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Research Report

Retrograde study of projections from the tuberomammillary nucleus to the mesopontine cholinergic complex in the rat

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ABSTRACT

The mesopontine cholinergic complex comprising the pedunculopontine (PPTg) and laterodorsal (LDTg) tegmental nuclei is a key component of ascending reticular activating system. The brainstem cholinergic nuclei are yet under the control of descending projections from hypothalamic arousal centers such as the hypocretinergic, lateral hypothalamus (LH), and the histaminergic, tuberomammillary nucleus (TMN). The present study was designed to determine the differential projection pattern as well as the laterality of descending TMN projections to the PPTg and LDTg. Our results showed that each TMN subdivision provided differential projections to the mesopontine complex. The majority of PPTg-projecting neurons were located in ventrolateral TMN mainly at its subpial border, whereas LDTg-projecting cells were in dorsomedial TMN with a few in the medial border of the ventrolateral subdivision. For both PPTg and LDTg cases, retrogradely labeled neurons were more pronounced in each TMN subdivision ipsilateral to the injection site. The light microscopic observation also indicated that hypocretinergic, terminal-like boutons formed close appositions to somata as well as proximal dendrites of PPTg- or LDTg-projecting TMN neurons. Taken together, the present observations suggested that each TMN subdivision provide differential projections to the mesopontine cholinergic complex and that the LH, via the TMN, provides indirect, descending projections to the complex as well.

1. Introduction

Together with noradrenergic locus coeruleus (LC) and serotonergic dorsal raphe (DR) nucleus, the mesopontine cholinergic complex comprising the pedunculopontine (PPTg) and laterodorsal (LDTg) tegmental nuclei plays a major role in ascending reticular activating system (Steriade, 1991; Saper et al., 2005). The ascending, cholinergic projection is actively involved in cortical arousal via its relay stations at various thalamic nuclei as well as basal forebrain targets (Satoh and Fibiger, 1986; Hallanger and Wainer, 1988; Semba et al., 1990; Lin et al., 1996). In fact, the PPTg and LDTg provide differential, ascending projections in that the former projects selectively to extrapyramidal structures and the superior colliculus, while the latter provides inputs preferentially to anterior thalamus and rostral basal forebrain (Woolf and Butcher, 1986; Hallanger et al., 1987).

The reciprocal interaction between hypothalamic arousal centers and brainstem monoaminergic/cholinergic nuclei during various sleep–arousal states has been well established (Semba and Fibiger, 1992; Saper et al., 2001). Similar to

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hypocretinergic lateral hypothalamus (LH), histaminergic tuberomammillary nucleus (TMN) in the posterior hypothalamus provides descending input to brainstem monoaminergic/cholinergic nuclei (Ko et al., 2003; Gerashchenko et al., 2004; Lu et al., 2006). Anterograde-tracing study reported that histamine-immunoreactive fibers with terminal boutons exist in the LDTg and LC of the cat (Schwartz et al., 1986; Panula et al., 1989). Electrophysiological study demonstrated that histaminergic descending afferents activate H_1 receptors situated on cholinergic neurons and that the interactions between histaminergic and cholinergic neurons constitute an important circuit in cortical activation during wakefulness (Lin et al., 1996).

The present study was designed to determine the regional distribution of PPTg- or LDTg-projecting cells within subdivisions of the TMN. It is the first retrograde-tracing study examining TMN projections to the mesopontine complex and is also a sequel to our previous reports concerning TMN projections to the brainstem monoaminergic nuclei (Lee et al., 2005b, 2008). Iontophoretic or pressure injection of retrograde tracers into the PPTg or LDTg was performed to determine the differential distribution as well as the laterality of the descending projection. Likewise, the possibility that the projection was under the control of hypocretinergic LH was also investigated using a combination of retrograde tracing and hypocretin (HCRT) immunostaining.

