

ORIGINAL ARTICLE

The Subnuclear Distribution of 5-HT_{1A} Receptors in the Human Nucleus of the Solitary Tract and Selected Structures of the Caudal Medulla

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ABSTRACT The distribution of 5-HT_{1A} receptors in the subnuclei of the human caudal nucleus of solitary tract and adjacent structures in the dorsal vagal complex was studied using [^{3}H]8-OH-DPAT, a highly selective 5-HT_{1A} receptor agonist. The highest binding of the labeled ligand was found in the dorsal motor nucleus of the vagus, followed by the medial, intermediate, and subpostremal subnuclei of the nucleus of solitary tract. Previous animal studies suggest an important role for these structures in the regulation of visceral function, particularly for the gastrointestinal and cardiovascular systems. The results of this study suggest the possibility of an analogous role for 5-HT_{1A} receptors in the regulation of these autonomic pathways in humans as well.

INTRODUCTION

The dorsal medial medulla is part of the neural network regulating the respiratory, gastrointestinal, and cardiovascular systems. Along with the nucleus of solitary tract (NTS), the area postrema (AP) and the dorsal motor nucleus of the vagus (DMN) compose an anatomically and functionally integrated structure, the dorsal vagal complex. Within the human dorsal medulla, at the caudal end of the fourth ventricle, the NTS is composed of ten subnuclei integrating afferents from the vagal, glossopharyngeal, and trigeminal nerves. The subnuclear organization of the human caudal (visceral) NTS recently has been redefined (1). The application of this cytoarchitectural map of the human NTS will facilitate better comparisons between humans and non-human mammals.

The localization of neurotransmitter systems within the human NTS may help to define the neurochemical basis of visceral regulation. Serotonin (also termed 5-hydroxytryptamine or 5-HT) is one of several neurotransmitters that has significant binding in the medulla (2). There are multiple serotonin receptor subtypes (3,4). Pedigo et al. (5) characterized the 5-HT_{1A} subtype and its high affinity for the ligand 8-hydroxy-2-(di-N-propylamino) tetraline (8-OH-DPAT), a highly selective 5-HT_{1A} receptor agonist (6-8). Serotonin has been implicated in the regulation of many autonomic pathways, including respiration (9), cardiovascular function (9), and swallowing (10). However, the role of each 5-HT receptor subtype in the neural control of visceral function remains to be fully characterized.

The anatomical distribution of the 5-HT_{1A} receptor subtype has been previously studied in the NTS of the rat (2,11-13), cat (14) and human brain (15,16). The current study examines the distribution of 5-HT_{1A} receptors in the human caudal NTS, and the adjacent DMN and AP, using the tritiated ligand 8-OH-DPAT. The focus of this study is the relative levels of 5-HT_{1A}

