Tyrosine Hydroxylase Neurons in the Rat Dorsal Raphe Nucleus: A Morphological and Topographical Immunohistochemical study

Jameie SB*, Behzadi G**, Noyan MH***

Abstract

Background: The dorsal raphe nucleus (DRN) is one of the main serotonergic nuclei in the mammalian nervous system which is involved in many functions. The DRN mainly consists of the largest number of serotonergic neurons intermingled with catecholamine containing neurons. In order to define the morphology and location of tyrosine hydroxylase immunoreactive (TH-ir) cells in different areas of DRN we performed TH immunohistochemistry for the adult rat DRN.

Material & Methods: Adult Sprague-Dawley male rats (250-300gr) were used in this study. Following transcardially perfusion-fixation with aldehyde solutions, the brain stems were removed. By using vibrotome, 40mM thick coronal sections were cut and then processed for TH immunohistochemistry. Sections were mounted and coverslipped and studied by light microscopy.

Results: TH positive neurons were observed mainly in the rostral up to the end of the central part of DRN. Four types of TH-ir neurons with different distribution were observed in the nucleus. Among the total counted cells, the ovoid and polygonal neurons respectively showed the highest and lowest density in DRN.

Discussion: The TH positive neurons in DRN that could be dopaminergic in nature may participate in their major projections to the different areas received putative dopaminergic inputs from DRN, i.e. the striatum, hippocampus and the basal forebrain. None of the serotonergic projections from DRN influence the functions of these areas.

Key words: **Dorsal Raphe Nucleus, Tyrosine-hydroxylase, Immunohistochemistry, Dopaminergic neurons**

* Department of Anatomy- Cellular & Molecular Research Center, Iran University of Medical Sciences- Tehran, Iran

** Department of Physiology- Shahid Beheshti University of Medical Sciences- Tehran- Iran

*** Department of Anatomy and Cell Biology- University of Saskatchewan- Saskatoon- Canada.

Introduction

The dorsal raphe nucleus (DRN) is the largest single collection of neurons containing serotonin in the entire brain[1, 2, 3, 4]. DRN is thought to be involved in a wide variety of complex physiological and behavioral processes, such as autonomic functions, vigilance states, sleep, analgesia, mood, affect, learning, movement and memory[5, 6, 7,8,9]. The fact that the DRN is a major source of different neurotransmitter based innervation to the trigeminal somatosensory system, cerebral cortex, basal ganglia, medial prefrontal cortex, nucleus accumbens, striatum, hippocampus, basal forebrain, hypothalamus and thalamus[4,10,11,12,13, 14,15,16,17], where many of these functions are integrated, makes this relatively small brainstem nucleus particularly attractive. Neurons of DRN produce a wide variety of neurotransmitter- related molecules other than 5-HT, including dopamine, glutamate, GABA, substance P (SP) and enkephalin. There are some reports on the morphology and localization of the different types of non-serotenergic immunoreactive neurons encountered throughout the DRN[18]. By using histofluorescence techniques, the presence of catecholamine- containing neurons in rat DRN has been shown[19, 20]. SP, GABA and glutamate immunoreactive neurons have been shown to be distributed throughout rostrocaudal extent in DRN[18]. These various molecules are produced either by distinct neurons in DRN or are coexpressed with other chemical markers in different combinations. The present TH immunohistochemical study was undertaken to determine the morphology and distribution of Tyrosine Hydroxylase Immunoreactive (TH-ir) neurons in different areas of the rat DRN.

Materials and Methods

Ten adult male Sprague- Dawley rats (250-300 g) were used in this study. The animals were maintained on a 14:10 hour lighting schedule with food, water and libitum. All the animals were anesthetized with intraperitoneal injection of sodium pentobarbital (45 mg/kg) and

transcardially perfused with 300ml of heparinized saline solution (0.9%), followed by 350 ml of fixative solution containing 4.0% paraformaldehyde, 0.5 % glutaraldehyde and 0.2 % picric acid in phosphate buffer (PB; 0.1M, pH 7.4). Afterwards, the brains were removed and postfixed in the same fixative at 4°C overnight. By using vibrotome, transverse serial sections of 40μ thickness of the brain stem were cut throughout the rostrocaudal extent of the DRN. The sections were first perincubated in a solution containing 10% of normal serum and 0.4% of Triton X-100 (Sigma) for 30 minutes at the room temperature and then incubated with rabbit TH anti-serum (Chemicon Inc 1:500) for 16 hours at the room temperature. The primary antibody were diluted in a solution of phosphate buffer sodium (PBS) containing 0.1% Triton X-100. After this incubation, the sections were rinsed in PBS and reincubated for 2 hours at the room temperature with biotinylated goat anti-rabbit IgG (Vector) diluted 1:50 in a PBS solution containing 0.1% Triton X-100. This was followed by extensive washing in PBS and incubation in the avidin-biotin- peroxidase complex (ABC kit; Vectostain 1:100 in PBS) for 1 hour at the room temperature. After a 30 minute rinse in PBS and Tris buffer (0.05 M, pH 7.6), the bound peroxidase was revealed by placing sections in a solution containing 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, 0.05%) and H2O2 (0.005%) in Tris buffer for 10 minutes. This chromogen produces a brown and diffuse reaction product in the immunoreactive elements. The reaction was stopped by repeated washes in PBS. All sections were mounted on gelatin-coated slides, dried overnight, dehydrated in serial ascending concentrations of alcohol and coverslipped. Observation was done by using light microscopy.

