Department of Anatomy (Gross Anatomy and Neuroanatomy)

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General Summary

Our department's research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, the organization of neuronal networks and their development are investigated to elucidate brain function and diseases using immunocytochemistry, electron microscopy, in situ hybridization histochemistry, single-cell tracer injection, and patch-clamp electrophysiology. Our primary interests are the quantitative architecture and dynamics of microcircuits and their relationships. In gross anatomical research, the functional importance of variations in organ systems is explored by examining cadavers and animals.

Research Activities

Pattern differentiation of excitatory and inhibitory synaptic inputs on distinct neuronal types in the rat caudal nucleus of the tractus solitarius

Region-and size-specific neuronal organizations of the caudal nucleus of the tractus solitarius (cNTS) were investigated, after which excitatory and inhibitory synaptic input patterns onto specific cell types were analyzed with patch-clamp recording and immunoelectron microscopy. The cell-size distribution and numerical density of cNTS neurons were examined in subregions at levels of the area postrema. In the subpostremal and dorsomedial subnuclei, characterized by the presence of dense glutamatergic and sparse GABAergic somata, small calbindin neurons constituted 42% of all cells. The medial subnucleus contained large numbers of glutamatergic, GABAergic, and catecholaminergic somata, and large tyrosine hydroxylase–containing cells constituted 13% of cells in this region. Small neurons (<150 μm^2) represented about 80% of the cell population of the cNTS. Predominant excitatory postsynaptic currents were observed in the adult small neurons, whereas inhibitory postsynaptic currents were more evident in larger neurons, irrespective of subnuclear location. This distinct differentiation of postsynaptic current patterns was not evident in neonates. GABAergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%) in adults. These results indicate that differential synaptic input patterns were developmentally established in distinct small and large neurons.

Local axonal arborization patterns of distinct neuronal types in the cNTS

Neurons in the cNTS are heterogeneous in cell size (50 to 450 μm^2 in somal area) and other morphologic characteristics. For a more objective classification of cNTS neurons, their morphologic features were analyzed quantitatively on the basis of reconstructed bio-

cytin-filled cells after whole-cell patch-clamp recordings. On the basis of the patterns of axonal branching behaviors, cNTS cells could be classified into 2 groups: smaller cells (mean somal area, 94.1 μm^2; range, 62-120 μm^2; n=22) and larger cells (mean somal area, 245 μm^2; range, 142-411 μm^2; n=23). Extensive axonal arborization with numerous possible synaptic boutons was specifically associated with smaller neurons, whereas larger cells possessed few or no axon collaterals, suggesting their distinct roles as local circuit neurons (or interneurons) and projection neurons, respectively. With regard to somatodendritic characteristics, the following correlations with cell size were found: 1) smaller cells had larger form factors than did larger cells ($P<0.05$); and 2) larger neurons had more extensive dendritic arborization, expressed by total dendritic length ($P<0.01$) and the number of dendritic branching points ($P<0.01$), than did smaller cells. These findings suggest that small cNTS neurons contribute specifically to the integration of input information generated in the local circuits, whereas large neurons convey the integrated information to other autonomic brain regions.

Postnatal development of GABAergic axon terminals in the rat cNTS

The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the cNTS plays a pivotal role in cardiorespiratory reflexes, we know little about the formation of the local neural network in the cNTS. In the present study, we focused on GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organizations in the rat cNTS immunocytochemically at both the light and electron microscopic levels. The counting of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminals in the cNTS was constant until the second postnatal week and that GABAergic axon terminals were reorganized around postnatal day 10 (P10). Electron microscopic observation revealed that most GABAergic axon terminals formed axosomatic synapses on neurons with smaller soma (smaller neurons) at P2 to P4 but that the number of axosomatic synapses decreased considerably after P8. Orphan GABAergic boutons were present specifically near somata of smaller neurons at P10, and the number of axodendritic synapses on thicker dendrites decreased gradually during postnatal development. These results show that GABAergic axon terminals detach from the somata of smaller neurons during the second postnatal week. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to the reorganization of the local network within the cNTS from the neonatal type to the adult type. These postnatal changes in the cNTS local network might be a prerequisite for cardiorespiratory reflexes of the adult type.

Activity-dependent reorganization of local circuitry in the developing visceral sensory system

Neural activity during critical periods could fine-tune functional synaptic connections. The activation of *N*-methyl-D-aspartate (NMDA) receptors is implicated in this process, and their blockade leads to disruption of normal circuit formation. This phenomenon has been thoroughly studied in several neural systems, including the somatosensory system, but has not yet been evidenced in the visceral sensory system. Ultrastruc-

tural analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic currents of cNTS cells revealed that developmental changes in the synaptic organizations were blocked by MK-801, an NMDA receptor antagonist, when administered on P5 to P8, a presumed critical period for the visceral sensory system. Normal synapse reorganization during postnatal development dictates undifferentiated neonatal cNTS neurons, in terms of synaptic input patterns measured with electron microscopy and electrophysiology, into 2 cell groups: small and large cells under far stronger excitatory and inhibitory influence, respectively. Blockade by MK-801 during the critical period might leave adult neurons wired in undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

