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Research Report

Inhibitory and excitatory amino acid neurotransmitters are utilized by the projection from the dorsal deep mesencephalic nucleus to the sublaterodorsal nucleus REM sleep induction zone

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ABSTRACT

The sublaterodorsal nucleus (SLD) in the pons of the rat is a locus supporting short-latency induction of a REM sleep-like state following local application of a $GABA_A$ receptor antagonist or kainate, glutamate receptor agonist. One putatively relevant source of these neurotransmitters is from the region of the deep mesencephalic nucleus (DpMe) just ventrolateral to the periaquiductal gray, termed the dorsal DpMe (dDpMe). Here, the amino acid neurotransmitter innervation of SLD from dDpMe was studied utilizing anterograde tract-tracing with biotinylated dextranamine (BDA) and fluorescence immunohistochemistry visualized with laser scanning confocal microscopy. Both markers for inhibitory and excitatory amino acid neurotransmitters were found in varicose axon fibers in SLD originating from dDpMe. Vesicular glutamate transporter2 (VGLUT2) represented the largest number of anterogradely labeled varicosities followed by vesicular GABA transporter (VGAT). Numerous VGAT and VGLUT2 labeled varicosities were observed apposed to dDpMe-labeled axon fibers indicating both excitatory and inhibitory presynaptic, local modulation within the SLD. Some double-labeled BDA/VGAT varicosities were seen apposed to small somata labeled for glutamate consistent with being presynaptic to the phenotype of REM sleep-active SLD neurons. Results found support the current theoretical framework of the interaction of dDpMe and SLD in control of REM sleep, while also indicating operation of mechanisms with a greater level of complexity.

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Abbreviations: BDA, Biotinylated dextran amine; CTb, Cholera toxin b-subunit; DpMe, Deep mesencephalic nucleus; dDpMe, Dorsal DpMe; GABA, γ-aminobutyric acid; $GABA_AR$, $GABA_A$ receptor; GAD67, Glutamic acid decarboxylase-67; GLYT2, Glycine transporter-2; LC, Locus caeruleus; NREM, Non-REM; PAG, Periaquiductal gray; REM, Rapid eye movement; S.E.M., Standard error of the mean; SLD, Sublaterodorsal nucleus; VGAT, Vesicular GABA transporter; VGLUT2, Vesicular glutamate transporter-2; vlPAG, Ventrolateral PAG

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1. Introduction

The neural mechanisms subserving rapid eye movement (REM) sleep have proven remarkably resistant to elucidation despite application of a wide variety of neuroscience techniques. Historically, a critical role for the cholinergic system was proposed based mainly on the result in cat of direct, local intracerebral administration of muscarinic agonists to sites in the pons able to induce a short latency onset and prolonged duration of REM sleep episodes (Baghdoyan et al., 1987; Vanni-Mercier et al., 1989). Sites of high sensitivity were identified in the pontine reticular formation as well as a site ventral to the locus coeruleus (LC) termed the peri-LCα (Baghdoyan et al., 1987; Vanni-Mercier et al., 1989). Questions of species generalizability of these mechanisms arose due to the failure to replicate these findings in rodent models. Sites in the caudal aspects of the rostral pontine reticular formation of rat were found to induce a long-lasting increase in REM sleep with cholinergic agonists and other neurotransmitter systems' ligands, but no evidence for a short latency "triggering" of the state had been reported in this species (Bourgin et al., 1995; Marks and Birabil, 1998).

More recently it has been found that $GABA_A$ receptor ($GABA_AR$) antagonists injected into the area of the peri-LCα of cat induced REM sleep similar to muscarinic agonists, but with shorter duration of effect (<2 h) (Xi et al., 1999). This finding was followed by the first demonstration of short latency induction of a REM sleep-like state in head-restrained rat with iontophoretic application of $GABA_AR$ antagonists, gabazine and bicuculline, into the site homologous to peri-LCα, termed the rostral sublaterodorsal nucleus (SLD) (Boissard et al., 2002). Application of cholinergic agonists induced wakefulness and failed to increase REM sleep indicating limits to the cross-species homology. Short latency onset of a REM sleep-like state has been replicated with pressure injection of bicuculine in the SLD of the freely moving rat (Pollock and Mistlberger, 2003).

