Immunohistochemical study on the distribution of ion channels in rat trigeminal sensory nucleus

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국문초록

흰쥐 삼차신경 감각핵에 존재하는 이온통로의 분포에 관한 면역조직화학적 연구

박호영 · 최기운 · 최호영
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삼차신경절의 뉴론이 구강악안면영역에서의 촉각, 압각, 온도각 및 통각 등 다양한 감각을 중추신경계로 전달하는 역할을 하는 것은 주지의 사실이다. 이러한 신경전달에 있어서 이온통로는 감각정보를 전달하는데 핵심적인 역할을 수행한다. 이 중 소디움 통로는 활동전위의 발생에 중요하며, 칼슘 통로는 시냅스 전도에 있어서 필수적인 역할을 수행하고, 포타슘 통로는 안정막전압의 유지 및 재분극에 관여한다. 최근에 여러 가지의 이온통로들의 뇌조직내의 분포에 관한 연구가 시작되고 있는데 삼차신경의 일차구심뉴론이 종지하는 삼차신경핵 즉 삼차신경 척수감각핵, 삼차신경 주감각핵, 삼차신경 중뇌핵 및 삼차신경 운동핵에 존재하는 이온통로에 관한 연구는 매우 희소하여 본 연구에서는 흰쥐의 삼차신경핵에 존재하는 소디움, 칼슘 및 포타슘 이온통로들을 면역조직화학적 방법으로 조사하여 다음과 같은 결과를 얻었다.

(1) 소디움 통로는 삼차신경 척수감각핵, 삼차신경 주감각핵 및 삼차신경 운동핵 모두에서 강하게 염색되었다.
(2) 칼슘 통로는 삼차신경 척수감각핵에서는 N-type 통로가 중등도로 염색되었으며, P/Q-type 통로는 약하게 염색되었으나 R-type 통로는 거의 염색되지 않았다. 삼차신경 주감각핵에서는 P/Q-type 통로가 매우 약하게 염색 되었다.
(3) 포타슘 통로는 삼차신경 척수감각핵과 삼차신경 주감각핵에서 inwardly rectifying 포타슘 통로(Kir 2.1)가 중등도로 염색되었고, voltage-gated 포타슘 통로(Kv 4.2)가 약하게 염색되었으며, BKCa는 그 염색 정도가 매우 약하게 나타났다.

이상의 결과를 종합해 볼 때 삼차신경 감각핵에는 소디움 통로의 분포가 가장 많았으며, 칼슘통로에서는 N-type이, 포타슘 통로 중에는 inwardly rectifying 통로(Kir 2.1)가 가장 많이 분포함을 관찰할 수 있었다.

주요어 : 삼차신경 감각핵, 면역조직화학법, 소디움 통로, 칼슘 통로, 포타슘 통로

I. INTRODUCTION

Trigeminal nerves transmit various sensory information such as touch, pressure, pain and temperature sensations from the oromaxillofacial region to the central nervous system. The central ends terminate in the trigeminal principal nucleus and the trigeminal spinal nucleus and transmit the information through the trigeminal mesencephalic nucleus. Therefore three nucleus i.e. the trigeminal principal nucleus, the trigeminal spinal nucleus and the trigeminal mesencephalic nucleus serve as the first gates to enter sensory information, so the nucleus have fundamental roles in sensory reception in the oromaxillofacial region.

Ion channels of the sensory neurons play a pivotal role in the generation and transmission of sensory information. Generally the Na+ channels are

involved in the rapid generation of action potentials. Intracellular recordings in the mammalian TRG neurons have shown that three types of action potentials exist; (1) fast spikes, most of which were completely blocked by the external application of tetrodotoxin (TTX), (2) Co2+-and TTX-resistant humped spikes (Puil et al. 1986) and (3) slowly decaying TTX-resistant and Cd2+-sensitive action potentials (Galdzicki et al. 1990). These reports suggest that voltage-dependent sodium current (INa) may be involved in the electrical activities of the TRG neurons.

A proper understanding of many CNS diseases and disorders requires identification of the ion channels underlying them and knowledge of their normal physiological roles. Recently, considerable progress has been made in understanding certain forms of epilepsy (Biervert et al. 1998; De Zeeuw et al. 1995; Smart et al. 1998), and episodic ataxia (Browne et al. 1994), both of which may be caused by mutations in genes encoding voltage-gated K+ (Kv) channels. Kv channels are crucial in a wide variety of processes, including action potential preparation and lymphocyte activation, and play as essential role in neurons where they regulate the resting membrane potential, impact dendritic excitability, control the frequency and duration of action potentials, and modulate neurotransmitter release (Hille 1992).

Calcium flux through voltage-gated calcium channels has been shown to be essential for a host of important cellular functions in the nervous system. These include neurite outgrowth, gene regulation, neuronal excitability, plasticity, and neurotransmitter release. A variety of calcium channels has been described in different types of tissue (Bean 1989; Scott et al. 1991). Commonly, calcium currents (ICa) are grouped into three types on the basis of the finding of Nowycky et al. (1985) in chick dorsal root ganglia (DRG) (Fox et al. 1987a, b). They described a transient (T-type), or low voltage- activated (LVA), current; a sustained (L-type), or high voltage-activated (HVA), current; and a third current, termed an N-type, that exhibited kinetic properties between the T-type and the L-type current. Recently a fourth type, known as the P-type, has been described in Purkinje cells (Llinas et al. 1989; Regan et al. 1991).