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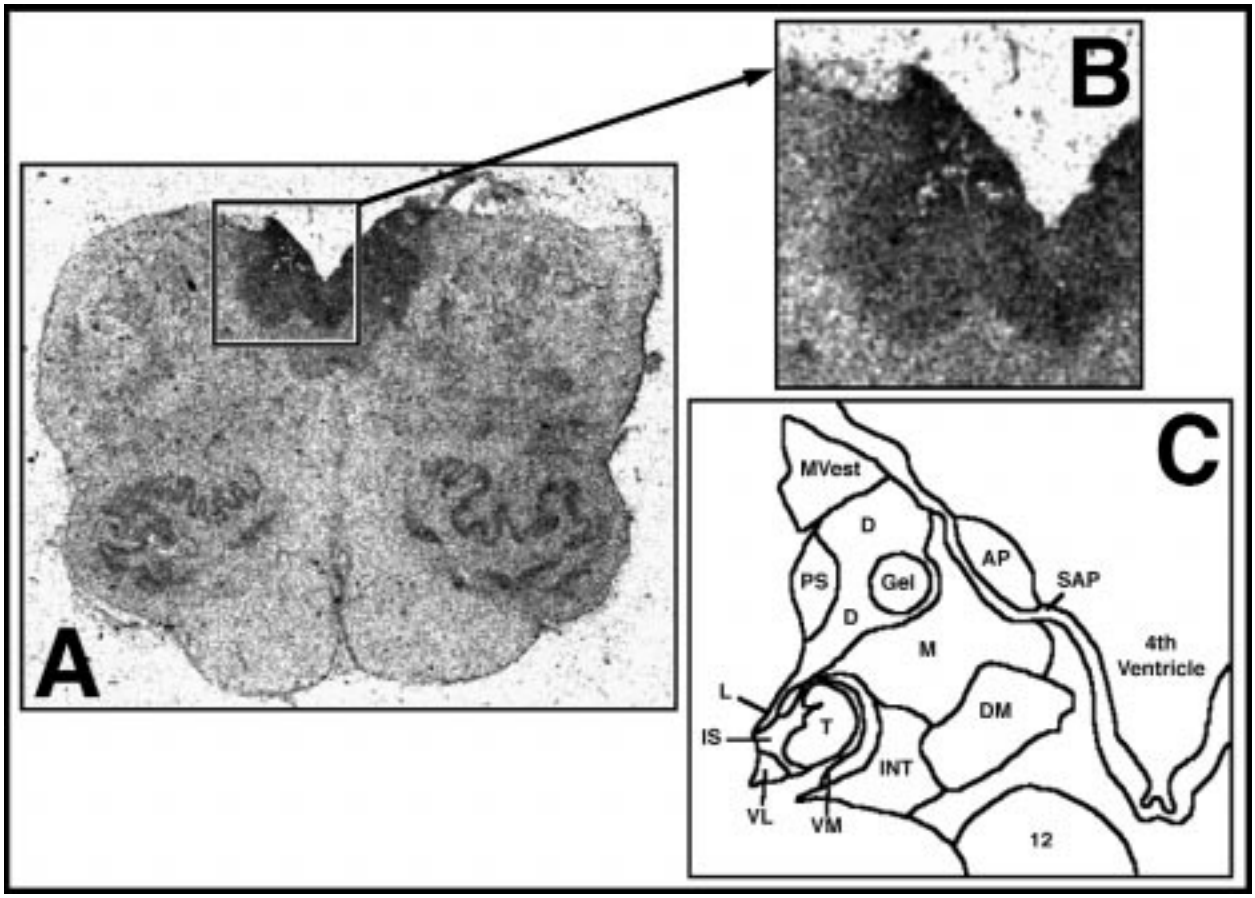

Figure 1. A and B: Autoradiogram of [^{3}H] 8-OH-DPAT binding in the human dorsal vagal complex at the level of the area postrema. Areas with the highest binding densities are denoted by dark gray, intermediate binding by moderate gray, and lightest binding by light gray. C: Schematic of the dorsal vagal complex indicating subnuclei relevant to study. **T:** tract of the nucleus of the solitary tract (NST); **VL:** ventrolateral subnuclei of the NST; **L:** lateral subnuclei of the NST; **IS:** interstitial subnuclei of the NST; **INT:** intermediate subnuclei of the NST; **VM:** ventromedial subnuclei of the NST; **D:** dorsal subnuclei of the NST; **M:** medial subnuclei of the NST; **Gel:** substantia gelatinosus subnuclei of the NST; **AP:** area postrema; **SAP:** subpostremal subnuclei of the NST; **DM:** dorsal medial nucleus of the vagus; **12:** hypoglossal nucleus; **MVest:** medial vestibular nucleus.

receptors in the subnuclei of the human caudal NTS, to better understand their putative involvement in autonomic visceral regulation.

