Understanding the regional specificity of neurons in the trigeminal subnucleus caudalis and spinal dorsal horn

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Neurons in the trigeminal subnucleus caudalis (Vc) and spinal cord dorsal horn (SDH) play important roles in modulating and relaying pain signals to the higher centers of the central nervous system (CNS). Morphologically, many aspects including a laminated structure, cytoarchitecture and cellular elements in these two central regions are very similar [1,2,3,4]. Most nociceptive afferents with a small-diameter terminate in the superficial laminae of Vc and SDH. Functionally, sensory neurons in Vc and SDH respectively receive nociceptive inputs from the orofacial and other somatic regions, and convey the inputs to higher brain centers [1,2,3,4,5]. According to the mechanoreceptive properties, sensory neurons in both the Vc and SDH are classified into three types. They are low-threshold mechanoreceptive (LTM), wide-dynamic range (WDR) and nociceptive-specific neurons (NS) [1,2,3,4,5]. Because of these similarities Vc has been considered as analogous to SDH [4,6]. However, some differences between Vc and SDH have also been reported. For example, the distribution pattern of substance P (SP) and calcitonin gene related peptide (CGRP) in Vc of adult animals is different from that in SDH. In SDH there is no dual representation of peripheral regions, which can be found in rostral and caudal Vc. A transitional zone found between Vc and trigeminal subnucleus interpolaris plays a special role in sensorimotor function. However, no such region is found in the spinal system [5,7,8]. CNS neurons are regionalized. They are organized as groups through their circuitry. Although it is known that differences in functions among these groups in the adult CNS are predetermined during the development of the neuroepithelium [9,10,11,12,13], understanding how the regional specificity is developed in the CNS is still a big challenge in neuroscience research. It has recently been found that ninhydrin-reacting small molecules released locally are involved in the region-specific regulation of neuronal development in cultured Vc and SDH neurons [14,15]. In this review we focused on present knowledge about the region-specific regulation of neuronal development in these two CNS regions associated with the transmission of nociceptive signals.

Keywords: pain; regionalization; development; spinal cord; trigeminal subnucleus caudalis

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Introduction

The subnucleus caudalis of the spinal tract nucleus (Vc) in the dorsal region of the medulla represents the most caudal penetration of the trigeminal system [3, 4, 16]. Second-order sensory neurons in Vc and spinal dorsal horn (SDH) receive primary afferent inputs from the orofacial and other somatic areas of the body, respectively. Many morphological and functional similarities are noted between Vc and SDH. For example, nociceptive afferents with small-diameters terminate mostly in the superficial laminae [1, 2, 3, 4, 5]. There are three types of somatic sensory neurons found in both the Vc and SDH. They respond only to innocuous stimuli (e.g., tactile) (LTM neurons), or only to noxious stimuli (NS neurons); or to both innocuous and noxious stimulation (WDR neurons). Nociceptive neurons are located in the superficial and deeper laminae and relay nociceptive signals to the higher centers of brain [1, 2, 3, 4, 5]. The activity of nociceptive neurons in both the Vc and SDH have been found to be modulated by glial cells [17, 18]. Although some differences between Vc and SDH have been found (e.g. in the distribution pattern of CGRP and SP afferent fibers, and in the dual representation of peripheral areas) [5, 7, 8], Vc has been considered as analogous to SDH [4, 6].

Developmental differences of cultured Vc versus SDH neurons

Recent studies [14, 15] have demonstrated that when compared with cultured neurons from the SDH or hippocampus (Hip) of rat embryos (18 days gestation), Vc neurons grow much slower, there are a fewer number of primary processes and the length of the processes are shorter. Two weeks after the culture plating, the processes of Hip and SDH neurons appear merged and form networks while Vc neurons still remain separated [14, 15]. Electrophysiological data demonstrate that all neurons at day 14 in culture from Hip, SDH or Vc show whole-cell responses to glutamate in a concentration-dependent manner. Compared with the current density of SDH or Hip neurons, however, the density recorded in Vc neurons is significantly lower [14, 15]. Furthermore, less than 30% of recorded Vc neurons show spontaneous miniature excitatory post-synaptic currents (mEPSCs) which can be found in over 80% of recorded SDH and Hip neurons. The mean frequency and peak amplitude of mEPSCs recorded in cultured Vc neurons are around 1 Hz and 25 pA. Most mEPSCs recorded in Vc are mediated by both AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid) and NMDA (N-methyl-D-aspartate) receptors. The AMPA and NMDA-mediated components have decay time constants around 5 ms and 90 ms, respectively. These parameters are similar to those in SDH and Hip neurons [14, 15, 19, 20, 21].

