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2．研 究 テ ー マ

中枢神経系の局所神経回路の解析：特に三叉神経核・脊髄の局所回路に関する研究

3．成 果 の 概 要（100字程度）

中枢神経系をニューロンレベルからボトムアップに理解しようとするときに、決定的に欠けている情報は「局所神経回路」の詳細である。今回の研究では中国第四軍医大学李雲慶教授の解剖学教研室と協力して、中枢神経系（特に三叉神経系）の局所回路を明らかにしたいと考え、共同研究を行った。李雲慶教授は脳幹のA5領域にあるノルアドレナリン作動性ニューロンが、黒質からGABA作動性入力を受け、三叉神経運動核に投射していることを発見し、同研究室の李金蓮教授は三叉神経中脳路核に中枢性および末梢性のグルタミン酸作動性入力が小胞性グルタミン酸トランスポータ１を使用していることを明らかにした。

4．研 究 組 織

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— 日中医学協会助成事業 —

中枢神経系の局所神経回路の解析：特に三叉神経核・脊髄の局所回路に関する研究

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要旨

中枢神経系をニューロンレベルからボトムアップに理解しようとするときに、決定的に欠けている情報は「局所神経回路」の詳細である。今回の研究では中国第四軍医大学李雲慶教授の解剖学教研室と協力して、中枢神経系（特に三叉神経系）の局所回路を明らかにしたいと考え、共同研究を行った。李雲慶教授は脳幹のA5領域にあるノルアドレナリン作動性ニューロンが、黒質からGABA作動性入力を受け、三叉神経運動核に投射していることを発見し、同研究室の李金蓮教授は三叉神経中脳路核に中枢性および末梢性のグルタミン酸作動性入力が小胞性グルタミン酸トランスポータ1を使用していることを明らかにした。

Key Words　局所神経回路，三叉神経運動核，三叉神経中脳路核，小胞性グルタミン酸トランスポータ1

報告

Demonstration of GABAergic and noradrenergic neurons in the A5 region receiving GABAergic afferent fibers from the substantia nigra and projecting to the trigeminal motor nucleus in the rat

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Orofacial movements, such as mastication, deglutition, suck, pronunciation, are complex functions. They are controlled by motor neurons in the brainstem, while the activities of the motor neurons in the brainstem are regulated by cerebral motor cortex through the corticobulbar tract. In recent years, it has been also found that the majority of the descending fibers in the corticobulbar tract do not terminate directly onto the motor neurons within the motor nuclei, but to the regions containing pre-motor neurons, such as lateral portion (parvicellular part) of the reticular formation in the brainstem, parabrachial nuclei, trigeminal sensory nuclei, raphe nucleus, substantia nigra (SN), etc. These regions are also called pre-motor neuron pool.

Anatomical studies have demonstrated that neurons in the lateral part of the substantia nigra (SNl) project to the reticular formation around the trigeminal motor nucleus (Vmo), especially to the region between the Vmo and dorsal and medial to the superior olive complex, this area is also called A5 region. A5 region belongs to the pontine parvicellular reticular formation and contains pre-motor neuron and is occupied by a few sparsely distributed medium to large noradrenalin (NA)-containing neurons and many small GABAergic neurons. Whether GABAergic neurons in the SNl send their descending fibers to the A5 region and GABAergic and NAergic neurons in the A5 region, in turn, project to the Vmo are still uncovered. In order to investigate the control mechanisms for the orofacial movements, in the present study, the neural tract tracing methods combined with immunohistochemical staining for GABA and dopamine beta-hydrolase (DBH), a chemical marker for noradrenergic neurons, were used in the rat. The main results were as follows:

(1) Immunohistochemical staining for GABA and DBH revealed that: ① there were GABA-like immunoreactive (-LI) neurons in the SNl and A5 region; ② DBH-LI neurons were also seen in the A5 region, locus coeruleus (LC) and sublocus coeruleus region; ③ GABA-LI terminals were dense found in the Vmo, while DBH-LI terminals were scattered in the Vmo.

(2) After injecting tetromethyl rhodamine-dextran amine (TMR-DA) into the unilateral A5 region, a few TMR-DA retrogradely labeled neurons were observed chiefly in the ipsilateral side of the SNl. Of the TMR-DA retrogradely labeled neurons, 21.2% (68/321) of them showed GABA-like immunoreactivity.

(3) Injecting TMR-DA into the unilateral Vmo, we encountered some TMR-DA retrogradely labeled neurons principally in the ipsilateral side of the parvicellular reticular formation, trigeminal sensory nuclei, parabrachial nuclei, raphe nucleus, superior trigeminal nucleus, SN, paratrigeminal region, LC, sublocus coeruleus region, A5 region, etc. Among the TMR-DA retrogradely labeled neurons in the A5 region, 32.8% (42/128) or 16.1% (18/112) of them exhibited GABA- or DBH-like immunoreactivities, respectively.