METHODS

Unfixed human brainstem was obtained at autopsy from 9 subjects, ranging in age from 18 to 71 years, who died without any known history at neurological or psychiatric dysfunction. Toxicology studies showed that subjects were not taking any drugs just prior to death that might have had impact upon the binding characteristics of serotonin receptors. Macroscopic inspection of the brains at autopsy did not reveal any gross pathology. All brains were removed within 28 hours of death. Following blocking, the medullas were rapidly frozen in isopentane and dry ice to reduce freezing artifacts. The tissue was then stored at -70°C until sectioning. Twenty micron sections were cut using a Jung Frigocut 2800E cryostat and mounted on acid scrubbed gel-coated slides. Total binding was performed by incubating the slide mounted sections for 1 hour at 25°C in a buffer (50 mM Tris HCI, 5 mM $CaCl_2$, pH 7.4) containing 2 nM [^{3}H] 8-OH-DPAT (NEN Dupont, S.A.162.9 Ci/mmol) (2). Non-specific binding was determined by incubating consecutive sections in the same solution as above and also containing 1 µM 8-OH-DPAT. The sections were then washed in buffer at 4°C for 5 minutes and briefly dipped in 4°C distilled water. The slides were rapidly dried in a stream of cool air.

The dried slides were attached to a sheet of cardboard, placed adjacent to tritium sensitive film (Ultrafilm, LKB, Bromma, Sweden), and seated in an x-ray cassette for 28 days at 4°C. Receptor quantification was performed with a Macintosh computer-assisted image analysis system (NIH Image 1.52b). The slide-mounted sections were stained with cresyl violet for anatomical study, and each section was traced by hand

Table 1. Specific binding of [^{3}H] 8-OH-DPAT to structures in the dorsal vagal complex.

Structure	Specific Binding fmol/mg (mean ± s.e.m.)
tractus of the NTS	10.64 ± 0.50
ventrolateral subnucleus of the NTS	11.69 ± 0.69
lateral subnucleus of the NTS	10.68 ± 0.50
interstitial subnucleus of the NTS	13.26 ± 0.73
intermediate subnucleus of the NTS	16.10 ± 1.05
ventromedial subnucleus of the NTS	14.51 ± 1.03
dorsal subnucleus of the NTS	14.22 ± 1.17
medial subnucleus of the NTS	16.67 ± 1.35
substantia gelatinosus subnucleus of the NTS	15.52 ± 1.88
area postrema	15.07 ± 1.60
subpostremal subnucleus of the NTS	16.30 ± 1.42
dorsal motor nucleus of the vagus	17.82 ± 1.18
hypoglossal nucleus	14.08 ± 0.78

for use as a template for anatomical localization on its corresponding autoradiographic image. Optical densities were converted to fmol/mg of tissue using tritiated polymer standards (Amersham). Specific binding was calculated by subtracting non-specific binding from total binding within each region of interest.

RESULTS

The density of 5-HT_{1A} receptors in the human caudal NTS was found to be low overall, with the results listed in Table 1 and depicted in Figure 1. The highest 5-HT_{1A} receptor binding in the dorsal vagal complex was seen in the DMN. High receptor binding (16-18 fmol/mg) was found in the medial, subpostremal, and intermediate subnucleus of the NTS. An intermediate level of binding (14-16 fmol/mg) was found in the substantia gelatinosus, ventromedial, and dorsal subnuclei of the NTS, and the area postrema and hypoglossal nuclei. The lowest level of binding (10-14 fmol/mg) was found in the interstitial, ventrolateral, and lateral subnuclei, and the tract itself. Relative significance of mean specific binding between the subnuclei is depicted in Table 2. Non-specific mean binding was 20% of the total binding and homogeneous throughout the gray matter of the medulla. There was no correlation between binding densities and subject age, post-mortem interval, or freezer storage time (data not shown).

DISCUSSION

This study quantified the relative distribution of 5-HT_{1A} receptors in the ten subnuclei of the human caudal NTS and the adjacent area postrema, dorsal motor nucleus of the vagus, and the hypoglossal nucleus. Highest receptor densities were found in the DMN, and the medial, intermediate, and subpostremal subnuclei of the NTS. Intermediate levels of binding were found in the substantia gelatinosus, ventromedial, and dorsal subnuclei of the NTS, and the area postrema and hypoglossal nuclei. Lower receptor binding levels were seen in the interstitial, ventrolateral, and lateral subnuclei of the NTS, and the tract itself.