There are several techniques to study the ion channels. The first is to isolate the ion channels from the cell membrane, and to characterize the nature and biochemical properties of them. The second is to measure the ionic currents through the ion channels by the patch clamp technique. And the third is to observe the localization of the ion channels by staining them with antibodies.

The main goals of this study were (1) to observe some kinds of ion channels (2) to observe the localized distribution of the ion channels in the trigeminal principal nucleus, the trigeminal spinal nucleus by immunohistochemical method.

Ⅱ. MATERIALS & METHODS

1. Peroxidase Immunostaining

Sprague-Dawley rats of Postnatal 0 day were sacrificed by decapitation and fixed without perfusion in 4% paraformaldehyde in 0.1M Na-phosphate buffer, pH 7.4. Brains were removed and stored at 4℃ overnight in the same fixative, followed by storage for 2-3 days at 4℃ in 30% sucrose, 0.13M phosphate buffer. Frozen serial sections were cut at 30㎛ in the coronal orientation on a Minotome Plus microtome. Sections were pretreated with 0.3% hydrogen peroxide in 0.1M PBS containing 0.3% Triton X-100 (PBST) for 30min at room temperature to inhibit endogenous peroxidase activity. After washing with 0.1M PBST, the sections incubated in PBS containing 3% normal goat serum, 2g/l BSA and 0.1% Triton X-100 for 30-45min. Polyclonal anti-Kv 4.2, BKCa, Kir 2.1 1A, 1B, E (product no. APC-023, 021, 026 and ACC-001, 002, 006 ; Alomone Labs, Jerusalem, Israel) and anti-Na+ channel, type I (product no. #06-649 ; Argonex company) were used as primary antibodies at a dilution of 1:100 for 24 h at 4℃. After primary incubation, sections were given three 10-min washes in 0.3% Triton X-100 in PBS and then processed for peroxidase immunostaining using Vector Laboratories. Sections were incubated for 1h at room temperature in Vector goat anti-rabbit Ig-G. Three 10-min washes in 0.3% Triton X-100 in PBS. Sections were incubated under the same conditions in ABC solution. After three 10-min washes in

0.3% Triton X-100 in PBS, sections were transferred into Tris-buffered saline. Sections were incubated in 3' 3- diaminobenzidine substrate for 5-10 min before the reaction was stopped in distilled water.

2. Fluorescent Immunostaining

Sprague-Dawley rats of Postnatal 0 day were sacrificed by decapitation and fixed without perfusion in 4% paraformaldehyde in 0.1M Na-phosphate buffer, pH 7.4. Brains were removed and stored at 4℃ overnight in the same fixative, followed by storage for 2-3 days at 4℃ in 30% sucrose, 0.13 M phosphate buffer. Frozen serial sections were cut at 30㎛ in the coronal orientation on a Minotome Plus microtome. Sections were washed in PBS containing 0.3% Triton X-100 for 30min. After washing with 0.1M PBST, the sections incubated in PBS containing 3% normal goat serum, 2g/l BSA and 0.1% Triton X-100 for 30-45 min. Polyclonal anti-Kv 4.2, BKCa, Kir 2.1 1A, 1B, E (product no. APC-023, 021, 026 and ACC-001, 002, 006 ; Alomone Labs, Jerusalem, Israel) and anti-Na+ channel, type I (product no. #06-649 ; Argonex company) were used as primary antibodies at a dilution of 1:100 for 24h at 4℃. Sections were incubated for 1h at room temperature in PBS containing anti-rabbit fluorescein-labeled IgG (dilution of 1: 100, KPL Europe). After three 10-min washes in PBS and examined with epifluorescence microscopy.

Ⅲ. RESULTS

1. Sodium Channels

Sodium channels exist on the membrane of the soma in the trigeminal spinal nucleus, trigeminal principal nucleus, trigeminal mesencephalic nucleus, and trigeminal motor nucleus were immunohistochemically stained with the antibody for the sodium channels. Because the primary antibodies used in this experiment are not specific for the TTX-s or TTX-r sodium channels - those are able to bind to TTX-s and TTX-r sodium channels, TTX-s and TTX-r sodium channels on the membrane of TRG neurons could not be separated from each other. Sodium channels of the membrane in the nucleus were strongly stained with the antibodies. There is no difference in the degree of the staining among the tested nucleus.

2. Calcium Channels

1) N-type calcium channels

In this experiment, it is noted that there was a moderate staining of the N-type calcium channels of the boutons and somata of trigeminal spinal nucleus compared to other part of the brain. N-type calcium channels were moderately stained in the trigeminal principal nucleus.

2) P/Q-type calcium channels

In this immunohistochemical study, P/Q-type calcium channels were weakly stained in trigeminal spinal nucleus. And a very weak staining for the P/Q-type calcium channels was observed in the trigeminal principal nucleus.

3) R-type calcium channels

R-type calcium channel was not observed in trigeminal spinal nucleus and trigeminal principal nucleus.

Table 1. Immunofluorescence for sodium channels and calcium channels

	Na+ channel	N-type calcium channel	P/Q-type calcium channel	R-type calcium channel
Trigeminal spinal nucleus	+++	++	+	-
Trigeminal principal nucleus	++	+		-

high +++, moderate ++, weak +, very weak±, negative -

Table 2. Immunofluorescence for potassium channels

	Kir 2.1	Kv 4.2	BKCa
Trigeminal spinal nucleus	+ +	+	
Trigeminal principal nucleus	+		-

high +++, moderate ++, weak +, very weak±, negative -

3. Potassium Channels

1) Inwardly rectifying potassium channels

In this experiment, moderate staining of the Kir 2.1 in the trigeminal principal nucleus were observed. On the other hand, staining of the Kir 2.1 was weak trigeminal spinal nucleus.

