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Research report

Retrograde study of projections from the tuberomammillary nucleus to the dorsal raphe and the locus coeruleus in the rat

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Abstract

In the first series of experiments, a retrograde tracer, WGA-apo-HRP-gold (WG), was injected into the dorsal raphe (DR) or the locus coeruleus (LC) and adenosine deaminase immunostaining was subsequently performed for the tuberomammillary nucleus (TMN) in order to investigate projections from the TMN to the two brainstem monoaminergic nuclei. Following rostral DR injections, the majority of retrogradely labeled cells were located in the dorsomedial and ventrolateral subdivisions of the TMN. At middle DR levels, midline injections resulted in labeling mainly in the ventrolateral subdivision, whereas lateral wing injections produced labeling mostly in ventral and caudal TMN subdivisions. When injections were made in the caudal DR, only a few cells were observed along the rostro-caudal extent of the TMN. On the other hand, following rostral LC injections, labeled neurons were observed mainly in ventrolateral and ventral subdivisions of TMN. For principal LC injections, labeled cells were observed mostly in ventrolateral, ventral, and caudal TMN subdivisions, whereas for injections at caudal pole of LC, only a few cells were located along the rostro-caudal extent of the TMN. In the second series of experiments, an iontophoretic injection of fluorogold (FG) into the DR was paired with a pressure injection of WG into the LC to investigate the collateral distribution of TMN axonal fibers to DR and LC. Double-labeled cells were observed in ventrolateral, ventral, and caudal TMN subdivisions. The present study indicated that there exists a robust projection from the TMN to the DR or the LC and that some TMN neurons have axon collaterals projecting to both DR and LC. The TMN neurons with such axon collaterals might provide simultaneous, possibly more efficient, way of controlling the brainstem monoaminergic nuclei, thus influencing various sleep and arousal states of the animal.

Theme: Neural basis of behavior
Topic: Monoamines and behavior

Keywords: Tuberomammillary nucleus; Dorsal raphe; Locus coeruleus; Histamine cells; Serotonin cells; Norepinephrine cells

1. Introduction

The tuberomammillary nucleus (TMN) in the posterior hypothalamus consists of several clusters of histaminergic cells: (1) a dorsomedial group, adjacent to the mammillary recess of the 3rd ventricle, (2) ventrolateral and ventral groups, laminae of tightly-packed cells located at the ventral, sub-pial surface, and (3) a caudal group, located lateral to the lateral mammillary nucleus [40,42,53]. Neurons in the TMN as well as other two brainstem monoaminergic nuclei, the dorsal raphe (DR) and the locus coeruleus (LC), have common characteristics in that they exhibit maximal rates of discharge during waking, decrease their firing during non-rapid eye movement (NREM) sleep, and virtually become quiescent during rapid eye movement (REM) sleep [1,2,11,28,41,46,48]. The three monoaminergic nuclei are also similar in that they serve as major arousal centers and project extensively to the cerebral cortex [18,33,34].

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Using an anterograde tracing method, Steininger et al. [43] provided evidence for sub-regional organization of preoptic area projections to arousal-related monoaminergic cell groups; for example, ventrolateral preoptic nucleus (VLPO) provides a robust innervation to the histaminergic tuberomammillary nucleus (TMN), whereas VLPO/medial preoptic area and VLPO/lateral preoptic region send dense projections to the LC and the DR, respectively. The ventral portions of the medial preoptic area and the lateral preoptic nucleus correspond to dorsomedial and dorsolateral regions of the VLPO, respectively, which are collectively called "extended VLPO" by Lu et al. [26]. A functional differentiation between the VLPO and the extended VLPO has been suggested such that: (1) during NREM sleep, the sleep-active neurons in the VLPO might inhibit the activity of cells in the TMN, DR, and LC by releasing GABA and galanin; and (2) during the transitions from NREM to REM sleep, the firing of DR and LC is further decreased because of recruitment of inhibitory neurons in the extended VLPO [26,37,38,45]. Thus, one implication of the above findings is that TMN communicates with DR and LC during various sleep–waking states of the animal. Further support for this hypothesis derives from the fact that histamine-like immunoreactive fibers have been observed in the DR and the LC [13,22] and the TMN, in return, receives noradrenergic and serotonergic inputs from brainstem regions [7]. The present report is the first extensive, retrograde study of the projections from subdivisions of the TMN to those of the DR and/or the LC. A retrograde tracer, WGA-apo-HRP-gold, was localized within specific subdivisions of the DR or the LC and subsequent adenosine deaminase (ADA) immunostaining was performed for TMN sections to identify neurons projecting to the DR or the LC. Collateral projections of TMN axonal fibers to the DR and LC were also analyzed by making paired injections of fluorogold (FG) into the DR and WG into the LC.

