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Research report

Effect of estrogen on vagal afferent projections to the brainstem in the female

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ABSTRACT

The effects of 17β-estradiol (E) on the distribution and density of brainstem projections of small or large diameter primary vagal afferents were investigated in Wistar rats using transganglionic transport of wheat germ agglutinin- (WGA; preferentially transported by non-myelinated afferent C-fibers; 2%), or cholera toxin B-subunit- (CTB, 5%; preferentially transported by large myelinated afferent A-fibers) conjugated horseradish peroxidase (HRP) in combination with the tetramethylbenzidine method in age matched ovariectomized (OVX) only or OVX and treated with E (OVX+E; 30 pg/ml plasma) females for 12 weeks. Additionally, these projections were compared to aged matched males. Unilateral micro-injection of WGA-HRP into the nodose ganglion resulted in dense anterograde labeling bilaterally, with an ipsilateral predominance in several subnuclei of the nucleus of the solitary tract (NTS) and in area postrema that was greatest in OVX+E animals compared to OVX only and males. Moderately dense anterograde labeling was also observed in paratrigeminal nucleus (PAT) of the OVX+E animals. CTB-HRP produced less dense anterograde labeling in the NTS complex, but had a wider distribution within the brainstem including the area postrema, dorsal motor nucleus of the vagus, PAT, the nucleus ambiguus complex and ventrolateral medulla in all groups. The distribution of

Abbreviations: 4V, 4th ventricle; 10N, vagus nerve; 12M, hypoglossal nucleus; ap, area postrema; Ambc, nucleus ambiguus, pars compacta; Ambv, nucleus ambiguus, pars ventralis; cc, central canal; Com, commissural subnucleus of NTS complex; CS, calamus scriptorius; Cu, cuneate nucleu; cuf, cuneate fasciculus; CVLM, caudal ventrolateral medulla; dcs, dorsal corticospinal tract; dsc, dorsal spinocerebellar tract; DMV, dorsal motor nucleus of the vagus; DMSp5, spinal nucleus of the trigeminal, dorsomedial part; E, 17β-estradiol (1,3,5[10] Estradiene-3,17β-diol); ECu, external cuneate nucleus; FN, facial nucleus; GR, gracile nucleus; GRN, gigantocellular reticular nucleus; icp, inferior cerebellar peduncle; ION, inferior olive nucleus; In, nucleus intercalatus; LRt, lateral reticular nucleus; MdD, medullary reticular nucleus, dorsal part; mlf, medial longitudinal fasciculus; MvD, medullary reticular nucleus, ventral part; MV, medial vestibular nucleus; NTS, nucleus of the solitary tract; NIS, nucleus intercalatus; OVX, ovariectomy; PAT, paratrigeminal nucleus; PeHA, Perihypoglossal area; PGRNl, paragigantocellular reticular nucleus, lateral part; PRP, nucleus prepositus; py, pyramidal tract; pyd, pyramidal decussatio; rs, rubrospinal tract; RVLM, rostral ventrolateral medulla; Sc, central subnucleus of NTS complex; Sdl, dorsolateral subnucleus of NTS complex; Sg, subnucleus gelatinosus of NTS complex; Slnt, intermediate subnucleus of NTS complex; Sm, medial subnucleus of NTS complex; Sni, interstitial subnucleus; sp5, spinal tract of the trigeminal nerve; Sp5C, spinal nucleus of the trigeminal, caudal part; Sp5I, spinal nucleus of the trigeminal, interpolar part; St, solitary trac; Svl, ventrolateral subnucleus of NTS complex; TMB, tetramethylbenzidine; VLM, ventrolateral medulla; vsc, ventral spinocerebellar tract; X, nucleus X

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CTB-HRP anterograde labeling was densest in OVX+E, less dense in OVX only females and least dense in male rats. Little, if any, labeling was found within PAT in males using either WGA-or CTB-HRP. Taken together, these data suggest that small, non-myelinated (WGA-labeled) and large myelinated (CTB-labeled) diameter vagal afferents projecting to brainstem autonomic areas are differentially affected by circulating levels of estrogen. These effects of estrogen on connectivity may contribute to the sex differences observed in central autonomic mechanisms between gender, and in females with and without estrogen.