2) Voltage-gated potassium channels

In this study, there was weak staining for the Kv 4.2 in trigeminal spinal nucleus. The Kv 4.2 channels were very weakly stained in the trigeminal principal nucleus.

3) Ca2+-activated K+ channels

In this experiment, the intensity of staining for the BKCa channels was weak in the trigeminal principal nucleus and the trigeminal spinal nucleus.

Ⅳ. DISCUSSION

Trigeminal sensory nerves relay mechanical, thermal, chemical and proprioceptive information from craniofacial regions. Trigeminal sensory nerves act at the focal level to maintain the integrity of craniofacial tissues through trophic influences and serve the whole animal through reflexes that protect other external sensory and internal homeostatic systems from damaging environmental changes.

There are some nuclei concerning with the trigeminal nerves: trigeminal spinal nucleus, trigeminal principal nucleus, trigeminal mesencephalic nucleus and trigeminal motor nucleus. It has been 50 years since Olszewski (1950) divided the trigeminal spinal nucleus into oralis, interpolaris and caudalis subdivisions and nearly 25 years since Gobel et al. (1977) detailed the structural homologies between caudalis and the spinal dorsal horn. While these landmark studis have shaped discussions of the special relationship between caudalis and craniofacial pain (Renehan and Jaquin 1993; Sessle 2000), this useful homology may need revision since recent evidence suggests that select portions of caudalis are organized differently form spinal systems. The subnucleus caudalis, the largest subdivision of the trigeminal spinal nucleus, consists of an elongated laminated portion that merges without clear boundaries with the cervical dorsal horn, while the rostral caudalis is displaced medially by the caudal tip of interpolaris to form a distinctive transition region. The ventral interpolaris/caudalis transition region, which is well described in rodents, consists of rostral caudalis with its fragmented laminar appearance, intersttial islands of neurons embedded in the trigeminal spinal tract, and a ventral crescent-shaped region of caudal interpolaris (Phelan and Falls 1989).

Spinal motor neurons are the final integration point for electrical signals that initiate and control skeletal muscle contraction. And trigeminal motor nucleus controls masticatory muscles. Several neuromuscular diseases result from dysfunction of the motor neurons. In at least two cases, Ca2+ channels are implicated in the disease process. Lambert-Eaton myasthenic syndrome is caused by circulating antibodies against presynaptic Ca2+ channels (Engel 1991; Sher et al. 1993). These antibodies reduce the level of presynaptic Ca2+ current and the efficiency of neurotransmitter release (Lang et al. 1983; Kim 1985). Amyotrophic lateral sclerosis (ALS) is caused by progressive death of motor neurons (Appel and Stefani 1991). One current hypothesis for the etiology of ALS implicates Ca2+ channels in motor neurons (Appel et al. 1991, 1993; Delbono 1991, 1993; Smith et al. 1992; Uchitel et al. 1992; Morton et al. 1994; Rowland 1994). These results mean that ion channels play a critical role to function in normal and disease states.

The mesencephalic trigeminal nucleus contains somata of first order neurons associate with the proprioceptors of the head regions. Proprioceptive afferents of law closing masticatory muscles, tooth mechanoceptors and extraocular muscles have been identified in the trigeminal mesencephalic nucleus (Alvarado- Mallart et al. 1975; Buisseret-Delmas et

al. 1990; Cody et al. 1972; Jerge 1963; Matesz 1981). Masticatory neurons are distributed throughout the whole rostralcaudal extent of the trigeminal mesencephalic nucleus, while the periodontal and extraocular muscle sensory neurons are situated mainly in the caudal part of the nucleus (Dessem and Taylor 1989). The trigeminal mesencephalic nucleus neurons resemble dorsal root ganglion cells, but a noticeable different is the presence of axosomatic synaptic boutons (Copray et al. 1990; Liem et al. 1992)

Sodium currents through the sodium channels were first recorded by Hodgkin and Huxley, who use voltage clamp techniques to demonstrate the three key features that have come to characterize the sodium channel: (1) voltage- dependent activation, (2) rapid inactivation, and (3) selective ion conductance (Hodgkin and Huxley 1952). Detailed analysis of sodium channel function during the 1960s and 1970s using the voltage clamp method applied to invertebrate giant axons and vertebrate myelinated nerve fibers yielded mechanistic models for sodium channel function (Armstrong 1981; Hille 1984). These functional studies predicted that sodium channels would be rare membrane proteins, difficult to identify and to isolate from the many other proteins of excitable membranes. During the 1970s, a new line of research emerged, focusing on development of methods for molecular analysis of sodium channels. Biochemical methods for measurement of ion flux through sodium channels, high affinity binding of neurotoxins to sodium channels, and detergent solubilization and purification of sodium channel proteins labeled by neurotoxins were progressively developed (Ritchie and Rogart 1977; Catterall 1980). These biochemical approaches led to discovery of the sodium channel protein in 1980. Photoaffinity labeling with a photoreactive derivative of an -scorpion toxin identified the principal -subunit and the auxiliary 1 subunit of brain sodium channels (Beneski and Catterall 1980). Immediately thereafter, partial purification of tetrodotoxin binding proteins from electric eel electroplax revealed a correlation between tetrodotoxin binding activity and a protein of ~270 kDa (Agnew et al. 1980). Subsquent purification studies showed that the sodium channel from mammalian brain is a complex of (260kDa), 1 (36kDa), and 2 (33kDa) subunits (Hartshorne and Catterall 1981; Hartshorne et al. 1982), the tetrodotoxin binding component of the sodium channel purified from eel electroplax is a single protein of 270 kDa (Miller et al. 1983), and the sodium channel from skeletal muscle is a complex of and 1 subunits (Barchi 1983).