(4) After injecting biotinylated dextranaine (BDA) into the SNl and wheat germ agglutinin-horseradish peroxidase (WGA-HRP) into the Vmo, respectively, the following connections were observed in the A5 region under the laser scanning confocal microscope (LSCM) and electron microscope (EM): ① BDA anterogradely labeled fibers and terminals, some of them also showed GABA-like immunoreactivity, contacted with WGA-HRP retrogradely labeled neuronal cell bodies and dendritic processes; ②BDA anterogradely labeled fibers and terminals contacted with WGA-HRP retrogradely labeled neuronal cell bodies and dendritic processes, some of the WGA-HRP labeled neurons also exhibited GABA- or DBH-like immunoreactivities; ③ BDA anterogradely labeled fibers and terminals, some of them also showed GABA-like immunoreactivity, made symmetric axo-somatic and axo-dendritic synapses with WGA-HRP retrogradely labeled neuronal cell bodies and dendritic processes; ④ BDA anterogradely labeled fibers and terminals formed symmetric or asymmetric axo-somatic and axo-dendritic synapses with WGA-HRP retrogradely labeled neuronal cell bodies and dendritic processes, some of the WGA-HRP labeled neurons also exhibited GABA- or DBH-like immunoreactivities.

(5) After iontophoretic injecting *phaseolus vulgaris* agglutinin-L subunit (PHA-L) into the A5 region and pressure injecting WGA-HRP into the masticatory muscles, such as masseter, temporalis, etc., respectively, the following connections were observed in the Vmo under the LSCM and EM: ① PHA-L anterogradely labeled fibers and terminals, some of them also showed GABA- or DBH-like immunoreactivity, contacted with WGA-HRP retrogradely labeled neuronal cell bodies and dendritic processes; ② PHA-L anterogradely labeled fibers and terminals, some of them also showed GABA-like immunoreactivity, constituted symmetric synapses with WGA-HRP retrogradely labeled neurons; ③ PHA-L anterogradely labeled fibers and terminals, some of them also showed DBH-like immunoreactivity, made asymmetric axo-somatic and axo-dendritic synapses with WGA-HRP retrogradely labeled neuronal cell bodies and dendritic processes.

These results indicate that: ① GABAergic neurons in the SNl send descending projection fibers to the A5 region; ② GABAergic and noradrenergic neurons in the A5 region project to the Vmo; ③ GABAergic descending fibers from the SNl make symmetric or asymmetric synapses, respectively, with GABA- or NA-containing pre-motor neurons in the A5 region that send projection fibers to the motor neurons in the Vmo innervating the masticatory muscles.

Functional consideration: ① GABAergic neurons in the SNl might exert inhibitory influence upon the activities of GABAergic and noradrenergic pre-motor neurons in the A5 region through the descending projection fibers from SNl to the A5 region; ② GABAergic and noradrenergic neurons in the A5 region might exert the inhibitory and excitatory effects upon the activities of the Vmo motor neurons, respectively; ③ GABAergic descending fibers from the SNl might disinhibit the inhibitory influence of the GABAergic neurons in the A5 region upon the activities of Vmo motor neurons. These inhibitory and excitatory regulatory effects upon Vmo motor neurons might be very important for the modulatory mechanisms on the orofacial movements.

Vesicular glutamate transporter 1 (VGluT1) immunoreactivity in the central and peripheral endings in mesencephalic trigeminal nucleus of the rat

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The amino acid glutamate is a major excitatory neurotransmitter in the central nervous system (CNS) of mammals. In particular, sensory neurotransmission from primary afferents onto spinal or trigeminal neurons appears to be largely mediated by glutamate. Glutamate transport into synaptic vesicles is a prerequisite for the quantal release of glutamate at synapses. Recently, two vesicular glutamate transporters, VGLUT1 and VGLUT2, have been identified and shown that they can selectively accumulate glutamate and confer glutamate release properties to transfected cells or neurons. Varoqui et al. (2002) and Todd et al. (2003) reported that all low-threshold cutaneous and proprioceptive myelinated afferents fibers express VGLUT1, whereas some cutaneous afferents (which arborize in laminae III-VI) in the spinal cord also contain a low level of VGLUT2 and was associated with nociceptive afferents. Wu et al. (2004) have further been indicated that that VGLUT1 is expressed not only in the central axon terminals in the spinal cord but also in peripheral sensory endings of muscle-spindle afferents.

The mesencephalic trigeminal nucleus (Vmes) is composed of primary afferent neurons, sensory peripheral terminals of which are associated mainly with muscle spindles of the jaw-closing muscles and with periodontal mechanoreceptors of both maxillary and mandibular teeth, while the central terminals of which extend mainly the trigeminal motor neurons(Vmo) and superatrigminal nucleus(Vsup). Though our previous studies have also showed that VGLUT1 is expressed in primary afferent neurons of the dorsal root ganglion (Li et al, 2003a) as well as in those of the trigeminal ganglion (Li et al, 2003b), however, no VGLUT1 immunoreactivity has so far been demonstrated in the cell bodies of neurons, and peripheral and central terminals of the Vmes. In order to investigate the above questions, in the present study, in situ hybridization histochemistry for VGLUT1, the tract tracing methods combined with immunofluorecence histochemical staining for VGluT1 and cholera toxin B subunit (CTb, transganglionic transport tracer, which has been shown to be taken up selectively by these axons when injected into masseter nerves) were used in the newborn rat, young rat and adult rat. The main results were as following:

1. VGLUT1 immunoreactiviy in Vmes-neuron soma was detected in newborn rats (within 24 hours after birth) throughout the whole rostrocaudal extent of the Vmes. It was more intense in 3-day-old rats, then decreased gradually. In 11-day-old rats, VGLUT1 immunoreactivity still remained, but was very week at the caudal levels of the Vmes. No VGLUT1- immunoreactivity was detected in the Vmes of rats older than 11-day-old.