With the notable exception of the DMN, the present findings are in basic agreement with previous studies in humans and non-human mammals. In the rat, high levels of binding have been found in the central and intermediate subnuclei of the NTS (11,13). The rat central subnucleus is homologous to a portion of the medial subnucleus in humans (1). Thor et al. (12) also found high 5-HT_{1A} binding in the lateral and interstitial subnuclei of the rat, in disagreement with a study by

Table 2. Relative significance of mean specific binding in the human dorsal complex of the medulla.

	T	VL	L	IS	INT	VM	D	M	Gel	AP	SAP	DM	12
T	-				*	*	*	*	*	*	*	*	*
VL		-			*			*	*	*	*	*	
L			-		*	*	*	*	*	*	*	*	*
IS				-				*				*	
INT	*	*	*		-								
VM	*		*			-						*	
D	*		*				-					*	
M	*	*	*	*				-					
Gel	*	*	*						-				
AP	*	*	*							-			
SAP	*	*	*								-		
DM	*	*	*	*		*	*					-	*
12	*		*									*	-

* Significantly different at 95% using the Fisher PLSD. **T:** tract of the nucleus of the solitary tract (NST); **VL:** ventrolateral subnuclei of the NST; **L:** lateral subnuclei of the NST; **IS:** interstitial subnuclei of the NST; **INT:** intermediate subnuclei of the NST; **VM:** ventromedial subnuclei of the NST; **D:** dorsal subnuclei of the NST; **M:** medial subnuclei of the NST; **Gel:** substantia gelatinosus subnuclei of the NST; **AP:** area postrema; **SAP:** subpostremal subnuclei of the NST; **DM:** dorsal medial nucleus of the vagus; **12:** hypoglossal nucleus.

Mannaker and Verderame (11) and this human study. This discrepancy could be due to problems in anatomic localization as noted by Thor et al. (12) and to species differences.

In the present study, the DMN exhibited the highest relative density of 5-HT_{1A} receptors in the human dorsal vagal complex. Previous studies reported lower levels of binding in the DMN compared to the NTS (2,11-13,16,17). Within the DMN, a recent vagotomy study found that 5-HT_{1A} receptors reside on interneurons and other neuronal elements rather than vagal preganglionic motor neurons (18). Moreover, the current finding is consistent with functional studies of 5-HT_{1A} receptors in the DMN. Physiological investigations using 8-OH-DPAT in the rat, cat, and rabbit suggested the presence of functionally significant numbers of 5-HT_{1A} receptors in the DMN (14,19-21). 8-OH-DPAT microinjection into the right and left DMN caused bradycardia in rats and cats, thought to be secondary to increased vagal outflow to the heart (14,20,21). There was also an increase in central respiratory rate due to increased phrenic nerve activity from DMN stimulation with 8-OH-DPAT (20,21).

The DMN receives vagal efferents from the thoracic and abdominal viscera, and is the site of origin of preganglionic parasympathetic efferent fibers to the same (22,23). These findings suggest a primary role for 5-HT in the neural regulation of respiration. 5-HT_{1A} receptors located on central neural elements within this circuit may account for the effects of 8-OH-DPAT on cardiovascular and pulmonary function. Thus, if there is a preservation of function across species, 5-HT_{1A} receptors may regulate cardiac and respiratory function within the human DMN. Although the current findings, with respect to the relative binding in the DMN compared to the NTS, differ from other distribution studies, the current results are enhanced by the large number of subjects in the study and the consistency of the results with physiological studies of 5-HT_{1A} receptors in the DMN.

