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PRESYNAPTIC $GABA_B$ RECEPTORS DECREASE NEUROTRANSMITTER RELEASE IN VESTIBULAR NUCLEI NEURONS DURING VESTIBULAR COMPENSATION

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Abstract—Unilateral damage to the peripheral vestibular receptors precipitates a debilitating syndrome of oculomotor and balance deficits at rest, which extensively normalize during the first week after the lesion due to vestibular compensation. *In vivo* studies suggest that $GABA_B$ receptor activation facilitates recovery. However, the presynaptic or postsynaptic sites of action of $GABA_B$ receptors in vestibular nuclei neurons after lesions have not been determined. Accordingly, here presynaptic and postsynaptic $GABA_B$ receptor activity in principal cells of the tangential nucleus, a major avian vestibular nucleus, was investigated using patch-clamp recordings correlated with immunolabeling and confocal imaging of the $GABA_B$ receptor subunit-2 ($GABA_BR2$) in controls and operated chickens shortly after unilateral vestibular ganglionectomy (UVG). Baclofen, a $GABA_B$ agonist, generated no postsynaptic currents in principal cells in controls, which correlated with weak $GABA_BR2$ immunolabeling on principal cell surfaces. However, baclofen decreased miniature excitatory postsynaptic current (mEPSC) and GABAergic miniature inhibitory postsynaptic current (mIPSC) events in principal cells in controls, compensating and uncompensated chickens three days after UVG, indicating the presence of functional $GABA_B$ receptors on presynaptic terminals. Baclofen decreased GABAergic mIPSC frequency to the greatest extent in principal cells on the intact side of compensating chickens, with concurrent increases in $GABA_BR2$ pixel brightness and percentage overlap in synaptotagmin 2-labeled terminals. In uncompensated chickens, baclofen decreased mEPSC frequency to the greatest extent in principal cells on the intact side, with concurrent increases in $GABA_BR2$ pixel brightness and percentage overlap in synaptotagmin 1-labeled terminals. Altogether, these results revealed changes in presynaptic $GABA_B$ receptor function and expression which differed in compensating and uncompensated chickens shortly after UVG. This work supports an important role for $GABA_B$ autoreceptor-mediated inhibition in vestibular nuclei neurons on the intact side during early stages of vestibular compensation, and a role for $GABA_B$ heteroreceptor-mediated inhibition of glutamatergic terminals on the intact side in the failure to recover function.

Key words: vestibular deafferentation, synaptic plasticity, presynaptic receptors, excitation, inhibition.

INTRODUCTION

Gamma-amino-butyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain with multiple important and diverse functions during development (Ben-Ari, 2002; Owens and Kriegstein, 2002; Lujan et al., 2005) and after lesions in sensory systems (Hendry and Jones, 1986, 1988; Akhtar and Land, 1991; Rosier et al., 1995; Fuchs and Salazar, 1998; Moore et al., 2002) (for review see, Caspary et al., 2008). The two major types of GABA receptors, $GABA_A$ and $GABA_B$, are found on presynaptic and postsynaptic membranes. $GABA_A$ receptors function mainly as ligand-gated chloride channels generating fast synaptic inhibition, while $GABA_B$ receptors are G-protein-coupled metabotropic receptors which produce slow, prolonged inhibition. Presynaptic $GABA_B$ receptors form two functional subtypes, $GABA_B$ autoreceptors on GABAergic terminals and $GABA_B$ heteroreceptors positioned on non-GABAergic terminals (for review, see Bettler and Tiao, 2006).

The vestibular nuclei are composed of a diverse assortment of second-order vestibular neurons, with some of them functioning as reflex projection neurons transmitting signals from the peripheral vestibular sensory epithelium to motor neurons in the vestibular reflex pathways. In a wide range of species, GABA-mediated inhibition plays a critical role in regulating signal processing in the vestibular nuclei, since these neurons receive massive GABAergic inputs from the cerebellum, contralateral vestibular nuclei, inferior olivary nucleus and local interneurons (de Waele et al.,

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Abbreviations: ACSF, artificial cerebrospinal fluid; C1, C2, first cervical and second cervical spinal cord segments; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EGTA, ethylene glycol tetraacetic acid; GABA, gamma-amino-butyric acid; $GABA_BR2$, $GABA_B$ receptor subunit-2; Hepes, hydroxyethyl piperazineethanesulfonic; MAP2, microtubule-associated protein 2; mEPSCs, miniature excitatory postsynaptic currents; mIPSCs, miniature inhibitory postsynaptic currents; MVN, medial vestibular nucleus; NGS, normal goat serum; PBS, phosphate-buffered saline; PF, paraformaldehyde; Syt1, synaptotagmin 1; Syt2, synaptotagmin 2; TTX, tetrodotoxin; UL, unilateral labyrinthectomy; UVG, unilateral vestibular ganglionectomy; VOR, vestibuloocular reflex.

