Descending projections from the dorsolateral pontine tegmentum to the paramedian reticular nucleus of the caudal medulla in the cat

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We examined whether the dorsolateral pontine cholinergic cells project to the paramedian reticular nucleus (PRN) of the caudal medulla. In 3 cats, wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was injected into the PRN and we noted cells in the dorsolateral pons that contained the HRP reaction product, cells that were immunolabelled for choline acetyltransferase (ChAT), and cells that contained the HRP reaction product and were ChAT positive. We found cholinergic projections from the Pedunculopontine tegmental and laterodorsal tegmental nuclei to the PRN. This finding is consistent with studies indicating a cholinceptive region in the medial medulla mediating suppression of muscle tone. Our results demonstrate that this medullary region has monosynaptic input from pontine neurons implicated in generating the atonia of rapid eye movement sleep.

INTRODUCTION

Magoun and Rhines first noted that electrical stimulation of the medial medulla produced a suppression of electromyographic activity in decerebrate cats. Subsequently, it was found that such stimulation produced inhibitory postsynaptic potentials in both flexor and extensor motoneurons. A projection from dorsolateral pontine regions to the nucleus magnocellularis (NMC) of the rostral medial medulla has been described and it has been suggested that this pathway may be responsible for the loss of muscle tone which normally occurs during rapid eye movement (REM) sleep. The NMC has been shown to project directly to the spinal cord.

Recently, Lai and Siegel described another, more caudal region in the medulla, the paramedian reticular nucleus (PRN), which when electrically stimulated, produced loss of muscle activity in the decerebrate cat. They also found that microinfusion of acetylcholine into this region readily suppressed muscle tone. In this study we examined the brainstem source of cholinergic input into the PRN. Aside from cranial motor nuclei, the only other large concentrations of cholinergic cells in the brainstem are located in the dorsolateral pontine tegmentum. In this study we placed wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) into the PRN and determined whether the cholinergic neurons from the dorsolateral pontine tegmentum innervated the PRN.

MATERIALS AND METHODS

Three cats were deeply anesthetized with sodium pentobarbital (35 mg/kg) and bilateral (one cat) or unilateral (two cats) stereotaxic microinjections of WGA-HRP (0.5%, Sigma) dissolved in 0.9% saline were made into the PRN using a 1.0 µl Hamilton microsyringe. 0.05 µl of the retrograde tracer was injected at each site at a rate of 0.01 µl/min. The microsyringe was left in place for 15 min after the injection. After a 2-day period of survival, the cats were given an overdose of sodium pentobarbital and 15 min later given 3000 units of heparin intravenously. The brains were fixed by cardiac perfusion with 300 ml of 0.9% saline, 2 liters of 3% paraformaldehyde-0.1% glutaraldehyde in 0.1 M phosphate buffer (PB), and 2 liters of 10% sucrose in 0.1 M PB. The brains were blocked in the stereotaxic apparatus at the precollicular and pontomedullary levels, and then placed in 30% sucrose in 0.1 M PB solution at 4 °C until equilibrium was reached. Sections were cut at a thickness of 50 µm on an American Optical freezing microtome and immediately processed for visualization of the retrograde tracer.

All tissue sections were initially processed for HRP detection using tetramethylbenzidine (TMB) as the chromagen. The TMB was stabilized using the diaminobenzidine-cobalt chloride-hydrogen peroxide solution according to the method described by Rye et al. For the medullary region, alternate sections were mounted on gelatine-coated slides and counterstained with Neutral red for histological verification of the WGA-HRP injection site. The remaining series of medullary sections were mounted on gelatine-coated slides and after dehydration through a graded sequence of alcohol-xylene solutions, coverslipped with Permount. The pontine sections were processed as follows. One in four series of sections were mounted on gelatine-coated slides, and after dehydration,
examined under the light microscope for HRP-labelled cells. The remaining sections in the series were immunolabelled with polyclonal antibodies against choline acetyltransferase (Chemicon), the acetylcholine synthesizing enzyme, using the avidin-biotin immunocytochemical labelling procedure. Subsequently, the immunolabelled sections were mounted on gelatine coated slides and after dehydration through a graded series of alcohol-xylene solutions, the slides were coverslipped with Permount.

Fig. 1.

**Fig. 1.** Distribution of retrogradely labelled cells in the pons following injection of WGA-HRP into the paramedial reticular nucleus of the caudal medulla (inset). Solid circles represent neurons that were singly labelled with the HRP reaction product while open triangles represent neurons that were retrogradely labelled and were ChAT immunopositive. Insets: the WGA-HRP injection site is depicted by a heavy black area while the stippling shows the extent of the diffusion. The anterior-posterior planes are from Herman's atlas of the cat brainstem. Selected abbreviations: AQ, aqueduct; CNF, nucleus cuneiformis; FTC, central tegmental field; FTG, gigantocellular tegmental field; FTL, lateral tegmental field; FTP, perilemniscal tegmental field; IC, inferior colliculus; IP, interpeduncular nucleus; LC, locus ceruleus; PAG, periaqueductal area; SC, superior colliculus; PG, pontine gray.