Interpretation of the role of 5-HT_{1A} receptors in the human NTS and area postrema also depends upon the functional and anatomical homologies of these structures across species. In the rat and the cat, the intermediate nucleus receives pulmonary and tracheal afferents (24,25). Vagal sensory afferents to the ventral NTS, a subnucleus that receives prominent innervation from the respiratory viscera in the rat and cat (23,26), are serotonergic (27). These findings suggest that 5-HT_{1A} receptors in the human intermediate and ventrolateral subnuclei may modulate cardiorespiratory functions, in the rat, the subpostremal nucleus is important in the integration of cardiorespiratory efferents (24). Chemoreceptors and baroreceptors project to the subpostremal subnucleus as well as to the medial subnucleus of the rat (25). In rats, cats, and rabbits, the medial subnucleus also receives sensory fibers from baroreceptors in the aortic arch and carotid sinus, whereby it exerts effects upon the control of brood pressure (25). The 5-HT_{1A} receptors in the corresponding subnuclei in the human caudal NTS may help modulate respiration and blood pressure.

The dorsal vagal complex plays an important role in modulating gastrointestinal function, and may be part of the neural network regulating food intake. Relatively high [^{3}H] 8-OH-DPAT binding was seen in the human medial subnucleus of the NTS. The central subnucleus in the rat, which has partial homology to the medial subnucleus in the human NTS, receives esophageal afferents and may serve as a link to the compact formation of the nucleus ambiguus, which is the origin of motor neurons to the esophagus (26,28,29). Thus, the medial subnucleus in the human caudal NTS may play a significant role in swallowing and other functions of the upper alimentary canal. In the cat, the medial subnucleus is also the primary site of gastrointestinal afferent termination, particularly from the stomach (25). Serotonergic vagal sensory afferents from the nodose ganglia terminate in and immediately underneath the area postrema in the rat, suggesting a role for serotonergic receptors in these regions mediating gastrointestinal reflexes (27). Serotonin release is elevated in the dorsal vagal complex in fed compared to fasted rats (30). Furthermore, small increases of gastric motility can be produced by co-injections of serotonin and thyrotropin-releasing hormone into the dorsal vagal complex (31). Taken together, these studies suggest that 5-HT_{1A} receptors in the human medial subnucleus may modulate the function of the alimentary canal and play a role the complex regulation of feeding behavior.

Several other subnuclei within the NTS have notable levels of 5-HT_{1A} receptors. The role of these 5-HT_{1A} receptors in visceral functions can be inferred from the neuroanatomical studies in non-human mammals. Like the adjacent medial subnucleus, the intermediate subnucleus of the rat receives abundant gastrointestinal afferents, mainly from the upper alimentary canal. These afferents appear to regulate esophageal and gastric activities such as swallowing, gagging, and vomiting (24). Subcutaneous administration of 8-OH-DPAT blocks vomiting in the cat elicited by motion, cisplatin, and xylazine (32). This suggests that 5-HT_{1A} receptors located on neural elements such as the medial and intermediate subnuclei of the NTS and the area postrema may play an important role in the neural mediation of nausea and vomiting.

Finally, it should be noted that dendrites from the DMN, and axons from a variety of structures, including

but not limited to the area postrema, parabrachial nuclei, and hypothalamus, terminate in the NTS (33-37). Localization of 5-HT_{1A} receptors on these neuronal elements, rather than on neuron cell bodies in the structures studied, cannot be ruled out. Nevertheless, the presence of 5-HT_{1A} receptors within anatomical components of the dorsal vagal complex, whether on cell bodies, dendrites, or presynaptic terminals, suggests an important role for this receptor system in the function of each anatomical component.

In summary, this study has redefined the distribution of 5-HT_{1A} receptors in the human dorsal vagal complex. Relatively high levels of [^{3}H]8-OH-DPAT binding were found in the medial and intermediate subnuclei of the NTS, in accordance with previous reports. In contrast to previous reports, but in agreement with physiological studies, the present study noted high binding in the DMN. This binding pattern suggests an important role for 5-HT_{1A} receptors in the modulation of peripheral afferent input and vagal outflow to the gastrointestinal and cardiorespiratory systems.

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