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Synaptic Remodeling in the Vagal Nuclei Following Vagal-hypoglossal Nerve Anastomosis

ABSTRACT

Synaptic remodeling associated with neuronal and glial changes in the vagal dorsal motor nucleus (DMV) and nucleus ambiguous (NA) after vagal-hypoglossal nerve anastomosis (VHA). At 25 days postoperation (dpo), there were 50% fewer presynaptic boutons containing round vesicles (R) or round and large dense-cored synaptic vesicles (R+D) contacting HRP-labeled DMV motoneurons. The loss of R boutons was maintained throughout the remaining postoperative intervals up to 500 dpo, whereas R+D boutons were further reduced at 123 dpo but were restored at 315 dpo so that by 500 dpo, 71.4% of them had gained access to the DMV motoneurons. Boutons containing pleomorphic synaptic vesicles (P) were completely disconnected from the DMV motoneurons at 25 dpo and did not reappear even in long-term reinnervation stage. Loss and recovery of presynaptic boutons occurred in parallel with changes in astroglial ensheathment of the DMV motoneurons. However, the vagal efferent neurons in the NA had responded to VHA in a different manner. Firstly, the numbers of R, R+D, P or F (containing flattened synaptic vesicles) boutons contacting the NA motoneurons were markedly increased at 500 dpo. Secondly, the astroglial ensheathment was not evident in the NA. Thirdly, the extensive dendritic sprouting of the NA neurons as opposed to the dendritic retraction of the DMV neurons. The differences between NA and DMV may be attributed to the unique nature of the two nuclei to structures they normally supply and their different compatibility with the newly innervated target, viz. tongue skeletal musculature.

INTRODUCTION

Experiments using vagal-hypoglossal nerve anastomosis (VHA) have been carried out in rats by Flumerfelt et al. (1986) [8] and McWilliam et al. (1995) [33] and, more recently, in cats by us [5, 27, 28]. These studies [8, 33] had demonstrated that after VHA, the general visceral efferent (GVE) neurons in the dorsal motor nucleus of the vagus (DMV) can reinnervate the tongue musculature by sending their axon terminals to motor endplates initially occupied by the hypoglossal nerve [8]. Neuronal phenotype in the DMV was also altered from CGRP-negative to CGRP-positive [33]. Our study had extended this to show that in long-term VHA, the GVE neurons in the DMV exhibited signs of dendritic withdrawal and that they were ensheathed by hypertrophied astroglial processes [27, 28]. In light of the above, we suggested that the autonomic nature of the DMV motoneurons was not appropriate for the newly acquired target, viz. the tongue skeletal musculature, for voluntary movement and, hence, some chemical [33] and structural [5, 27, 28] modifications would be necessary for the regenerating DMV motoneurons to facilitate a better functional recovery after VHA. Furthermore, with the astroglial ensheathment induced by VHA, the regenerating DMV motoneurons could physically displace most, if not all, of the synaptic boutons related to autonomic function [27]. Our experimental results furthermore suggested that neural plastic changes in the DMV may be influenced by some retrogradely transported factors/signals derived from the new target organ, tongue muscles [27]. This view is

substantiated by the fact that unlike the dendritic withdrawal occurring in the DMV, motoneurons in the nucleus ambiguus (NA), another nucleus of the vagus nerve, exhibit a profound dendritic sprouting after VHA [5, 28]. In addition, the astroglial ensheathment observed in the DMV does not occur in the NA [27]. Moreover, the myelinated axons emanating from the NA, but not the DMV, acquire a better interaction with the motor end-plates initially occupied by the hypoglossal nerve [28].

The DMV is preganglionic autonomic in function and projects to postganglionic neurons that innervate the smooth muscles and glandular cells in the abdominal viscera as well as the heart and other supradiaphramatic structures [6, 9, 13, 20]. It is also known to receive extensive projections from the nucleus of solitary tract (NTS) which plays a pivotal role in the central visceromotor system [30, 37, 50]. Although the NA also contains some preganglionic autonomic neurons projecting to the ganglia associated with the heart, lungs, stomach and pancreas [3, 4, 23, 24], it resembles to a large extent a somatic nucleus such as the hypoglossal nucleus as it mainly innervates the branchiomeric skeletal musculature in the soft palate, pharynx, larynx and esophagus [3, 7, 21, 24]. In view of this unique nature, the two component nuclei, NA and DMV, of the vagus nerve would be ideal for comparison of the effect of VHA on synaptic remodeling. Since the VHA had reestablished a new neural pathway, it was hypothesized that retrogradely transported factors released by the new target, altered neuronal microenviroment and neural circuit may reset different plastic changes in the two nuclei. Morphometric analyses were thus performed by electron microscopy to further elucidate in what way the NA and DMV neurons would exhibit differential neural plasticity.

