Immunohistochemical demonstration of regionally selective projections from locus coeruleus to the vestibular nuclei in rats

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Summary. This study describes a regionally selective projection of tyrosine hydroxylase and dopamine β-hydroxylase-immunoreactive fibers from locus coeruleus (LC) and the A4 region of nucleus subcoeruleus to the vestibular nuclear complex in Long-Evans and Sprague-Dawley rats. These fibers travel in two distinct pathways. A lateral descending noradrenergic bundle provides input from LC to the superior vestibular nucleus (SVN), the cochlear nuclei, and the cerebellar cortex. A medial descending noradrenergic bundle provides input to the lateral vestibular nucleus (LVN), medial vestibular nucleus (MVN), and the inferior vestibular nucleus (IVN) before continuing on to the cochlear and cerebellar nuclei. The terminal plexus of these fibers varies markedly across these vestibular nuclear regions. Immunoreactive axons form a dense plexus around somata and proximal dendrites of Deiters’ neurons in dorsal LVN. The axon plexus is less dense in SVN and ventral LVN, and relatively sparse in MVN and IVN. This regional selectivity of noradrenergic innervation suggests that central adrenergic systems may selectively modulate vestibulospinal reflexes at the level of the vestibular nuclear complex.

Key words: Vestibular nuclei – Locus coeruleus – Dopamine β-hydroxylase – Tyrosine hydroxylase – Rat

Introduction

Locus coeruleus (LC) and nucleus subcoeruleus are a major source of noradrenergic innervation throughout the neuraxis (Dahlström and Fuxe 1964; Fuxe 1965; Pickel et al. 1974; Swanson and Hartman 1975; Moore and Bloom 1979; Foote et al. 1983; Moore and Card 1984). Activity of these adrenergic neurons has been linked with arousal and attention, affective disorders, learning and memory, sleep, anxiety, nociception, and cerebral blood flow (Remond et al. 1976; Aston-Jones et al. 1984; Charney et al. 1990). Neuronal spike activity in LC increases during tasks requiring attention to sensory stimuli and decreases during internally oriented activities such as feeding, grooming, and sleeping (Foote et al. 1983; Aston-Jones et al. 1984; Jacobs 1986). There is also evidence that noradrenergic input from LC is involved in vestibular information processing. Neurons in LC receive projections from the vestibular nuclei (Cedarbaum and Aghajanian 1978; Fung et al. 1987b) and respond to natural stimulation of both the vestibular end organs and neck proprioceptors (Barnes et al. 1989; Manzoni et al. 1989; Pompeiano et al. 1990). In addition, unilateral LC lesions produce asymmetric postural responses in decerebrate cats (D’Ascanio et al. 1989a). Thus, it has been suggested that adrenergic activity is an important contributor to the performance of vestibulospinal pathways (D’Ascanio et al. 1989a,b; Pompeiano 1989; Pompeiano et al. 1990).

There is a relative paucity of information about the sites of action of norepinephrine (NE) in vestibular pathways. It has been proposed that adrenergic influences on vestibulospinal pathways are mediated by a direct projection of the coeruleospinal tract to both spinal motoneurons and Renshaw cells (Fung et al. 1987a; Pompeiano 1989). Other evidence suggests the possibility of more direct effects of NE on these circuits. Applicable levels of NE have been detected in the vestibular nuclei (Versteeg et al. 1975; Palkovits and Brownstein 1989), and d-amphetamine and NE appear to act at alpha adrenoreceptors to increase the firing rate of motion-sensitive neurons in the lateral vestibular nucleus (LVN; Kirsten et al. 1974; Kirsten and Sharma 1976). Despite this evidence of a physiologic role of NE in the vestibular nuclei, the anatomic evidence regarding the sources and distribution of adrenergic input is contradictory. Although original histofluorescence studies reported a lack of catecholaminergic terminals in the vestibular nuclei (Fuxe 1965), the results from subsequent studies have ranged from an absence of adrenergic content in vestibular nuclear complex to a substantial adrenergic content (Versteeg et al. 1975; Moore and Bloom 1979; Moore...
1982; Moore and Card 1984; Fritschy and Grzanna 1989; Steinbusch 1991). This communication presents immunohistochemical evidence of a regionally selective projection from LC and nucleus subcoeruleus to the vestibular nuclei in albino and pigmented rats. Since the innervation forms a dense plexus around Deiters' neurons in the LVN, these data are consistent with a direct noradrenergic effect of LC on vestibulospinal projection neurons in LVN.

Materials and Methods

Fourteen adult male rats (nine Sprague-Dawley and five Long-Evans, 250-350 g body weight) were housed singly in suspended cages at 22°C with a 12-h light and 12-h dark cycle and ad libitum access to food and water. Rats were killed by pentobarbital sodium overdose (100-150 mg/kg i.p.) and perfused transcardially with 50 mM phosphate-buffered isotonic saline (PBS, pH 7.3-7.4) followed by a paraformaldehyde-lysine-periodate (PLP) fixative solution (McLean and Nakane 1974). The brains were cryoprotected for at least 3 days at 4°C in a phosphate-buffered 4% paraformaldehyde solution containing 30% sucrose. Two of the brains were sectioned in the transverse plane at 60-80 μm on a vibratome. The remaining brains were sectioned at 40 μm in the horizontal or transverse plane on a sliding microtome equipped with a dry ice freezing stage. Sections were stored for short periods of time (less than 4 days) at 4°C until used for the immunohistochemical procedures. For longer term storage, free-floating sections were placed in a solution containing 30% PBS, 30% ethylene glycol, and 30% sucrose, and stored at 20°C.