2. Materials and methods

A total of 44 Sprague–Dawley rats including both sexes and ranging in weight from 300 to 350 g were used in this study. Prior to surgery, each rat was anesthetized with an intraperitoneal injection of chloral hydrate (3.6% in distilled water, 1 ml/100 g body weight). All animals used in this study were treated according to guidelines approved by the institutional animal care and use committee and conformed to the NIH guidelines on care and use of animals in research.

2.1. DR injection sites

The skull around bony lambda was removed and the superior sagittal sinus ligated with surgical sutures rostrally and caudally. Angiovasectomy was performed between suture points in order to expose the cerebral fissure at midline. Based on the atlas of Paxinos and Watson [32], the rostral DR was approached for sites between 0.5–1.0 rostral to bony lambda at the depth of 5.2–5.4 mm. The midline at middle DR levels was targeted for sites between 0–0.5 mm caudal to the lambda at the depth of 5.6–5.8 mm, whereas the lateral wing subdivision was approached from 0.3–0.4 mm lateral to the midline at the depth of 5.4–5.6 mm. The caudal DR was targeted 1.0–1.5 mm caudal to the lambda at the depth of 6.0–6.2 mm.

2.2. LC injection sites

Rats were placed in a stereotaxic apparatus. The incisor bar was set at −10.0 mm, thus tilting the skull surface 10–20° downward from the horizontal plane. Based on the atlas of Paxinos and Watson [32], the rostral LC was targeted 1.1 mm lateral from the midline, 0.2–0.4 mm caudal to the posterior border of the transverse sinus at the depth of 5.5–5.7 mm, whereas the principal LC was 1.2 mm lateral from the midline, 0.6–0.8 mm caudal to the sinus at the depth of 5.6–5.8 mm. Finally, the caudal pole of LC was at 1.0–1.2 mm caudal to the sinus, 1.2 mm lateral from the midline, and 5.7–5.9 mm deep from the dural surface.

2.3. WG injection

The WG was synthesized using inactivated WGA-HRP (Sigma, L-0390) and 10 or 20 nm (Sigma, G1527 or G1652) colloidal gold [4]. The injection apparatus consisted of a glass micropipette (tip diameter, 10–15 μm) hydraulically linked to a 2.0 μl Hamilton syringe. We injected 0.02 μl of WG nine times (a total of 0.18 μl) at 3-min intervals into a single site within the DR or the LC and took out the pipette 30 min after the first injection trial.

2.4. FG injection

To examine the collateral distribution of TMN axonal fibers to the DR and the LC, WG was pressure-injected into subdivisions of the LC, whereas FG was iontophoretically injected into midline at middle DR levels. The opposite combination was also performed but proved to be inappropriate because FG produced an extensive injection site with a mediolateral dimension of 1300–1500 μm; the LC, however, is a nucleus with a tight mediolateral dimension (150-350 μm). A solution of 1% FG (Fluorochrome Inc.) was prepared in saline, drawn into the tip of a glass micropipette (tip diameter, 10–15 μm) via capillary action, and deposited within the DR nuclei using a 2 μA alternating current applied on a 5-s duty cycle for 10–20 min through a silver lead wire (Stoelting, 50880) inserted in the pipette.

2.5. Perfusion–fixation and silver enhancement reaction

After a survival period of 48–72 h following tracer injections, the animals were perfused using 150 ml of saline followed by 600 ml of fixative containing 4% paraformal-