Under the light microscope we could identify cells that contained the HRP reaction product only, cells that were choline acetyltransferase (ChAT) positive, or those cells that contained both HRP and were ChAT positive. The location of the HRP-positive, and the double-labelled HRP + ChAT-positive cells was plotted using a drawing tube attached to a Zeiss microscope. The drawings were then transferred to an IBM-PC computer using a computer-assisted design program (Prodesign, American Small Business Computers, Pryor, OK) and final plots were made on a plotter (Hewlett-Packard 7475A). The location and counts of HRP- and HRP + ChAT-positive cells were determined from all sections that were immunolabelled with the ChAT antibody. Sections that were processed for visualization of the HRP reaction product were used to confirm the presence of retrogradely labelled cells.

**RESULTS**

Figs. 1-3 summarize the distribution of retrogradely labelled cells in the dorsolateral pontine tegmentum. Bilateral injection of the retrograde tracer into the medial portions of the caudal medulla (Fig. 1, inset) or
cholinergic input from 1.3% of LDT cells while the dorsal and ventral PRN received 0.5% and 1.4%, respectively. Thus, only about 0.5-1.5% of dorsolateral pontine cholinergic cells project to the PRN. Previously it has been shown that the major portion of the projection of the LDT and PPT is ascending.\(^{2,4,14,17,23,25,31,35,36}\)

**DISCUSSION**

This study demonstrated that the cholinergic cells from the LDT and PPT project to the area of PRN in the caudal medulla from where electrical stimulation and microinfusion of acetylcholine and cholinergic agonists readily produce loss of muscle tone in decerebrate cats. Therefore, these findings provide anatomical support for the study by Lai and Siegel.\(^9\) These results are also in agreement with the most recent findings of projections from the PPT to the caudal medulla by Rye et al. in the rat.\(^19\) Thus, the cholinergic projection from the dorsolateral pons to the PRN may be part of the pathway producing the suppression of muscle tone seen in the decerebrate cat after systemic as well as local administration of cholinergic agonists. This pathway may also be involved in mediating the sudden loss of muscle tone (cataplexy) which occurs in narcolepsy. Besides cataplectic attacks, narcolepsy is characterized by excessive daytime sleepiness, direct transitions from waking to REM sleep and hypnagogic hallucinations. It has been suggested that some of the systems of narcolepsy, such as cataplexy and sleep-onset REM sleep, may be due to hyperactive brainstem cholinergic mechanisms.\(^26\)

Support for the role of cholinergic mechanisms in atonia is derived from studies where microinjection of cholinergic agonists into the dorsolateral and medial pons produces a profound suppression of muscle tone in cats.\(^6,16,27,28\) Electrical and cholinergic stimulation of the midline pons produces loss of muscle tone in cats.\(^18\) Recently, we have determined that the cholinergic-induced loss of muscle tone in the pons may be mediated by the M<sub>2</sub> muscarinic receptor.\(^33\) It has been postulated for some time that the cholinergic-induced inhibition of muscle tone may be mediated by cholinergic as well as cholinceptive pontine cells.\(^21,26\) In this study we found in the midline pons (raphe pontis) and medial and dorsolateral pons, numerous retrogradely labelled cells which were not ChAT immunopositive. These cells were in areas where infusion of cholinergic agonists readily triggers atonia and REM sleep, and therefore, these cells might be hypothesized to be cholinceptive. The medial pontine cholinceptive cells receive input from the LDT-PPT cholinergic neurons and therefore, atonia may be mediated by a polysynaptic pathway involving the LDT/PPT, medial pons and PRN. Some of the axons may descend within the medial longitudinal fasciculus.\(^18\)

Besides cholinergic agonists, glutamate has also been found to evoke atonia when injected into the regions ventral to the locus ceruleus.\(^19\) Lai and Siegel suggest that the glutamate-sensitive/glutamatergic neurons induce atonia by impinging on cells in the NMC.\(^9\) Electrical stimulation and microinjections of glutamate into the NMC readily evoke atonia in decerebrate cats and this region corresponds to the Magoun and Rhines inhibitory center.\(^7\)

Thus, we hypothesize that loss of muscle tone may be due to both poly- and mono-synaptic pathways which involve activation of glutamate-sensitive and cholinergic/cholinceptive cells in the ventral and lateral portions of the dorsolateral and medial pons. The pontine glutaminergic neurons act via the NMC and the cholinergic/cholinceptive neurons exert their influence via pontine cholinceptive neurons and via the PRN. Both the NMC and PRN have been shown to have massive projections to the spinal cord.\(^1,3,22\)

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