The calcium channels are known to be involved in the pacemaker depolarization, Ca2+- dependent secretion and the activation of Ca2+-dependent ionic conductance in neuronal preparations, although linking a specific type of ICa to a particular cellular process may be difficult (Tsien et al. 1988). It may be certain, however, that the subtypes of ICa contribute in different ways to the transmission of sensory signals of different modalities in primary afferent neurons. The subtypes of ICa are known to show diameter-dependent variation in acutely isolated rat DRG neurons, i. e., medium diameter neurons had a large amount of T-type ICa, whereas significantly large proportion of the whole-cell ICa was L-type in the small cells (Scroggs and Fox 1991, 1992), which shaped action potentials differently and may affect, therefore, significantly to the sensory transmission. In most cases, multiple calcium channel types coexist in the same neuron and it has been speculated that they may contribute toward different neuronal functions, possibly depending on their localization (Doughty et al. 1998; Wu et al. 1999).

Previous work on L-type and N-type calcium channels has demonstrated different localizations in specific areas of the neuronal surface. L-type channels have been found in the neuronal somata and proximal dendrites of rat brain, spinal cord, and retina (Westenbroek et al. 1990; Ahlijanian et al. 1990), which is consistent with a role in mediating the increases in intracellular calcium occurring in the cell body in response to excitatory inputs to the dendrites. N-type channels have been detected at the release face of presynaptic nerve terminals at the frog neuromuscular junction (Robitaille et al. 1990; Cohen et al. 1991; Torri Tarelli et al. 1991), in the presynaptic calyx-type nerve terminals of chick ciliary ganglia, and in the medial nucleus of the trapezoid body in rat (Haydon et al. 1994; Wu et al.

1999). These data, together with a number of functional findings (Miller 1990; Dunlap et al. 1995; Tsien et al. 1995; Stanley 1997), clearly indicate that N-type channels play a role in supporting neurotransmitter release. In this experiment, it is noted that there was a moderate staining of the N-type calcium channels of the boutons and somata of upper and lower trigeminal spinal nucleus compared to other part of the brain.

After the initial description of P-type calcium channels in Purkinje cells (Llinas et al. 1989; Mintz et al. 1992) and the subsequent designation of the Q-type calcium channels as a separate channel category (Randall and Tsien 1995), concern has arisen regarding the distinction between them. P/Q-type calcium channels are widely expressed in the mammalian brain in a predominantly presynaptic distribution (Westenbroek et al. 1995), and have a important role in modulating neurotransmitter release (Regehr and Mintz 1994). In this immunohistochemical study, P/Q-type calcium channels were weakly stained in the upper trigeminal spinal nucleus. And a very weak staining for the P/Q-type calcium channels was observed in the trigeminal principal nucleus. R-type calcium channel was not observed in the upper trigeminal spinal nucleus.

Inwardly rectifying potassium channels (Kir) control the resting K+ conductance in many excitable and non-excitable cells (Hille 1992). They mediate a high K+ conductance at the K+ reversal potential and at voltages slightly positive to the K+ reversal potential. Thus Kir have a stabilizing effect on the resting potential. Whenever Kir are regulated by second messengers, this represents a highly effect mechanism of controlling excitability (Nilius et al. 1993). Various subtypes of the Kir family have been cloned and heterologously expressed in order to study their functional properties. One of the Kir, Kir 2.1 is widely distributed in various tissues. Kir 2.1 is found in mammalian lens epithelial cells (Cooper et al. 1991), ventricular myocytes (Josephson 1998), endothelial cells (Silver and DeCoursey 1990), and oocytes (Ruppersberg and Fakler 1996). In this experiment, moderate staining of the Kir 2.1 in the trigeminal principal nucleus were observed. On the other hand, staining of the Kir 2.1 was weak in the trigeminal spinal nucleus.

Much interest has been generated recently in the potassium channel subunit Kv 4.2 for its critical role in regulating membrane excitability. Several lines of evidence suggest that Kv 4.2 underlies the A-type current (IA) in neurons (Serodio et al. 1996; Song et al. 1998) as well as the transient outward current (Ito) in cardiac ventricular myocytes (Barry et al. 1998). In particular, Tkatch, et al. (2000) have found that Kv 4.2 mRNA abundance and A-type current amplitude are linearly related in the striatum. In the hippocampus, such A-type current has been found to dampen back-propagation of action potentials in the distal dendrites of CA1 pyramidal neurons, thereby setting up the capacity to modify the excitatory postsynaptic potential or back-propagating action potentials following synaptic activity (Hoffman et al. 1997). Immunohistochemical studies show that Kv 4.2 protein localizes abundantly to the hippocampus (Sheng et al. 1992) with particular localization to the neuronal soma and dendrites (Maletic- Savatic et al. 1995), structures in which modulating membrane excitability could have important implications for long-term potentiation and memory. Furthermore, ultrastructural studies in supraoptic neurons have shown that Kv 4.2 is localized to the postsynaptic membrane directly across from the presynaptic terminal (Alonso and Widmer 1997). In this study, there was weak staining for the Kv 4.2 in trigeminal spinal nucleus.