Results

I: Distribution of TH-ir neurons

The morphology, localization and distribution of TH immunoreactive neurons encountered the rostrocaudal extent of the DRN were examined in ten rats used in this study. Among all immunostained neurons, only those with well delineated nucleus or clearly visible processes were mapped and counted. The morphological characteristics and patterns of distribution of the TH positive neurons in the DRN were remarkably similar in all the animals examined in this study. Thus the overall topographical distribution of the TH immunoreactive neurons in DRN can be considered as highly representative of the TH positive neurons throughout the rostrocaudal extension of the nucleus. To describe the distribution of the TH-ir neurons in DRN we used the nomenclature reported by Baker et al and Tork et al based on immunohistochemical and cytoarchitectonic of DRN[3,21]. According to their definition, six subnuclei including median or intrerfasicular, ventral, dorsal, ventrolateral, lateral and caudal exist in DRN. The first five subnuclei are mostly confined to the midbrain portion of DRN and the caudal subnucleus extends with the pontine portion of the nucleus. It is worth noting that a clear boundary could not be seen between the different partitions of the nucleus[21].

Numerous TH-positive neurons were observed mainly in rostral up to end of the central part of the nucleus that is corresponding to the midbrain portion of the nucleus described previously (Fig. 1A). They lie among a highly stained neuropil (Fig. 1B).Although some neurons of this type are occasionally seen within other parts of the nucleus. The most rostrally located TH- ir neurons were seen at the caudal level of the oculomotor nerve nucleus. The TH-ir cells located between the two MLF belonging to the median and ventral subnuclei (Fig. 1C, D). Subaqueductal TH-ir neurons are bipolar with processes that surround the cerebral aqueduct. Few TH-ir cells are also dispersed more laterally in the midbrain tegmentum. The population of the TH positive neurons is maximally developed at the level of the caudal part of the trochlear nerve nucleus, where most immunoreactive neurons extend along the midline from the dorsal aspect of the MLF to the ventral border of cerebral aqueduct (Fig. 2E). The caudal portion of the nucleus is devoid of TH-ir neurons.

II: Morphology of TH-ir neurons

Among the total TH-ir neurons of DRN, three different types of neuronal population including bipolar fusiform, round and multipolar perikarya have been revealed. Most of the TH-ir cells were small (about 18μm) bipolar fusiform cells (Fig. 2E, F) which mostly located in the dorsal and ventral part of the center of DRN i.e. midbrain portion of the nucleus. These cells often emit thin, long and varicose processes that branch at a considerable distance from the cell body (Fig. 2E). The round type neurons were mainly dispersed among the dense population of fusiform neurons (Fig. 2E, G). A few large (about 32μm) immunostained multipolar neurons can also be seen scattered in all reacted areas of DRN (Fig. 2E, G).

Fig. 1- Photomicrographs of TH-immunoreactive neurons in the dorsal raphe nucleus. A, lower power view of neurons seen at the anteroposterior level. B-D, higher power views of neurons in the periaquedactal gray beneath the cerebral aqueduct (B), and the median subnucleus (C, D). Scale bars= 500 μm (A), 250 μm (B) and 100 μm (C & D).

