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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：特に三叉神経の痛覚入力に関する研究.

3．日本滞在日程

２００２年１月１０日、名古屋空港着
２００２年１月１０日～４月１０日（下記日時以外）
京都大学医学研究科高次脳形態学教室にて研究に従事。
２００２年３月２７日～２８日
東京都神経研に研究打ち合わせ旅行。
２００２年３月２９日～３１日
第１０７回解剖学会総会・全国学術集会に出席。
２００２年４月１０日、名古屋空港発

4．研究報告書

別紙報告書作成要領に準じ、添付の用紙で研究報告書を作成して下さい。

研究発表中または研究中の本人のスナップ写真を添付して下さい。

※決算報告書（書式自由）を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

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—日中医学友好協会助成事業—

中枢神経系の局所神経回路の解析：特に三叉神経の痛覚入力に関する研究

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要　旨

By using immunohistochemical staining technique, retrograde neuronal tracing methods and electron microscopy, we investigated the local circuitry of the medullary dorsal horn (MDH), which is a brainstem center for pain information transmission.

1) The distribution of preprotachykinin B (PPTB)-, neurotensin (NT)-immunoreactive neuronal cell bodies in the MDH was examined. PPTB-LI neuronal cell bodies were mainly found in lamina II and less frequently in laminae I and III, and NT-LI neuronal cell bodies were mainly found in lamina II and, to a lesser extent, in lamina I. Most of PPTB- or NT-producing lamina II neurons were considered to be local circuit or intrinsic neurons in the MDH. 2) About 30% of PPTB-immunoreactive neurons showed immunoreactivity for calretinin, or calbindin, but not for parvalbumin. More than 50% of PPTB-immunoreactive neurons in lamina I displayed immunoreactivity for neurotensin. No PPTB-immunoreactive neurons expressed substance P receptor, but some PPTB-immunoreactive neurons in laminae I and II displayed NK3 receptor, which is a receptor for neurokinin B, the mature product of PPTB. This suggest the autoreceptor-like function of NK3 in PPTB-producing neurons. 3) Most PPTB-positive neurons responded to the painful stimulation by FOS expression after formalin injection into the oro-facial region, indicating that the PPTB-positive neurons in the MDH were involved in nociceptive information transmission. 4) About 50% of NT-positive neurons in lamina I expressed substance P receptor. Formalin stimulation in the oro-facial region induced FOS in most NT-positive lamina I neurons. 5) Under electron microscopy, the majority of the NT-containing terminals made symmetric synapses with the substance P receptor-expressing neuronal cell bodies and dendrites in lamina I. 6) Some NT-immunoreactive terminals in lamina I formed symmetric type of synapses on the dendrites of lamina I projection neurons to the thalamus or lateral parabrachial nucleus. The results of 5 and 6 indicates that NT-producing neurons have inhibitory effects on MDH projection neurons. 6) Under the electron-microscopic level with double immunolabeling methods, we observed that glutamic acid decarboxylase-, glycine transporter 2-, or serotonin-containing axonal terminals formed symmetric synapses with substance P receptor-expressing neuronal cell bodies and dendrites in lamina I of the MDH.

All these findings revealed the involvement of PPTB- or NT-producing neurons in local or intrinsic regulation of nociceptive information transmission in the MDH. Although the exact roles of PPTB- and NT-producing local circuit neurons have not been clarified yet, the present morphological analysis of those local circuit neurons will help the understanding of the mechanism for nociceptive information transmission in the MDH.

Key Words: Nociception, Medullary dorsal horn, Local circuit, Preprotachykinin B, Neurotensin

緒　言：

Medullary dorsal horn (MDH), which is also called the caudal subnucleus of the spinal trigeminal nucleus (Vc), receives both primary afferent fibers that transmit noxious information from the oro-facial region and projection fibers from the middle-line structures of the brainstem which regulate the nociceptive information. Unlike the spinal dorsal horn, which

is structurally and functionally identical to the MDH, the chemical architecture of the MDH has not been systemically investigated yet. This time, we have done some research works on the chemical architecture of the MDH by using immunohistochemical staining combined with neuronal tracer methods, and by double or triple immunofluorescence labeling method.

対象と方法：

Male Wistar rats were used in the present studies. Under deep anesthesia, some rats were injected with neuronal tracers for retrograde labeling, or formalin for painful stimulation. The rats were fixed and immunostained as described in the references (参考文献参照).

結 果 および 考 察：

1. Chemical architecture of the medullary dorsal horn

(1) Preprotachykinin B (PPTB)

By using immunohistochemical staining technique, we observed a quite special distribution pattern of PPTB-like immunoreactive (PPTB-LI) neuronal cell bodies. PPTB-LI neuronal cell bodies were mainly found in lamina II, especially in its inner part (IIi) where sparsely distributed PPTB-LI neurons formed a line which separates laminae II and III. In laminae I and III, only few PPTB-LI neuronal cell bodies were seen. PPTB-LI terminals were densely observed in laminae II and I.