Ca2+-activated K+ channels (Kca) are present in a remarkable variety of animal cells, where they integrate changes in intracellular Ca2+ concentration with changes in membrane potential. They are involved in many physiologic processes including regulation of secretion, smooth muscle tone, and control of action potential shape and firing pattern in excitable cells (Marty 1989; Vergara et al. 1998). BKCa can be divided into three main subfamilies based on their electrophysiological, pharmacological, and molecular profiles: large- conductance Ca2+-activated K+ channels, intermediate- conductance Ca2+-activated K+ channels, and small-conductance Ca2+-activated K+ channels (Vergara et al. 1998). Large-conductance Ca2+- activated K+ channels (BKCa) are found in a variety of both elec-

trically excitable and non-excitable cells (Jan and Jan 1997). Their activities are triggered by membrane depolarization and enhanced by cytosolic Ca2+, providing a link between the metabolic and electrical state of cells. The physiological roles of BKCa channels have been examined in several tissues. In smooth muscle in which they are particularly abundant, BKCa channels play a key role in setting the pace of contractile activity (Nelson et al. 1995), and in neurons, they are involved in regulation of transmitter release and repolarization of action potentials (Kaczorowski et al. 1996). In this experiment, the intensity of staining for the BKCa channels was weak in the trigeminal principal nucleus and the trigeminal spinal nucleus.

In this immunohistochemical study, thus, we could observe (1) the sodium channels in the trigeminal spinal nucleus and the trigeminal principal nucleus (2) calcium channels: N-type in the trigeminal spinal nucleus, and P/Q-type in the trigeminal spinal nucleus and the trigeminal principal nucleus (2) the potassium channels: inwardly rectifying potassium (Kir 2.1) channels in the trigeminal principal nucleus and the trigeminal spinal nucleus, voltage- gated potassium (Kv 4.2) channels in the trigeminal spinal nucleus, and very weak staining for the large-conductance calcium- activated potassium (BKCa) channels in the trigeminal spinal nucleus and the trigeminal principal nucleus.

These results means as follow.

1. Sodium channels play important role as nociceptor in trigeminal spinal nucleus, inducer of action potential in trigeminal principal nucleus.
2. N-type channels were stained stronger than other calcium channels. N-type channels play a role in supporting neurotransmitter release. We can estimate fibers or bodies of neuron synapse in both nucleus therefore neurotransmitter release is important in these nucleus.
3. There were weak staining for potassium channels. In the case of BKCa, it was repored that the channel was mainly distributed in smooth muscle. Previous work it was estimated that role of Kv 4.2 channel was related to long-term memory. So Kv 4.2 protein localized abundantly to the hippocampus (Sheng et al.1992) but in our study, the protein rare localized to sensory nucleus.

Ⅴ. CONCLUSION

Trigeminal sensory nerves relay mechanical, thermal, chemical and proprioceptive information from craniofacial regions. Trigeminal sensory nerves act at the focal level to maintain the integrity of craniofacial tissues through trophic influences and serve the whole animal through reflexes that protect other external sensory and internal homeostatic systems from damaging environmental changes.

Ion channels of the sensory neurons play a pivotal role in the generation and transmission of sensory information. Generally the Na+ channels are involved in the rapid generation of action potentials. Calcium influx triggers a number of cellular processes, including muscle contraction, second-messenger activation cascade, regulation of axonal guidance, control of neurotransmitter release. Generally potassium channels are involved in repolarization after depolarization, setting the pace of contractile activity, regulation of neurotransmitter release, and modulating membrane excitability.

The main goals of this study were (1) to observe some kinds of ion channels (2) to observe the localized distribution of the ion channels in the trigeminal principal nucleus, the trigeminal spinal nucleus and the trigeminal mesencephalic nucleus by immunohistochemical method.

In this immunohistochemical study we observed as follow.

1. We observed the sodium channels in the trigeminal spinal nucleus and the trigeminal principal nucleus. We suggested that sodium channels played the role as nociceptor in trigeminal spinal nucleus, inducer of action potential in trigeminal principal nucleus.
2. We observed the calcium channels: N-type in the trigeminal spinal nucleus, and P/Q-type in the trigeminal spinal nucleus and trigeminal principal nucleus. N-type channels were stained stronger than other calcium channels. N-type channels play a role in supporting neurotransmitter release. We can estimate fibers or bodies of neuron synapse in both nucleus therefore neurotransmit-

ter release is important in these nucleus.

3. We observed the potassium channels: inwardly rectifying potassium (Kir 2.1) channels in the trigeminal principal nucleus and the trigeminal spinal nucleus, voltage- gated potassium (Kv 4.2) channels in the trigeminal spinal nucleus, and very weak staining for the large-conductance calcium- activated potassium (BKCa) channels in the trigeminal spinal nucleus and trigeminal principal nucleus.

At present, it is obviously difficult to recognize the distinct ion channel types and their function in trigeminal sensory nucleus. Based on our results we can propose possible model systems in which a detailed electrophysiological and pharmacological analysis may help clarify the functional role of ion channels with a defined subunit composition in trigeminal sensory nucleus. Additional studies will be required to explore the roles and distributions of ion channels in trigeminal sensory nucleus in relation to their subunit composition and their positions.