Furthermore, a higher degree of cell death occurs in Vc than in SDH. Co-labeling of TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) with TuJ1, NeuN or GFAP antibody shows that more mature neurons undergo cell death in Vc when compared with that in SDH. However, cell death in young neurons or glial cells appears similar in these two regions [14, 15].

To understand the mechanisms underlying the differences observed in these regions, studies effects of different culture media e.g. neurobasal medium supplemented with B27, β-FGF2 and L-glutamine (which minimizes the growth of glial cells (<15%) [22]) or MEM supplemented with 10% fetal bovine serum and insulin were examined. Changing the culture medium or altering the concentration of β-FGF2 or insulin added into the medium does not produce significant effects on the difference in cell survival between Vc and SDH [14, 15]. However, co-culture of Vc with SDH tissues significantly enhances the growth of Vc neurons. The Vc neurons have more and longer processes, and higher glutamate-mediated current density when compared with Vc neurons not in the co-culture. The number of Vc neurons showing spontaneous mEPSC activity is also increased. In contrast, co-culture of SDH with Vc tissue has significantly depressed the growth of SDH neurons. The SDH neurons have less and shorter processes, and reduced glutamate-mediated current density when compared with SDH neurons not in the co-culture. The number of SDH neurons showing spontaneous mEPSC activity is also reduced [14, 15]. These data strongly suggest that some factor(s) are released in culture, which may cause the differences in neuronal development observed in Vc versus SDH.

Many factors such as growth factors [23, 24, 25, 26, 27, 28, 29, 30, 31] and non-protein small molecules e.g. ninhydrin-reacting factors (NF) [14, 15, 32, 33] have been reported to be involved in the regulation of neuronal growth. Since altering the concentration of β-FGF2 or insulin added into the culture media does not produce any significant effects on the differences in neuronal growth observed in Vc versus SDH, effects of non-protein small molecules such as ninhydrin-reacting factors (NF) isolated from Vc, SDH or Hip-conditioned medium were examined [14, 15].

Two ninhydrin fractions (NF1 and NF2) were isolated from media used for cell culture by column chromatography using Sephadex G-25 followed by G-15. Every fraction isolated was frozen, lyophilized and dissolved in cultured medium (75 µL/mL) to test on cultured cortical neurons [14, 15]. When compared to those in cortical cultures without any treatment the Hip- or SDH-NF1 isolated from Hip- or SDH-conditioned culture medium significantly enhances the
growth and viability of cultured cortical cells. However, treatment with the Vc-NF1 does not have such effects.

No significant change in cell growth or viability in cortical cells can be found following application of the Hip- or SDH-NF2, whereas the growth and viability of cortical cells are inhibited by the Vc-NF2; only 20% of the cells are alive in cortical cultures following treatment with the Vc-NF2 for 24 hours. Application of either NF1 or NF2 isolated from non-conditioned culture medium, or non-ninhydrin-reacting fractions isolated from conditioned culture media produces no significant effect. These findings have strongly suggested that ninhydrin-reacting small molecules released locally may be important factors involved in the region-specific regulation of neuronal development in Vc versus SDH.

To confirm that the region-specific release of NF1 and NF2 from cultured Vc, SDH or Hip of embryonic rats may also occur in postnatal animals in vivo, these fractions were isolated from conditioned media used for culture of Vc, SDH or Hip slices, which were prepared from rats at day 1 after birth [14, 15]. Consistently, the experimental data show that NF1 isolated from the medium used for Hip or SDH slice culture promote the growth of cells in cortical cultures. NF1 isolated from the medium used for Vc slice culture does not have such effect. NF2 isolated from the medium used for Hip or SDH slice culture does not produce any significant changes in cell growth of cortical cultures while isolated Vc-NF2 inhibits cell growth and viability. These findings not only exclude a possibility that the differences observed in neuronal development of SDH versus Vc might be produced by the different methods used for culture, but also demonstrate that Vc-NF2 and SDH-NF1 are critically involved in the region-specific regulation of neuronal development in Vc versus SDH ex vivo.

Regionalization in the CNS

One of the earliest events during the development of the CNS is the formation of regional patterning of the neural tube along anteroposterior and dorsoventral axes. At this stage, hundreds of different neuronal subtypes are developed from a single layer of neuroepithelial progenitor cells (see reviews [11, 12, 13]). The structure of the patterning is found to be intrinsically encoded in progenitors [13, 34, 35]. Although afferent connections may also affect the development of area specificity in a neuronal activity-dependent manner [13, 36], the area identity has been established before the appearance of any functional diversification [36, 37, 38, 39].

A high degree of plasticity in cell fate choices is found in neural progenitors [40, 41, 42, 43]. Recent data have shown that mature neurons can be developed from multiple cell sources including fibroblasts modified with the technique of “direct reprogramming” or “trans differentiation” [40, 41, 42, 43, 44]. The sequential generation observed in projection neurons in vivo has also been found in cultured neuron precursors [45]. Thus, genetic factors play a primary role in the process of neuronal development from progenitor cells, and the environmental factors which may act in vivo are secondary [11, 12, 13].