RESULTS AND DISCUSSION

This study has provided the first ultrastructural evidence of a dynamic ongoing remodeling process involving the pre- and postsynaptic elements of the DMV motoneurons at different reinnervation intervals after VHA. The principal findings are as follows: (1) compared with the control, there was a rapid decrease (55.4%) in the mean synapse frequency of R boutons on labeled somata and dendrites at 25 dpo. This remained relatively unchanged (52.5%) up to 500 dpo. On closer analysis, the loss of the boutons occurred mainly at somata and proximal dendrites in the long-term reinnervation stage; (2) in parallel with the R boutons, the mean synapse frequency of the R+D boutons was markedly decreased by 57.2% at 25 dpo and was further reduced by 72.2% at 123 dpo. Thereafter, R+D boutonal number increased so that there was only 28.6% reduction in the mean synapse frequency at 500 dpo. It is noteworthy that the percent increase in R+D boutons was greater on somata than on distal, secondary and proximal dendrites; (3) P bouton disappeared after VHA, and did not reappear; (4) the initiation, enhancement and consequent retraction of astroglial ensheathment around the regenerating DMV motoneurons as described previously [27] appeared to occur concurrently with loss and recovery of synaptic boutons; (5) the HRP labeling frequencies of distal and secondary dendrites were reduced in early reinnervation stage, but progressively increased with time. This is in contrast to that of somata and proximal dendrites and is in agreement with our previous observations of the initial massive reduction of dendrites of HRP-labeled DMV neurons [27,

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28]. The dendrites then regenerated giving rise to branches with time, but they never regained normal morphological features after VHA [27, 28]; and (6) there was considerable increase in spinous protrusions from the somata and large dendrites of the DMV motoneurons after VHA. In long-term survival animals, R+D boutons were observed on virtually all the spines that, in contrast, in the intact DMV were only postsynaptic to the R boutons.

On the other hand, the differential response of NA and DMV neurons to VHA treatment is unequivocal as shown in this study. For example, VHA resulted in a profound astroglial reaction in the DMV [27], a feature that was lacking in the NA. Astroglial hypertrophy has been reported in the monkey DMV following vagotomy [29], in which hypertrophied astrocytes along with activated microglial cells were involved in the phagocytosis of darkened dendrites and degenerating axon terminals. Short-term (4 months) and reversible ensheathment of facial motoneurons by astrocytic lamellar processes following facial nerve transection has also been described by Graeber and Kreutzberg [11, 12]. We suggested previously [27] that the astroglial ensheathment of DMV motoneurons following VHA was not involved in retrograde degenerative changes in injured neurons [11, 29], neither could it be considered a protective mechanism during neuronal regeneration [12]. It was reasoned that a similar astroglial reaction would also be observed in the NA if indeed the astroglial ensheathment were involved in the above functions. Thus, the occurrence of astroglial ensheathment in the DMV and the lack of it in NA may represent more than a phenomenon simply coupled with the retrograde axon reaction as described previously [11, 12, 29]. Indeed, the present results suggest that there may exist a more complex interaction of neuroplastic process between the central nuclei and their newly innervated peripheral target organ with time following VHA. We suggested earlier that astrocytes would play a key role in the synaptic stripping of inappropriate boutons related to autonomic function in the DMV [27]. The role of astrocytes in NA remains speculative in view of their very mild response. From a speculative point of view, the astrocytes in NA may facilitate a selective augmentation of appropriate boutons related to voluntary control of tongue movement for better overall functional recovery after VHA.

A salient feature in the NA in long-term reinnervation stage of VHA was the significant increase in distal dendrites of NA motoneurons as revealed by HRP labeling. This is in stark contrast to DMV neurons which showed signs of dendritic withdrawal [27], but is in agreement with our previous observations of the profuse dendritic sprouting of HRP-labeled NA neurons [5, 28]. It is well documented that parent neuronal interaction with targets determines the dendritic configuration. Depletion of this interaction such as by axotomy usually causes retraction of dendrites [4, 35, 38, 46]. Cat neck motoneurons on the contrary expand their dendritic trees probably due to the proximity of axotomy to soma [41], a feature observed in both invertebrates (helisoma, cricket and aplysia) [34, 40, 42] and vertebrates (sea lamprey) [14−16]. On the other hand, in the latter instance, the gross morphology, ultrastructure and trajectories of the sprouts resemble those of axons rather than dendrites [16]. In the present VHA model, the interaction of vagal efferent neurons with their targets was shifted but not removed. The alteration of targets has resulted in retraction of dendrites of DMV neurons but expansion of those in the NA neurons. This novel finding of dual and totally different dendritic

responses in the two vagal nuclei suggests that it was not axotomy but peripheral nerve cross-anastomosis that is responsible for the massive dendritic branching in the NA. It also rules out the possibility that the location of nerve transection is a causative factor for the dendritic sprouting, otherwise the same phenomenon would have occurred in both vagal nuclei. Target-derived neurotrophic factors are known to promote the survival and differentiation of presynaptic neurons [26, 36]. Even the transmitter phenotype of a neuron can be altered by signals derived from the target [33, 39, 43]. Thus, the survival, nature, and function of a neuron may be critically dependent on retrograde cues from its postsynaptic associates. Our results indicate a much more successful neuronal regeneration in the NA than DMV following VHA, thereby suggesting that retrograde, transsynaptic and specific signals from tongue skeletal muscles to the two nuclei match the NA but not DMV motoneurons, thus triggering the selectivity during competitive reinnervation.