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사진부도 설명

Fig. 1(a). A cell stained with sodium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 1(b). A fluorescence micrograph showing stained sodium channels with sodium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 2(a). A cell stained with sodium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 2(b). A fluorescence micrograph showing stained sodium channels with sodium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 3(a). A cell stained with N-type calcium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 3(b). A fluorescence micrograph showing stained N-type calcium channels with calcium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 4(a). A cell stained with N-type calcium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 4(b). A fluorescence micrograph showing stained N-type calcium channels with calcium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 5(a). A cell stained with P/Q-type calcium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 5(b). A fluorescence micrograph showing stained P/Q-type calcium channels with calcium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 6(a). A cell stained with P/Q-type calcium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 6(b). A fluorescence micrograph showing stained P/Q-type calcium channels with calcium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 7(a). A cell stained with R-type calcium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 7(b). A fluorescence micrograph showing stained R-type calcium channels with calcium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 8(a). A cell stained with R-type calcium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 8(b). A fluorescence micrograph showing stained R-type calcium channels with calcium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 9(a). A cell stained with Kir 2.1 potassium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 9(b). A fluorescence micrograph showing stained potassium channels with Kir 2.1 potassium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 10(a). A cell stained with Kir 2.1 potassium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 10(b). A fluorescence micrograph showing stained potassium channels with Kir 2.1 potassium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 11(a). A cell stained with Kv 4.2 potassium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 11(b). A fluorescence micrograph showing stained potassium channels with Kv 4.2 potassium channel anti-

bodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 12(a). A cell stained with Kv 4.2 potassium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 12(b). A fluorescence micrograph showing stained potassium channels with Kv 4.2 potassium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 13(a). A cell stained with BKCa potassium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 13(b). A fluorescence micrograph showing stained potassium channels with BKCa potassium channel antibodies in the trigeminal spinal nucleus (indicated with an arrow)

Fig. 14(a). A cell stained with BKCa potassium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

Fig. 14(b). A fluorescence micrograph showing stained potassium channels with BKCa potassium channel antibodies in the trigeminal principal nucleus (indicated with an arrow)

사진부도 ①

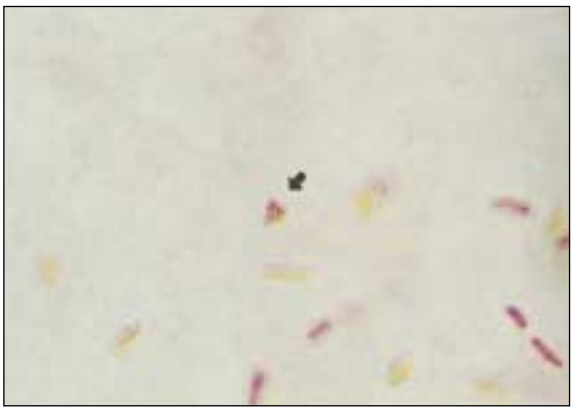
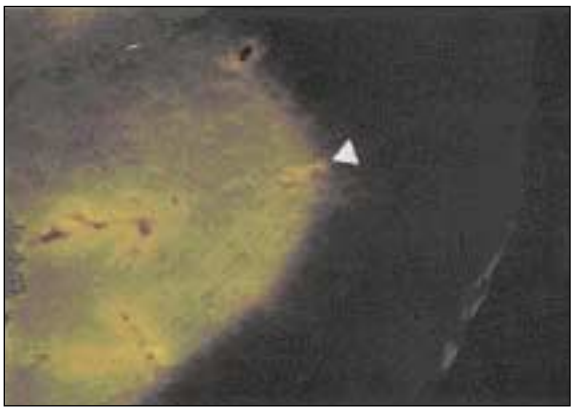


Fig. 1a.



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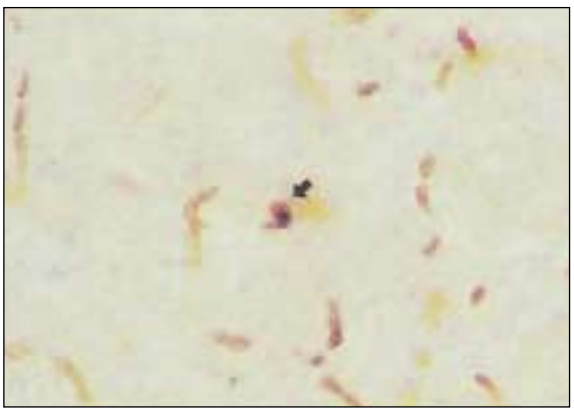

Fig. 2a.

Fig. 2b.

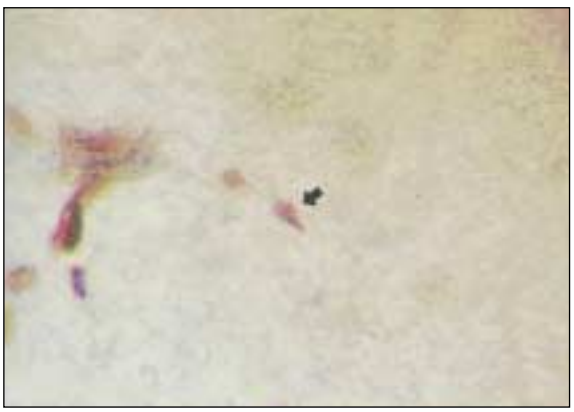

Fig. 3a.

Fig. 3b.

사진부도 ②

Fig. 4a.

Fig. 4b.

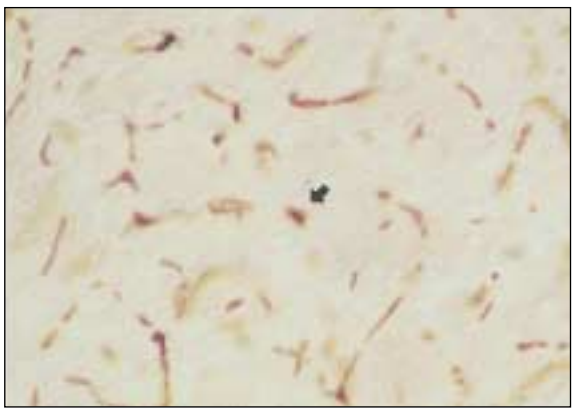

Fig. 5a.

