Clark et al., 2002, 2004; McDevitt et al., 2011; Hagan et al., 2012) to target neurons residing in a particular brain region.

Novel recombinant viral vectors are continuously developed to achieve greater control over transgene expression levels and cell-typespecificity (Lowenstein and Castro, 2002; Luo et al., 2008; Bouard et al., 2009; Papale et al., 2009; Zhang et al., 2010). Herpes simplex virus (HSV) has been commonly used for local manipulation of expression in specific brain targets and is advantageous in offering neuron-specific gene expression with a low immune and inflammatory response (Papale et al., 2009). In recent years, adeno-associated viruses (AAVs) with a double-floxed inverted open reading frame (DIO) have gained popularity. In these constructs, the transgene is inserted in the antisense orientation and flanked by two sets of lox sites; when Cre recombinase is present, an inversion occurs at the lox sites to flip and lock the transgene into the sense orientation (Atasoy et al., 2008). AAV-DIO viruses may be used in combination with transgenic animals expressing Cre in a cell-type-specific manner or with retrogradely transported, Cre-expressing canine adenovirus (CAV-Cre) to achieve greater specificity of transgene expression (Schnutgen et al., 2003; Saunders et al., 2012; Nair et al., 2013).

Previous work in our lab has used the neuron-specific HSV to increase 5-HT_{1B} receptor expression in the rat DRN and demonstrated that viral-mediated 5-HT_{1B} receptor expression localized correctly to axon terminals throughout the brain and increased autoreceptor activity (Clark et al., 2002, 2004; Hagan et al., 2012). This technique has permitted *in vivo* studies to elucidate the specific contribution of 5-HT_{1B} autoreceptors to emotional behaviors including fear, anxiety, depression, and stress. In rats, increasing expression of 5-HT_{1B} receptors in neurons in mid-rostrocaudal DRN decreased anxiety in the open field test, as well as fear potentiation of the startle response (Clark et al., 2002, 2004), but when the caudal DRN was targeted instead, expression of transgenic 5-HT_{1B} receptors had no effect on anxiety (McDevitt et al., 2011). Additionally, overexpression of 5-HT_{1B} receptors decreased fear expression in contextual fear conditioning but had no effect on acquisition; similarly, context-paired administration of a low dose of CP-94,253 to preferentially activate 5-HT_{1B} autoreceptors showed the same decrease in fear. Taken together, these data suggest a protective role of 5-HT_{1B} autoreceptors in these emotional behaviors, with differences along the rostrocaudal axis. Interestingly, these anxiolytic and fear-attenuating properties of 5-HT_{1B} autoreceptors are abolished when animals were exposed to stress or received the 5-HT_{1B} antagonist SB224289 prior to behavioral testing (Clark et al., 2002, 2004; McDevitt and Neumaier, 2011).

While it has been suggested that conditioned aversive stimuli activate the serotonergic system, which, in turn, projects to a variety of brain regions to elicit different behavioral responses (Deakin and Graeff, 1991), the specific circuits involved are still unknown. The amygdala is a brain area that receives strong serotonergic projections and is heavily implicated in fear and anxiety behaviors (Davis, 1992; Ebner et al., 2004; Lowry et al., 2005; Ciocchi et al., 2010; Haubensak et al., 2010; Herry et al., 2010; Johansen et al., 2010). Although approximately 10% of serotonergic neurons in the DRN project to the amygdala, nearly all of the neurons that project from the DRN to the amygdala are serotonergic (Ma et al., 1991); within the amygdala, serotonergic innervation is densest in the basolateral amygdala and more diffuse in the central amygdala (Vertes, 1991). However, it is still unclear how the serotonergic system acts in the amygdala in fear-related responses. One hypothesis proposed that the DRN-to-amygdala circuit is important for eliciting fear and anticipatory anxiety, while the DRN-to-periaqueductal gray circuit is important for preventing inappropriate freezing or fight-or-flight responses (Deakin and Graeff, 1991; Graeff et al., 1996). Because 5-HT_{1B} autoreceptors modulate serotonergic neurotransmission locally at the site of release, it is important to understand their function in specific circuits, as this may lead to greater insight about the role of serotonin in various brain regions.

To examine the selective contribution of 5-HT_{1B} autoreceptors to emotional behaviors, we designed and utilized two novel viral vectors. First, to overexpress 5-HT_{1B} autoreceptors in nerve terminals throughout the brain, we employed an HSV viral vector using the serotonin transporter (SERT) promoter to induce transgene expression only in serotonergic cells. Second, to overexpress 5-HT_{1B} autoreceptors in nerve terminals in one particular circuit, we used a conditional AAV-DIO viral vector in combination with CAV-Cre viruses or cell-type-specific Cre driver mouse lines, such as Pet1-Cre mice that use the serotonergic marker Pet-1 to selectively drive Cre expression. We harnessed these complementary techniques to further elucidate the role of 5-HT_{1B} autoreceptors in contextual fear conditioning. We hypothesized that 5-HT_{1B} autoreceptors attenuate fear expression but that their contribution to contextual fear conditioning is dependent on the specific circuit in which the 5-HT_{1B} autoreceptors are expressed.

EXPERIMENTAL PROCEDURES

Vector construction

To create a serotonin-specific viral gene expression system, we utilized a plasmid containing the 1.7-kb fragment of the human SERT promoter, which was received as a generous gift from Dr. Ove Wiborg. In rat raphe precursor cells, the 1250 bp proximal to the SERT gene shows the greatest promoter/enhancer activity