2. Results

Among 48 cases of LDTg ($n=25$) or PPTg ($n=23$) injections, cases with confined injection sites are depicted and analyzed histologically (Figs. 1–6) and quantitatively (Table 1). Representative examples of the PPTg (Fig. 1A, R252; B, R289) or LDTg (Fig. 1C, R253; D, R290) injection sites are shown. The iontophoretic injection of Fluorogold (FG) produced a homogeneous injection site with a spherical morphology (Fig. 1A and C). For fluorescence studies, we utilized rhodamine-coated latex microspheres (RCM) since our confocal microscope was not equipped with ultraviolet filter system necessary for FG identification. The RCM was pressure-injected, producing an irregularly-shaped clump at the injection site (Fig. 1B and D). The tissue sections at the injection center often exhibited the trajectory of glass micropipette (Fig. 1A and B).

2.1. *Regional distribution of PPTg- or LDTg-projecting TMN neurons*

The first set of experiments was performed to examine whether each subdivision of the TMN projected differentially to the PPTg or the LDTg. When iontophoretic injection of FG was targeted at the PPTg, the majority of double-labeled cells

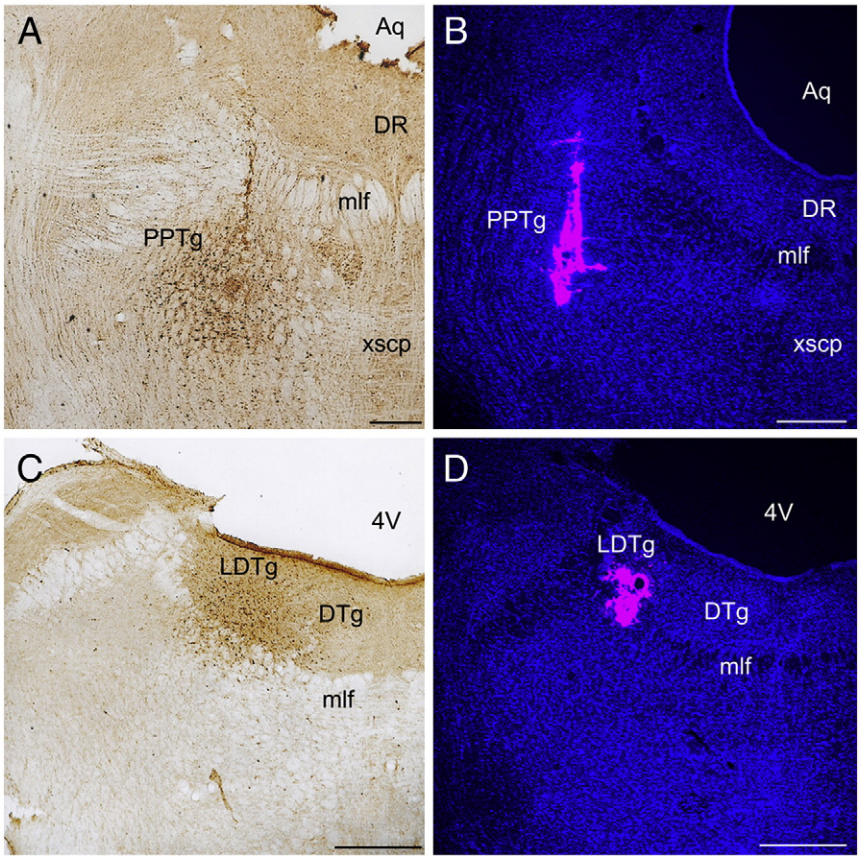

Fig. 1 – Representative examples of the PPTg (A, R252; B, R289) or LDTg (C, R253; D, R290) injection sites are depicted. Fluorogold (FG) was iontophoretically injected, whereas rhodamine-coated microspheres (RCM) were pressure-injected. 4V indicates fourth ventricle; Aq, cerebral aqueduct; DR, dorsal raphe nucleus; DTg, dorsal tegmental nucleus; mlf, medial longitudinal fasciculus; xscp, decussation of the superior cerebellar peduncle. Scale bars=500 μm.