By using double immunofluorescence staining method, about 30% of PPTB-LI neurons in IIi were found also showing immunoreactivities for calcium binding proteins, e.g. calbindin D-28k (CB) and calretinin (CR), but not for parvalbumin (PV). A few CB- and CR-immunoreactive neurons showed PPTB-like immunoreactivity. Over half of the PPTB-LI neurons in lamina I exhibited neurotensin (NT)-like immunoreactivity. Since neurokinin B, a mature peptide produced from PPTB, is the endogenous agonist of NK3 receptor, the relationship between PPTB and NK3 receptor was also investigated. Some PPTB-LI neurons in both lamina I and IIi were found to co-localize with NK3 receptor. PPTB-LI neurons did not show substance P receptor (SPR)-like immunoreactivity and only a few large neurons in the trigeminal ganglion showed PPTB-LI. After noxious stimulation induced by injecting formalin into the oro-facial region, c-fos gene expression products FOS protein were located in the nuclei of the majority of the PPTB-LI neurons, suggesting that PPTB-LI neurons might be mainly involved in the regulation of the oro-facial nociception. Now, the ultrastructural localizations of PPTB and NK3 receptor, e.g. auto-receptors of NK3 receptor on the pre-synaptic terminals, the distribution of NK3 receptor on the post-synaptic elements, the relationship and synaptic connections between NK3 receptor- and PPTB-immunopositive structures, have been carried out. We will soon get significant results.

(2) Neurotensin

Neurotensin (NT) is a previously found neuropeptide in the nervous system. Recently, it has been found that NT is closely related to the nociception. By using immunohistochemical staining technique, NT-LI neuronal cell bodies were mainly found in lamina II, especially in its inner part (IIi). In lamina I, a few quite large NT-LI neuronal cell bodies were observed. NT-LI neuronal cell bodies were hardly seen in lamina III. NT-LI terminals were densely observed in laminae I and II, especially in lamina II.

By using double immunofluorescence staining method, about half of the NT-LI neurons in lamina I were colocalized with substance P receptor (SPR). After noxious stimulation applied by injecting formalin into the oro-facial region, FOS protein were also located in the nuclei of the most of the NT-LI neurons in lamina I. Under the electron microscopic level, we observed that over 85% of the synapses formed by NT-immunopositive terminals were symmetric ones. By double-labeling technique, we also found that the majority of the NT-LI terminals made symmetric synapses with the neurons in lamina I which were labeled by injecting WGA-HRP in to the ventral posteromedial thalamic nucleus (VPM)

or lateral parabrachial region, including the Koellicker-Fuse nucleus. These results suggest that NT-LI neurons might be also mainly involved in the regulation of the oro-facial nociception, but not transmission.

2. Fiber connections

By using fluorescence retrograde tracing combined with immunofluorescent histochemical staining, we previously found that some preprodynorphin (PPD)-LI neurons in the MDH sent projection fibers to the VPM (ref. 90). This time, we have observed that FOS immunopositive PPD-LI neurons induced by oro-facial noxious stimulation projected to the lateral parabrachial region. This result suggests that PPD-LI neurons in the MDH are involved in the oro-facial noxious information transmission from the MDH to the lateral parabrachial region.

Neurons in the supratrigeminal nucleus (SupV) have been demonstrated belonging to the brainstem pre-motor neuron pool for the oro-facial motor nuclei, especially for the motor neurons in the trigeminal motor nucleus (Vm). In the Vm, we found that GABA and glycine (Gly) coexisted in some terminals which made close contacts with Vm motor neurons labeled by injecting WGA-HRP into the masseter muscle or masseter nerve. After injecting BDA into the SupV where some GABA- and Gly-containing neurons have been encountered, BDA anterogradely labeled axonal terminals have been found in Vm on both sides. By using immunofluorescent triple-staining technique, a few BDA-labeled terminals also showed immunoreactivities for both GABA and Gly. The present results indicate that GABA and Gly might coexist in some neurons within the SupV which project to the Vm on both sides. These results indicate that GABA and Gly might coexist in the neurons of the SupV and these GABA/Gly co-immunoreactive neurons might innervate the motor neurons in the Vm. Under the electron microscopic level, we have observed that GABA- or Gly-LI terminals constituted symmetric synaptic connections with the motor neurons in the Vm labeled by injecting WGA-HRP into the masseter nerve or masseter muscle. The synaptic connections between GABA/Gly coexisted terminals and Vm motor neurons are being investigated now.

According to their chemical natures, GABA neurons in the globus pallidus (GP) including interneurons and projection neurons could be divided into several subgroups. Among them, GABA/PV co-immunoreactive neurons belong to projection neurons. This time, we have studied the PV-containing projection from the GP to the reticular thalamic nucleus or subthalamic nucleus/entopeduncular nucleus. After injecting fluoro-gold (FG) into the reticular thalamic nucleus or subthalamic nucleus/entopeduncular nucleus, respectively, we observed that about 35% and 20% FG retrogradely labeled neurons in the GP showed PV-like immunoreactivities. No CB- and CR-containing GP neurons projecting to the reticular thalamic nucleus or subthalamic nucleus/entopeduncular nucleus.

3. Electron-microscopic (EM) studies

Under the EM level, we have observed that GAD-, glycine transporter 2 (GlyT2)-, serotonin (5-HT)-containing axonal terminals labeled by DAB reaction products formed symmetric synapses with SPR-LI neuronal cell bodies and dendrites labeled by 1.4 nm nano-gold enhanced by silver in lamina I. By the same double-labeling technique, we also found that the majority of the NT-LI terminals made symmetric synapses with the SPR-LI neuronal cell bodies and dendrites in lamina I. These results suggest that GABA, Gly, 5-HT and NT might have inhibitory effects on the SPR-LI neurons which receive noxious information from oro-facial region and transmit it to the higher centers of the central nervous system.

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