Recently, increasing amounts of data have also shown differences in non-neural cells e.g. glial cells in different brain regions [46, 47]. Glial cells include both astrocytes and microglia. Functional differences among astrocytes from different brain regions have been found in culture and transplantation experiments [48, 49, 50], which are related to the derivation of astrocytes from regionally patterned radial glial cells [50].

The spatial patterning, which creates a segmental template in the spinal cord, is organized by neural progenitors with position identities [50]. Astrocytes in the spinal cord are regionalized in a segmental template, and developed in a position-dependent manner [46, 51]. Through providing positional guidance cues, astrocytes are involved in the formation of neuronal circuits [46, 52]. Several genes in astrocytes expressed differentially in the dorsal versus ventral spinal cord are found to encode extracellular matrix components or axon and cell migration factors [53].

Questions remain to be answered

It has been reported that in SDH different subtypes of neurons e.g. projection neurons and interneurons may mature at different rates [54, 55]. However, whether interneurons and projection neurons mature at different rates in Vc remains unknown. Thus, whether the differences noted in the neuronal development and function of Vc versus SDH [14, 15] might be related to the different developmental stage of CNS neurons still needs to be investigated.

When compared with SDH or Hip, fewer trophic and more growth-inhibitory factors are locally released in Vc [14, 15]. This suggests that the locally released factors may play important roles in the regulation of neuronal maturation in different CNS regions. These findings have revealed novel insights for understanding how locally released factors are involved in the region-specific regulation of CNS regionalization and functional organization.

Since the differences in neuronal growth observed in Vc versus SDH are maintained in cultures either with MEM supplemented with 10% fetal bovine serum and insulin or with neurobasal medium with the addition of B27, β-FGF2 and L-glutamine, which significantly inhibits the growth of
The growth-trophic and growth-inhibitory molecules such as in SDH-NF1 and Vc-NF2 may be released from both neurons and glial cells. Thus, the details of what types of cells in Vc or SDH release these molecules and what kinds of intrinsic and extrinsic signals may be involved in regulation of the release of these molecules remains unknown.

Present data have strongly suggested that locally released Vc-NF2 and SDH-NF1 may play important roles in the regulation of neuronal growth ex vivo [14, 15]. To confirm region-specific regulation in SDH and Vc, studies comparing cell development in Vc versus SDH neurons in vivo are still needed. An important direction for future research is addressing whether and how these locally released factors in Vc and SDH are involved in the regulation of nociceptive and sensorimotor functions in vivo.

Interestingly, the elution and function profiles of NF1 and NF2 are found to be very similar to those of previously reported fractions V3A and V3 isolated from human plasma by using size exclusion chromatography on Sephadex G-25 followed by Sephadex G-15 [14, 15, 32, 33]. The fraction V3 may act like a insulin inhibitor whereas V3A has insulin-like activity [32, 33]. Myoinositol 1,2-cyclic phosphate and galactosamine are found in the fraction V3 and chiro-inositol and galactosamine in V3A. The myoinositol 1,2 cyclic phosphate group is found to play an essential role in the inhibitory action of fraction V3 [32, 33]. Application of V3 to cultured central neurons significantly inhibits neuronal growth while V3A promotes neuronal growth [36]. The same method used for isolating fractions V3A and V3 were utilized for isolating NF1 and NF2 from media used for culture of Vc and SDH. However, the chemical nature of the fractions NF1 and NF2 is still unknown. Thus, a major focus of future researches is to identify the chemical structures of the molecules in Vc-NF2 and SDH-NF1, which act to regulate neuronal growth in Vc and SDH, respectively. This study is required to develop agents which will selectively block the effect of Vc-NF2 or SDH-NF1. We believe that these studies will provide very useful approaches not only for investigating the region-specific regulation of neuronal development in Vc and SDH, but also for diagnosing and treating neurodegenerative diseases and pain.

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Conflicting interests

The authors have declared that no conflict of interests exists.

Author contributions

X.M.Y. designed research and wrote the manuscript, X.H.J. wrote the manuscript.

Abbreviations

CNS: central nervous system; Vc: trigeminal subnucleus caudalis; SDH: spinal cord dorsal horn; Hip: hippocampus; LTM: low-threshold mechanoreceptive; WDR: wide-dynamic range; NS: nociceptive-specific; SP: substance P; CGRP: calcitonin gene related peptide; mEPSCs: miniature excitatory post-synaptic currents; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA: N-methyl-D-aspartate; NF: ninhydrin-reacting factors.

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