Besides dendritic branching, growth changes were found at the presynaptic level after VHA. The NA motoneurons showed a significant increase in numbers of synaptic contacts due primarily to the increases of R+D, P and F boutons with a minor contribution from the R boutons. The different types of bouton associated with the various elements of the HRP-labeled NA neurons were also reorganized. On closer analysis, the percent increase in the synapse frequency of R, R+D and P boutons occurred mainly at distal dendrites, while that of F boutons occurred mainly at somata and large dendrites in the long-term reinnervation stage following VHA. It would appear from the above that the generation of new axon terminals and reorganization of synapse distribution to a large extent coincides with the expansion of dendritic trees and involves notably the distal dendrites of the regenerating NA motoneurons. In other words, the VHA has induced a distal shift in the relative distribution of synaptic boutons. It has been shown that the differentiation and shape of distal dendritic trees may be influenced by the impinging afferent fibers [44]. It is also known that peripheral control of the lingual musculature originates from temporomandibular joint receptors and sensory fibers contained in the trigeminal, glossopharyngeal and superior laryngeal nerves [31]. Axons from these nerves terminate in the NTS and sensory trigeminal nuclei, which in turn make monosynaptic or at least disynaptic contacts with the hypoglossal motoneurons [17, 47–49]. The NA, another key nucleus involved in the oral digestive behavior, can initiate and control the swallowing in response to intraoral stimuli. The neural apparatus mediating buccopharyngeal and esophageal phases of swallowing include peripheral sensory inputs via the pharyngeal and superior laryngeal branches of the vagus nerve, central relay neurons in the NTS, premotor neurons in the brainstem reticular formation, and bronchomotor neurons in the NA [1, 2, 22, 25]. The NTS also has direct synaptic connection with the NA motoneurons [18, 19, 47]. Since the present VHA has changed the peripheral sensory input pathway, i.e. sensation from the tongue instead of the esophagus, pharynx, larynx and thoracic and abdominal viscera directly to the NTS, it is speculated that the alteration in this neural circuit may have affected the dendritic configuration of NA motoneurons bringing about their sprouting.

It has been described that the synaptic location on a neuron is an important factor governing the shape of a propagating action potential [45]. Synapses on soma, proximal, and

secondary dendrites are thought to be more efficacious in influencing the neuronal outflow than those farther away on the distal dendrite. On the other hand, synaptic responses initiated in the most distal parts of the dendritic trees could exert a more precise control over the generation of impulses in the cell body and initial axonal segment. Thus, the presence of a relatively larger number of increased R, R+D and P boutons on the distal dendrites of NA motoneurons after VHA suggests that the regenerating NA neurons may enhance the precision of certain input information concerning intraoral stimulation or premotor control. In addition, the considerable increase in the synapse frequency of R+D, P and F boutons occurring at the somata and larger dendrites suggests that these regenerating NA neurons are probably more effective in modulating the nerve outputs for better execution of their newly acquired function, control of tongue movement.

As described above, VHA-induced growth changes include also sprouting of terminals onto the original neuronal elements and/or the newly formed dendrites and spines of NA motoneurons. The additional boutons must arise from the sprouting, i.e. proliferation of terminals, from the preexisting synapses or from the newly generated axons that might derive from sources not normally projecting to the NA motoneurons. For example, Travers and Norgren [47] have demonstrated that most of the premotor neurons projecting to the trigeminal, facial, ambiguus and hypoglossal motor nuclei of rats were from the brainstem reticular formation, frequently in areas adjacent to other synergetic motor nuclei. In other words, the reticular formation lateral to the hypoglossal nucleus and reticular structures surrounding the trigeminal motor nucleus projected to each of the four brainstem motor nuclei involved in oral-facial function [47]. In the NA of cats, it has been shown that the immunoreactivity of substance P is often associated with large dense-cored vesicles in terminals that are presynaptic to vagal efferent neurons [32]. These nerve terminals resemble the R+D boutons described in the present study. Substance P nerve terminals are also observed to make synaptic contacts with the hypoglossal motoneurons in cats, suggesting a role of this neuropeptide in the control of fine movements of the tongue [10]. In view of their similarity to premotor neuron pool, the possibility that the increased R+D boutons impinging on the NA motoneurons after VHA are derived from collaterals of the hypoglossal nucleus-projecting substance P nerve fibers should be considered.

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