Free-floating sections were rinsed (3 x 20 min, or 5 x 20 min if recovered from the ethylene glycol-sucrose solution) with PBS and then pretreated with a blocking solution of 0.1% Triton X-100 and 2% bovine serum albumin in PBS for at least 20 min. Primary antibodies were diluted in the blocking solution as follows: a polyclonal anti-tyrosine hydroxylase (anti-TH, generously provided by Dr. D. M. Kuhn) at dilutions of 1:1000-2000 (for discussion of specificity, see Billingsley et al. 1986), a polyclonal (Eugene Tech International) anti-dopamine-β-hydroxylase (anti-DβH) at dilutions of 1:1000-1500, and a monoclonal (Chemicon) anti-DβH at dilutions of 1:1000-1500. Sections were incubated with the primary antibodies overnight (average of 20 h) at 4°C. The immunoreactive structures were then visualized with standard avidin–biotin peroxidase complex (ABC) methods (Vectastain Elite Kit, Vector Laboratories, Burlingame, Calif., USA). The sections were rinsed with PBS, incubated with the appropriate biotinylated secondary antibody diluted 1:250 in PBS with 2% bovine serum albumin, rinsed with PBS, incubated with ABC reagent, rinsed again, and then placed in a solution of 0.01% H2O2 and 5 mg/ml diaminobenzidine in 0.05 M TRIS buffer. The reaction was followed by PBS rinse (3 x 10 min). Sections were then mounted on gelatin/chromalum-subbed slides, dehydrated through increasing concentrations of ethanol, finally into xylene, and then cover-slipped with Permount. Photomicrographs and camera lucida drawings were made to document the course of the coeruleovestibular fibers.

Results

Four major bundles of immunoreactive fibers exit LC and nucleus subcoeruleus (group A4). The two largest pathways, the dorsal and the coeruleobulbospinal noradrenergic bundles, are described extensively in previous publications (Dahlstrom and Fuxe 1964; Fuxe 1965; Loizou 1969; Olsen and Fuxe 1971; Pickel et al. 1974; Levitt and Moore 1979; Moore and Bloom 1979; Moore 1982; Foote et al. 1983; Moore and Card 1984; Jones and Yang 1985). The innervation of the cerebellum, cochlear nuclei, and vestibular nuclei, though, is provided by two other bundles of tyrosine hydroxylase (TH)- and dopamine β-hydroxylase (DβH)-immunoreactive axons that emerge from LC and group A4 (Figs. 1, 2). These pathways are termed the medial and lateral descending noradrenergic bundles.

The majority of immunoreactive fibers that enter the vestibular nuclear complex comprise the medial descending noradrenergic bundle. These fibers exit from the caudal margin of LC (Fig. 1B, C) and form a narrow sheet along the border of the fourth ventricle (Figs. 1B, 2), immediately superior to the medial vestibular nucleus (MVN). As the main fascicles curves laterally around the caudal border of LVN, axons enter the dorsal division of LVN and form a dense plexus of varicose fibers around the somata and proximal dendrites of Deiters' neurons (Figs. 2, 4). Axonal branches also contribute a less dense projection to the ventral division of LVN and a minor projection to MVN (Fig. 2). The main fiber bundle then continues laterally across the caudal margin of the inferior cerebellar peduncle to innervate the cochlear and cerebellar nuclei. This latter portion of the fiber bundle has been termed the "caudal cochlear bundle" in previous studies (Kromer and Moore 1980; Kössl et al. 1988).

The lateral descending noradrenergic bundle originates from both LC and nucleus subcoeruleus (group A4) and projects to the superior vestibular nucleus (SVN), cochlear nuclei, and the cerebellar cortex. The fibers exit the noradrenergic cell groups to form a diffuse sheet along the rostral border of SVN (Figs. 1A,C, 2), with axon collaterals ramifying within SVN. Although most fibers in this pathway travel dorsally along the superior cerebellar peduncle to the cerebellar cortex, some axonal branches continue laterally and caudally around SVN and enter the cochlear nuclei from a rostral approach. This pattern of cerebellar innervation was designated the cerebellar noradrenergic bundle (Olsen and Fuxe 1971) and the cochlear nuclear projection was termed the rostral cochlear bundle (Kromer and Moore 1980).

A salient feature of coeruleovestibular projections is their regional selectivity within the vestibular nuclear system. The innervation is

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Fig. 1A-C. Photomicrographs of descending pathways from locus coeruleus (LC) to the vestibular nuclei in horizontal sections of the rat brain. The orientation of the sections (R, rostral, M, medial) is shown in A. A Low-magnification photomicrograph of tyrosine hydroxylase- (TH)-immunoreactivity of LC. Note that descending fibers exit the rostral and intermediate borders of LC and a few traverse the superior cerebellar peduncle (SCP). The descending fibers exit at caudal to intermediate levels of LC; some of these fibers enter the superior vestibular nucleus (SVN) while others proceed caudally along the wall of the fourth ventricle (4r). B Photomicrograph of a horizontal section showing the medial descending noradrenergic bundle (arrow) along the border of the fourth ventricle (4r). C Higher magnification view of the caudal third of LC from A. Note the fibers of the lateral descending noradrenergic bundle (small arrows) that both curve laterally around and enter into SVN. The caudally directed fibers that join the medial descending pathway are indicated by large arrows.