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1995; Highstein and Holstein, 2006). Both $GABA_A$ and presynaptic and postsynaptic $GABA_B$ receptors are expressed in the mammalian vestibular nuclei (Gallagher et al., 1992; Holstein et al., 1992; Vibert et al., 1995; Eleore et al., 2005; Gliddon et al., 2005; Heskin-Sweezie et al., 2010). The tangential nucleus represents a major avian vestibular nucleus, composed primarily of the principal cells (80%), which are distinguished by large oval, glutamatergic cell bodies aligned in rows between the primary vestibular fibers near the lateral surface of the medulla oblongata (Peusner and Morest, 1977; Popratiloff and Peusner, 2011). The neurotransmitter phenotypes of the elongate (19%) and giant cells (1%) in the tangential nucleus have not been identified. Principal cells receive multiple inputs from diverse sources, including excitatory input from the canals, otoliths and high cervical spinal cord (C1–C2) (Gross, 1985; Cox and Peusner, 1990; Sato et al., 1997; Popratiloff et al., 2004). Although no local GABAergic or glycinergic interneurons are found in the tangential nucleus (Popratiloff and Peusner, 2011), the principal cells receive GABAergic inputs from the cerebellar flocculus. The neurotransmitter phenotype of inputs from the contralateral medial vestibular nucleus (MVN) (Cox and Peusner, 1990) has not been established. All the principal cells are vestibular reflex projection neurons whose axons project to the oculomotor nuclei and/or cervical spinal cord (Wold, 1978; Cox and Peusner, 1990; Gottesman-Davis and Peusner, 2010). Thus, the tangential nucleus differs from most vestibular nuclei in that their major output neurons have a distinctive morphology and all of them function as projection neurons within the vestibular reflex pathways. Previous amino acid immunolabeling studies reveal that GABAergic terminals compose about half of the synaptotagmin 1 (Syt1)-labeled terminals in the tangential nucleus (Popratiloff and Peusner, 2011). In voltage-clamp recordings, both $GABA_A$ and $GABA_B$ receptor-mediated currents are generated in principal cells from brain slices of 16-day-old chick embryos (E16) (Shao et al., 2003).

Unilateral peripheral vestibular lesions precipitate a complex syndrome of oculomotor and balance deficits (patients: Badke et al., 2002; chicken: Aldrich and Peusner, 2002; Shao et al., 2009, 2012) due to dysfunction of the vestibular sensorimotor reflex pathways (Llinas and Walton, 1979). In different species, the static symptoms usually normalize extensively during the first week after the lesion due to a process called vestibular compensation (Smith and Curthoys, 1989; Halmagyi et al., 2010). Immediately after unilateral vestibular ganglionectomy (UVG), four-day-old hatchling (H4) chickens show severe static symptoms, which generally start to recover by three days (Aldrich and Peusner, 2002). However, a significant portion of the operated chickens fails to recover by three days, the uncompensated chickens (47–48%; Shao et al., 2009, 2012). Presently, vestibular compensation is thought to involve functional reorganization of the synaptic connections in broadly distributed regions of the central nervous system which process vestibular signals, including the vestibular nuclei, cerebellum, inferior olivary nucleus, spinal cord, and visual system (Llinas and Walton, 1979; for review, see Dieringer, 1995). Research has focused on the vestibular nuclei because they must be intact for recovery to occur (Spiegel and Demetriades, 1925; Precht et al., 1966). While the exact cellular mechanisms underlying vestibular compensation are uncertain, it is thought that recovery from the static symptoms involves rebalancing the overall excitability in vestibular nuclei neurons bilaterally (for review, see Beraneck and Idoux, 2012; Lambert and Straka, 2012; Peusner et al., 2012). Major changes in the frequency and kinetics of glutamatergic and GABAergic spontaneous synaptic events occur in the vestibular nuclei neurons after peripheral vestibular lesions, suggesting that both presynaptic and postsynaptic factors participate in the recovery (Shao et al., 2012). Collectively, these properties must regulate rebalancing of neuronal excitability. However, the factors leading to changes in frequency and kinetics of spontaneous synaptic events are uncertain.

In general, behavioral studies support an important role for $GABA_B$ receptors in vestibular compensation, since systemic injection of the specific $GABA_B$ agonist, baclofen, decreases spontaneous nystagmus in rats after unilateral labyrinthectomy (UL) (Magnusson et al., 2000, 2002) and accelerates recovery of the postural reflexes in mice (Heskin-Sweezie et al., 2010). At present, the effect of $GABA_B$ receptors on specific excitatory and inhibitory inputs contacting vestibular reflex projection neurons has not been investigated after lesions. Accordingly, here the presynaptic or postsynaptic action of $GABA_B$ receptors in principal cells of the chick tangential nucleus were studied and distinguished unambiguously before and after UVG by correlating patch-clamp recordings with immunolabeling and high-resolution confocal imaging of these receptors.

EXPERIMENTAL PROCEDURES

Experimental animals

Cold, freshly fertilized, white Leghorn chick eggs (*Gallus gallus*) were purchased from CBT Farms (Chestertown, MD) or Charles River Spafas (North Franklin, CT) and maintained until hatching in an egg incubator equipped with circulating air, egg rotation unit, and temperature and humidity controls (model 1502, G.Q.F. Manufacturing Co., Savannah, GA). Twenty-four hours after hatching, chickens were identified as one-day-old hatchlings (H1). The chickens were housed in plastic cages equipped with heating lamps at the University's Animal Research Facility. The experiments were performed in accordance with protocols approved by the Institution of Animal Care and Use Committee of the George Washington University. The experiments also conformed to the guidelines of the National Research Council (2003) for the care and use of animals in research. Considerable care was taken to minimize the number of animals used in the experiments and their suffering.

These structure/function studies were performed on a total of 112 chickens. The electrophysiological experiments were undertaken on 97 chickens, including controls (n = 18),