Glial coverage of the small cell somata in the rat NTS during postnatal development
Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Previous studies have suggested that the number of axosomatic synapses on the small cells of the rat cNTS decreases toward the end of the first postnatal week. Astrocytes might be involved in this phenomenon. We used light and electron microscopy to examine the morphological development of astrocytic processes around the small cell soma in the rat cNTS. Structures within the cNTS positive for glial fibrillary acidic protein, glutamate-aspartate transporter, and glutamate transporter 1 became more intensely stained as development proceeded. Structures positive for glutamate-aspartate transporter encompassed calbindin-positive small cell somata after P10. Electron microscopic observations indicated that astrocytic processes encompass the small cell soma, whereas the number of axosomatic synapses decreases as development proceeds. The timing of glial coverage of the small cell soma appears to be consistent with the decrease in axosomatic synapses on the small cells. These observations suggest that astrocytes participate in regulating the decrease of axosomatic synapses on small cells in the cNTS during postnatal development.

Quantitative and immunohistochemical analysis of neuronal types in the mouse cNTS: Focus on GABAergic neurons
GABAergic neurons are major inhibitory interneurons that are widely distributed in the central nervous system. The cNTS, which plays key roles in respiratory, cardiovascular, and gastrointestinal functions, contains GABAergic neurons to regulate neuronal firing. In the present study, GABAergic neuronal organization was analyzed in relation to the location of subnuclei in the mouse cNTS. According to the differential expression of glutamate decarboxylase 67 (GAD67), vesicular glutamate transporter 2 (VGLUT2), calbindin, and tyrosine hydroxylase messenger RNAs, the cNTS was divided into 4 subnuclei: the subpostrema, dorsomedial, commissural, and medial subnuclei. The numerical density and size of soma in the 4 subnuclei were then quantified through analysis of an unbiased dissector. Calbindin-positive cells constituted subpopulations of small non-GABAergic neurons preferentially localized in the subpostrema subnucleus. Tyrosine hydroxylase-positive cells constituted large neurons preferentially localized in the medial subnucleus. GABAergic neurons constituted a subpopulation of small neurons, prefer-

entially localized in the commissural and medial subnuclei, which represented ≥ 50% of small cells in these subnuclei. Thus, the small GABAergic neurons were located around large tyrosine hydroxylase-positive cells in the ventrolateral portion of the cNTS. This finding, in combination with results of previous studies in the rat cNTS showing that large cells originate efferents from the cNTS, suggests that small GABAergic neurons in the commissural and medial subnuclei regulate output from the cNTS.

Postnatal development of axosomatic synapses in the rat NTS: Differences between dorsal and ventral subnuclei

Inhibitory axosomatic synapses effectively suppress the excitability of postsynaptic cells. Examining the development of inhibitory axosomatic synapses is important for understanding the maturation of information processing. The cNTS, which regulates the autonomic system, consists of several subnuclei. In the present study, the development of axosomatic synapses in the dorsal and ventral subnuclei was examined with electron microscopy. In dorsal subnuclei, the percentage of glutamate decarboxylase-positive terminals on the somata, the percentage of small cell somata with synapses, and the axosomatic synapse density markedly decreased from P5 to P10. In ventral subnuclei, the percentage of glutamate decarboxylase-positive terminals on the soma, the percentage of small or large cell somata with synapses, and the axosomatic synapse density were maintained or increased from P5 to P10. Thus, a decrease in inhibitory axosomatic synapses in dorsal subnuclei might facilitate maturation of fine receptive areas for peripheral inputs, whereas an increase in inhibitory axosomatic synapses in ventral subnuclei might facilitate the establishment of an effective regulation system for cNTS output.

Geometric and functional architecture of visceral sensory microcircuitry

Is microcircuit wiring designed deterministically or probabilistically? Does geometric architecture predict the functional dynamics of a given neuronal microcircuit? These questions were addressed in the visceral sensory microcircuit of the cNTS, which is generally thought to be homogeneous rather than laminar in cytoarchitecture. By means of in situ hybridization histochemistry and whole-cell patch-clamp recordings followed by neuronal reconstruction with biocytin filling, the anatomical and functional organization of NTS microcircuitry was quantified to determine associative relationships. The morphologic and chemical features of NTS neurons displayed different patterns of process arborization and subnuclear localization according to neuronal type: smaller cells featured presynaptic local axons, and GABAergic cells were aggregated specifically within the ventral NTS. The results suggest both a laminar organization and a spatial heterogeneity of NTS microcircuit connectivity. Geometric analysis of presynaptic and postsynaptic axodendritic arbor overlap of reconstructed neurons (according to parent somal distance) confirmed a heterogeneity of microcircuit connectivity that might underlie the differential functional dynamics along the dorsoventral axis. Functional dynamics in terms of spontaneous and evoked postsynaptic current patterns behaved in a strongly location-specific manner according to the geometric dimension and suggested a spatial laminar segregation of neuronal populations: a dorsal group of high excitation and a ventral group of balanced excitation and inhibition. Recurrent polysynaptic activity was also noted in a subpopu-

lation of the ventral group. Such geometric and functional laminar organization seems to provide the NTS microcircuit with both reverberation capability and a differentiated projection system for appropriate computation of visceral sensory information.

Publications

Negishi Y, Kawai Y. Geometric and functional architecture of visceral sensory microcircuitry. *Brain Struct Funct.* 2011; **216:** 17–30.

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