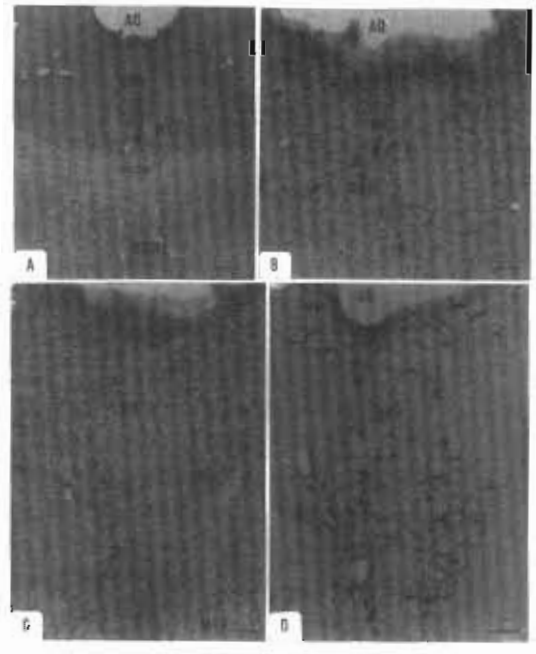
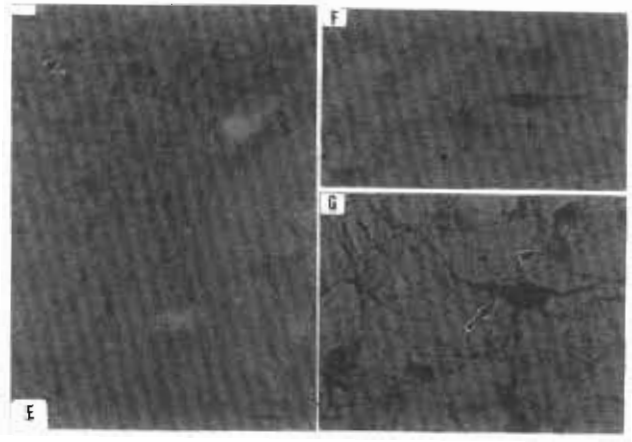


Fig. 2- Photomicrographs of TH-immunoreactive neurons in the dorsal raphe nucleus. E, higher power view of TH-ir neurons in the median and intrerfasicular portion of the nucleus. F, G, examples of TH-ir neurons in dorsal raphe nucleus, a bipolar fusiform (thin arrow) , a large, multipolar well immunostained (thick arrow) and a round weekly immunostained one(arrowhead). Scale bars= 250 μm (E), and 50 μm (F & G).



Discussiou

Immunohistochemical studies using different antibodies against certain neurotransmitters or their synthesizing enzyme have demonstrated the presence of non-serotonergic neurons in dorsal raphe nucleus [15, 19, 22, 23]. Although there are few reports concerning the presence of the TH-ir neurons in the different levels of DRN of rats[23, 24], cats[25], monkeys[18] and humans[26], morphology and the exact distribution of these chemospecific neurons in regards to the various divisions of the rat DRN have never been described. In the present study, one main cluster of TH-ir neurons located in the midbrain portion of DRN has been visualized. Chara et al. reported two subsets of TH-ir neurons in the squirrel monkey located in the same area as our data showed[18]. In another study it has been shown that the localization of dopaminergic cell bodies in the guinea-pig brain appeared similar to that in the rat brain[27]. The TH-ir neurons in the rat DRN were shown to be distinct from serotonergic neurons, which represent only one-third of the total neuronal population of the nucleus[9, 24]. Regarding to the nature of TH-ir neurons in DRN, it has been shown that these neurons do not express the enzyme dopamine-β-hydroxylase, a finding that supports the dopaminergic nature of these neurons[25, 27]. By using neural tracing techniques it is shown that dopaminergic neurons in the DRN, project to the striatum, nucleus accumbens, lateral septum and medial prefrontal cortex, but not to the substantia nigra[9,23]. It is also shown that there is a topographical distribution in the dopaminergic projections of DRN, i.e. the dopaminergic neurons projecting to the striatum are located in the midbrain division of the nucleus, whereas the nucleus aecumbens recieve dopaminergic fibers from the neurons locating ventral to the cerebral aqueduct and the less numerous dopaminergic neurons which project to the cortex and lateral septum are mainly distributed distal to those project to nucleus accumbens[23]. Common projections of DRN and ventral tegmental area in the rat have led to the probability that the dopaminergic neurons DRN are actually the extension of dopaminergic population of ventral tegmental area[23]. To approve this hypothesis further studies are needed.

Functional Consideration

Although dopamine-containing cell bodies were previously thought to be almost exclusively confined to the substantia nigra pars compacta, ventral tegmental area, and tuberoinfundibular system, our results indicate the existence and distribution of numerous dopaminergic neurons in the rat DRN, however the exact role of dopaminergic neurons of DRN and its projections are still unknown. Considerable evidence suggests that a dysfunction of the dopamine and serotonin neurotransmitter systems contributes to a diverse range of pathological conditions including schizophrenia, depression and drug abuse[28]. Tyrosine hydroxylase immunohistochemical study of the DRN of severely parkinsonian cats and monkeys revealed sever loss of dopaminergic neurons in this region. Cell loss was more extensive in the ventral portion of the nucleus with a relative sparing of neurons in the dorsal-most portions of the DRN[29]. Thus, findings in this study could have important implications for understanding and treatment of 5-HT and dopamine circuitry dysfunction in certain pathological conditions. Taken together, these findings also provide further evidence of neurochemical specificity and functional anatomical organization within the DR efferent projection system.

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