1. Introduction

The vagus nerve, composed of both efferent parasympathetic fibers and afferent sensory fibers, is well known to innervate a number of visceral organs that support cardiovascular, respiratory and gastrointestinal functions (Berthoud and Neuhuber, 2000). In addition, more recent studies have used stimulation of vagal afferent fibers in the treatment or prevention of a number of physiological and psychological disorders that include anxiety, depression, epilepsy, seizures, eating and cardiovascular disorders (Albert et al., 2015; Berntson et al., 2003; Chang et al., 2013; Galbarriatu et al., 2015; Hampel et al., 2015; Kawada et al., 1985; Klarer et al., 2014; Pardo et al., 2007; Rubio et al., 2014; Sudakov et al., 2012). These new experimental approaches to the treatment of disorders through vagal stimulation are based on the extensive anatomical and electrophysiological studies of the central projections of vagal afferent fibers. Vagal afferent projections have been studied in a variety of species using electrophysiological methods (Hermann et al., 1983; Ito, 1994; Kalia and Richter, 1985; Nosaka, 1986; Rudomin 1968; Yuan and Barber, 1993), axonal degeneration (Dubbeldam et al., 1979; Torvick, 1956) and autoradiograhic tract-tracing techniques (Beckstead and Norgren, 1979; Contreras et al., 1982), injections of biotinylated dextran amine (Shin et al., 2009), and the transganglionic transport of horseradish peroxidase (HRP) (Altschuler et al., 1989; Barry, 1987; Chazal et al., 1991; Chernicky et al., 1984; Ciriello et al.,, 1981; Culberson and Kimmel, 1972; Fitzakerley and Lucier, 1988; Gwyn et al., 1985; Hamilton and Norgren, 1984; Hermann et al., 1983; Kalia and Mesulam, 1980; Kalia and Sullivan, 1982; Katz and Karten, 1983; Lazar et al., 1992; Leslie et al., 1982; Maqbool et al., 1991; Pugh and Kalia, 1982; Ranson et al., 1993; Robertson et al., 1992; Rogers and Hermann, 1983; Scharoun et al., 1984; Shin and Loewy, 2009; Strain et al., 1990; Stuesse et al., 1984; Torrealba and Calderón, 1990; Wild et al., 1991; Xie et al., 1999).

The use of HRP as either a retrograde and/or anterograde tract-tracer has become one of the most frequently used neuroanatomical tract-tracing tools within both the peripheral and central nervous systems (Köbbert et al., 2000; Lanciego and Wouterlood, 2011; Mesulam, 1982; Ralston, 1990; van der Want et al., 1997; Wu et al., 2003). The advancement of the HRP tract-tracing technique following the conjugation of the free HRP to a variety of ligands such as wheat germ agglutinin (WGA) and cholera toxin B-subunit (CTB) (Köbbert et al., 2000; Mesulam, 1982; Mesulam and Rosene, 1979; Miceli and Malsbury, 1985; Ralston, 1990; Trojanowski and Schmidt, 1984; Wan et al., 1982, 1982b; Wang et al., 1998; Wu et al., 1999) has resulted in an enhanced anterograde tract-tracer that has been used extensively to elucidate neuronal connectivity (Gonatas et al., 1979; Mesulam, 1978; Stoeckel et al., 1977; Trojanowski et al., 1982; Trojanowski and Schmidt, 1984; Wan et al., 1982). Furthermore, the use of these HRP conjugates in combination with the use of the chromogen tetramethylbenzidine (TMB) for the visualization of the HRP has further increased the sensitivity of this neuronal tract-tracer (Mesulam, 1978; Mesulam and Rosene, 1979; Mesulam et al., 1980).

HRP normally is internalized by fluid phase endocytosis into neuronal cell bodies and damaged axons and dendritic processes as it has no specific affinity for neuronal cell surfaces (Silverstein et al., 1977). On the other hand, lectins and CTB undergo adsorptive endocytosis. WGA and CTB, after binding to cell surface specific oligosaccharides on the neuronal membrane (Gonatas, 1979; Gonatas et al., 1979; Pugh and Kalia, 1982; Robertson and Grant, 1985; Robertson et al., 1992; Trojanowski et al., 1981a, 1981b, 1982; Trojanowski and Schmidt, 1984; Wan et al., 1982), is followed by adsorptive endocytosis of the ligands into Golgi-endoplasmic reticulum-lysosome (Gonatas, 1979; Gonatas et al., 1979; Harper et al., 1980; Pugh and Kalia, 1982; Robertson and Grant, 1985; Robertson et al., 1992; Trojanowski et al., 1981a, 1981b, 1982; Trojanowski and Schmidt, 1984; Wan et al., 1982). The studies by Robertson and colleagues (Robertson and Grant, 1985; Robertson et al., 1992) have also provided evidence suggesting that WGA-HRP and CTB-HRP can be used to selectively map projections of different afferent fiber types within the peripheral nervous system. They, and others demonstrated that WGA-HRP was preferentially incorporated into small diameter non-myelinated C-fibers, while CTB-HRP was more preferentially transported by the larger diameter myelinated A-fibers (LaMotte et al., 1991; Kitchener et al., 1994; Robertson and Grant, 1985; Robertson et al., 1992). This is thought to be due to the finding that WGA has an affinity for the carbohydrate residues N-acetyl-D-glucosamine and sialic acid found on non-myelinated C-fibers (Nagata and Burger, 1974; Robertson and Grant, 1985), while CTB binds to the GM1 ganglioside (Cuatrecasas, 1973; Robertson and Grant, 1985) present only on myelinated fibers (Robertson and Grant, 1985).