2. VGLUT1 mRNA signals were detected in all Vmes-neurons soma throughout the whole rostrocaudal extent of the Vmes of newborn, young and adult rats. The hybridization with the sense RNA probe for VGLUT1 showed no hybridization signals.

3. In the longitudinal sections of the masseter muscles of adult rats, VGLUT1-immunopositive fibers ran through the capsule of muscle-spindle to form endings of ribbon-like spirals, which appeared to encircle the equatorial region of the intrafusal muscle fibers. Some VGLUT1- immunopositive fibers also formed knob-like endings on the equatarial regions of the intrafusal muscle fibers. VGLUT1-
immunoreactivity in the peripheral endings on the intrafusal muscle fibers was also detected in newborn rats; its intensity increased in older rats.

4. After injection CTb into the masseter nerve of the adult rats, the connections of CTb-labeled terminals and CTb-labeled neuronal cell bodies were observed in the Vmo and Vsup regions under the laser scanning microscope(LSCM) and electron

microscope(EM).

(1) Considerable number of CTb anterogradely labeled fibers and terminals (the central process of Vmes) were observed in the Vmo and Vsup regions after injecting CTb into the masseter nerve of the adult rats. At the same time, many of CTb retrogradely labeled neuronal cell bodies and dendrites were also found in the Vmo and Vsup regions.

(2) Under the laser scanning microscope, many terminals and varicose structures with CTb-labeled were scattered throughout the Vsup and Vmo. Varicose structures with CTb-labeled were seen frequently to be in close apposition to the CTb-labeled contours of cell bodies and dendrites, and the density of distribution of CTb-labeled varicosities on CTb-labeled contours did seem to be high. Almost all of CTb-labeled terminals showed VGluT1-like immuneoreactivity(-LI). Some colocalizated terminals of VGluT1-LI and CTb-labeling were frequently to be in close apposition to the CTb-labeled contours of cell bodies and dendrites.

(3)Under electron microscope, the following connection of the CTb-labeled terminals and CTb-labeled neuronal cell bodies or dendrites with VGLuT1-like immunoreactive terminals in the motor trigeminal nucleus (Vmo) were observed:

①CTb-labeled terminals containing small clear and spherical synaptic vesicles predominantly formed asymmetric synapses with the dendrites of the no-labeled neurons;

②VGLUT1-immunoreactive terminals containing small clear and spherical synaptic vesicles predominantly formed asymmetric synapses with the dendrites of CTb-labeled or no-labeled neurons;

③Some terminals of no-labeled neurons make the asymmetric or symmetric synapses with the dendrites of CTb-labeled neurons;

④CTb-labeled terminals predominantly formed asymmetric synapses with the dendrites or soma of the CTb-labeled Vmo neurons;

⑤CTb-labeled terminals showing VGLuT1-immunoreactivity (CTb/VGluT1) coming from Vmes predominantly formed asymmetric synapses with the dendrites or soma of the CTb-labeled Vmo neurons.

The present results indicate that: ①VGLUT1 is expressed in the peripheral endings supplying intrafusal muscles of muscle-spindles of the masseter muscle in both neonatal and adult rats; ②No VGLUT1-immunoreactivity was detected in the Vmes neuronal soma in rats older than 11-day-old, although VGLUT1 mRNA signals were detected in Vmes-neurons of adult rats. These results might be attributable to slower synthesis of VGLUT1 in the soma and/or faster transport of VGLUT1 from the soma to the peripheral and central endings in Vmes-neurons of older rats; ③VGluT1 is also expressed in the central endings of the Vmes-neurons of adult rats and mainly distributed in the motor trigeminal nucleus and supratrigeminal nucleus; ④Most of VGlUT1-positive terminals from Vmes neurons make asymmetric synapse contact with the cell bodies or dendrites of Vmo neurons innerveting the masticatory muscles.

Founctional considerations: ① The trigeminal proprioceptive sensory signals from the muscle-spindles of the masseter muscle might be transported to the Vmo neurons by the central terminals of the primary afferent neurons in the Vmes using excitatory glutamatergic transporters-VGLUT1; ②There are direct synapses contact between the VGLUT1-positive axonal terminals coming from the Vmes and the trigeminal motoneurons, suggesting that partly Vmo neurons might directly receive input proprioceptive sensory signals from the muscle-spindles of the masseter muscle, then produce masticatory muscles activities although there are lots of the GABAergic premotor interneurons neurons existing in the Vsup involved in the modulation of the masticatory muscles movements.

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