Fig. 5b.

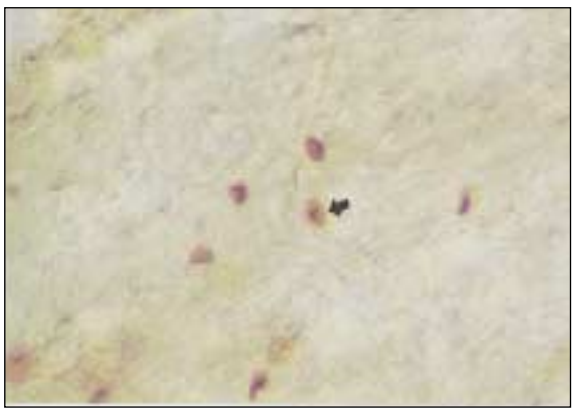

Fig. 6a.

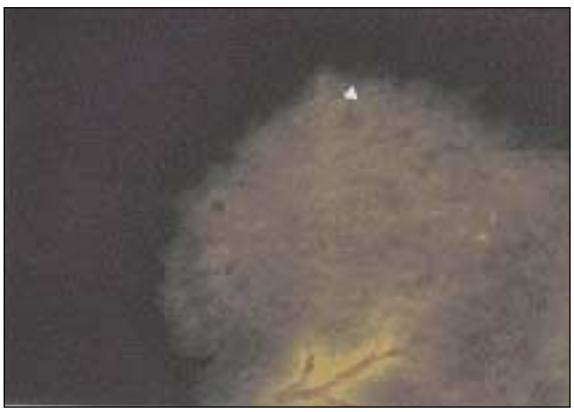

Fig. 6b.

사진부도 ③

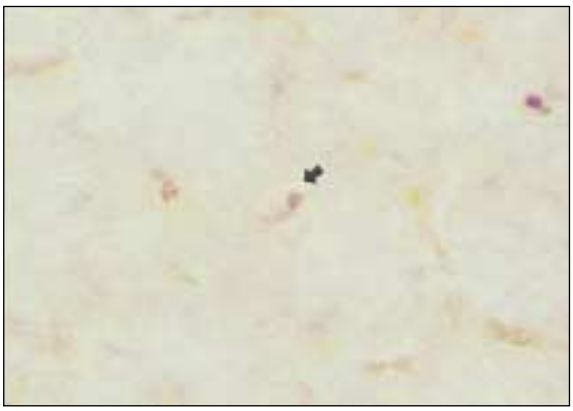

Fig. 7a.

Fig. 7b.

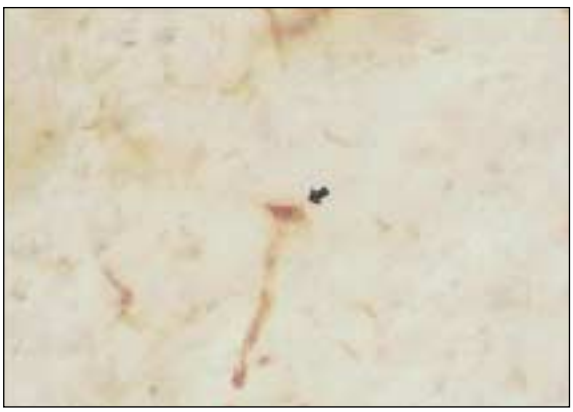

Fig. 8a.

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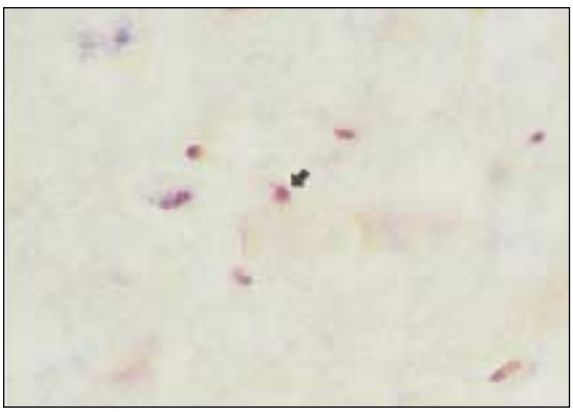

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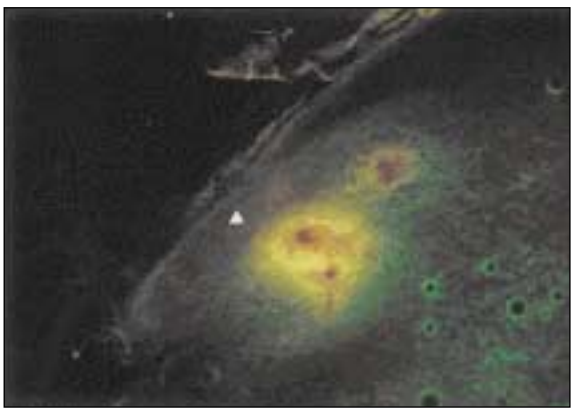


Fig. 9b.