The SLD contains neurons that fire selectively in REM sleep (termed, REM-on) (Boissard et al., 2002). Iontophoretic application of the excitatory, glutamate receptor agonist, kainate, in head-restrained rat, also triggered a REM sleep-like state within minutes of the start of its ejection (Boissard et al., 2002). Ibotenic acid lesions of the SLD destroying >90% of neurons resulted in about a 60% reduction in REM sleep (Lu et al., 2006). These findings are consistent with the concept that excitation of REM-on neurons in SLD is sufficient to initiate REM sleep (Boissard et al., 2002; Fort et al., 2009; Lu et al., 2006). This is further supported by the widespread projections of SLD neurons, both rostrally and caudally, that could be responsible for recruiting various neuronal populations subserving many processes of REM sleep (Boissard et al., 2002; Luppi et al., 2007; Lu et al., 2006; Vetrivelan et al., 2009) including the two major physiological indicators of REM sleep, muscle atonia and cortical activation.

The REM sleep-inducing effect of $GABA_AR$ antagonism in SLD was blocked by local application of kynurenic acid, a non-selective ionotropic glutamate receptor antagonist (Boissard et al., 2002). This is consistent with disinhibition of glutamate excitation being the action of $GABA_AR$ antagonists to induce REM sleep. A current model proposed by Luppi et al. (2012) posits the presence of a tonic glutamatergic excitation of SLD REM-on neurons. The state-selective, REM-on pattern of discharge by these neurons is posited to be produced by a potent GABAergic inhibition that is removed only during REM sleep. Application of sufficient kainate can overcome this inhibition and $GABA_AR$ antagonists disinhibit permitting the REM-on neurons to become active and induce REM sleep. This negative control by GABA in the SLD is viewed as one of the critical mechanisms in the distributed system of REM sleep control (Boissard et al., 2002; Fort et al., 2009; Luppi et al., 2007, 2012; Lu et al., 2006; Sapin et al., 2009).

The source of this GABA disinhibition in SLD is uncertain. The SLD receives GABAergic innervation from many sources including GABAergic neurons intrinsic to the SLD (Boissard et al., 2003; Lu et al., 2006; Sapin et al., 2009). Found prominently were neurons in regions of the midbrain, pontine and medullary reticular formation retrogradely labeled from SLD and also coexpressing a marker for GABA. Less numerous, additional retrogradely labeled GABAergic neurons were found in: the area of the lateral hypothalamus; substantia nigra reticulata; pedunculo pontine tegmental nucleus; and raphe magnus.

Drawing on homology with the cat, it has long been known that bilateral electrolytic lesions in the area of the midbrain reticular formation ventral to and including the ventrolateral periaqueductal gray (vlPAG) at the level of the ponto-mesencephalic isthmus resulted in an increase of REM sleep up to 400% lasting several days (Petitjean et al., 1975). This strongly implicates the presence of a mechanism inhibitory to REM sleep consistent with a GABAergic innervation. Inhibiting neuronal activity with bilateral injections of the potent inhibitory $GABA_AR$ agonist muscimol into this area also increased REM sleep, though more modestly (Sastre et al., 1996). More recently, studies utilizing ibotenic acid lesions and iontophoreticly applied unilateral muscimol ejections have replicated REM sleep increases in the rat (Lu et al., 2006; Sapin et al., 2009). Bilateral injections of muscimol also resulted in large REM sleep increases in guinea pig (Vanini et al., 2007). In rat, the midbrain reticular formation, termed the deep mesencephalic nucleus (DpMe) expressed a high number of GABA neurons projecting to SLD (Boissard et al., 2003) and an indirect measure of activation, c-Fos expression, indicated that a higher number of GABAergic neurons adjacent to the vlPAG, termed the dorsal DpMe (dDpMe), were activated when REM sleep was deprived compared to a condition of increased REM sleep during recovery from REM sleep deprivation (Sapin et al., 2009). Taken together, these findings are consistent with the dDpMe as one area providing GABAergic innervation to the SLD in modulation of the REM-on neurons important to REM sleep generation.

To demonstrate this role for the dDpMe in control of REM sleep, additional data are required, such as the specific activity patterns of GABAergic neurons projecting to SLD and whether they directly innervate REM-on neurons. Preliminary to addressing these questions, we sought here to examine the anatomy of the amino-acid neurotransmitter innervation of SLD from the dDpMe. Immunohistological markers for inhibitory and excitatory amino acid vesicular transporters were coupled with anterograde axonal tracing and imaged with fluorescence confocal laser scanning