사진부도 ④

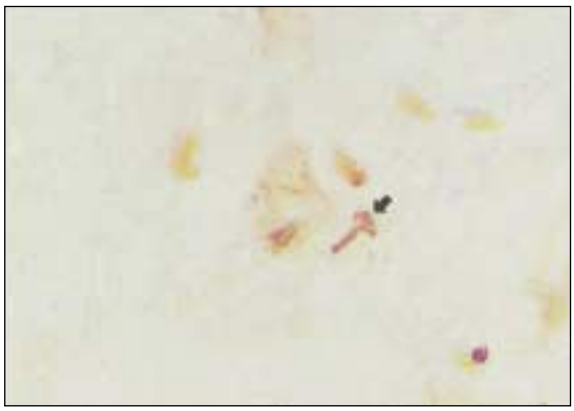


Fig. 10a.

Fig. 10b.

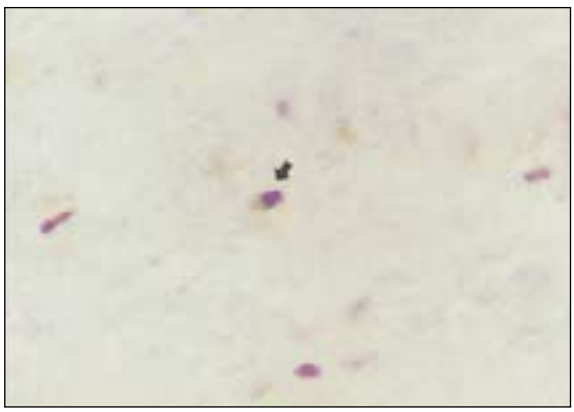
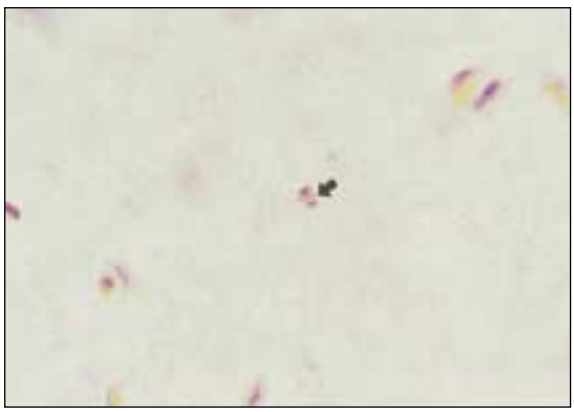


Fig. 11a.

Fig. 11b.



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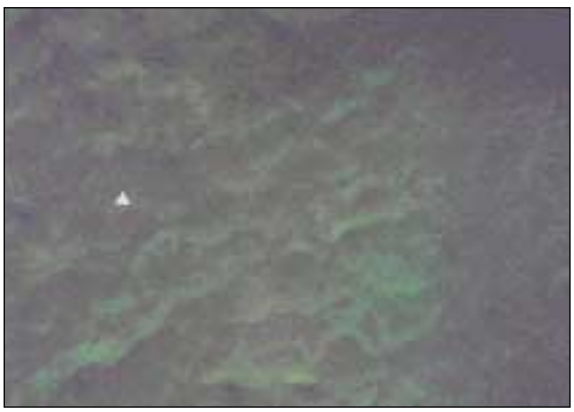
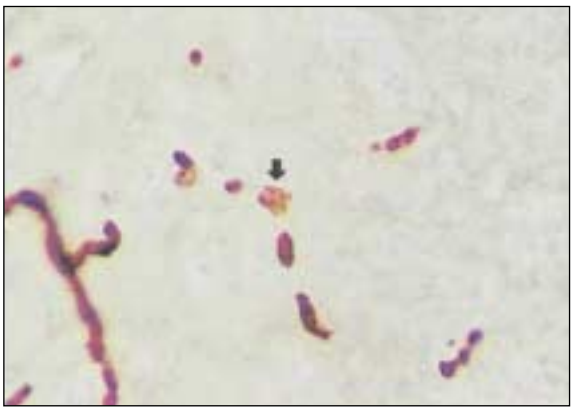


Fig. 12b.

사진부도 ⑤



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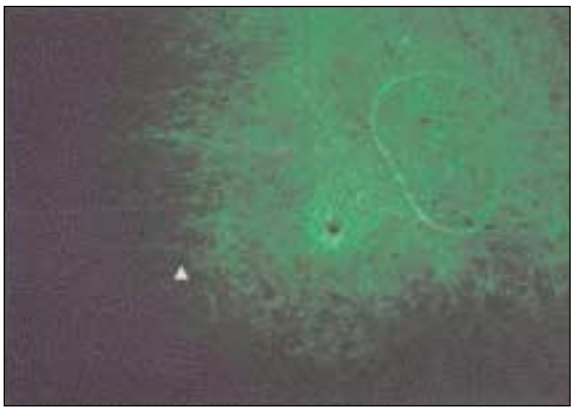

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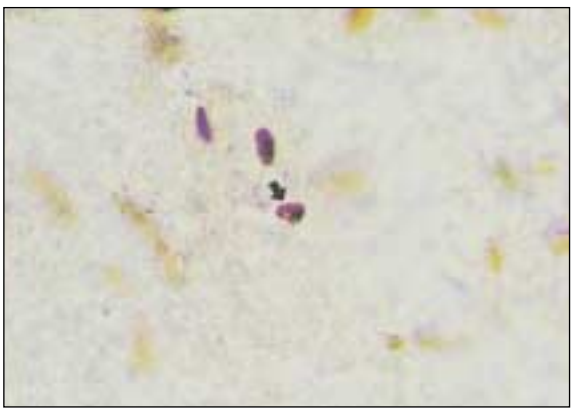

Fig. 14a.

Fig. 14b.