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We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system, mainly using the visual systems of chicks and mice. This research covers many developmental events including the patterning of the retina, axonal navigation, branching and targeting, synapse formation, refinement and plasticity. The scope of our interests also encompasses the mechanisms for various functions of the mature brain, including sensation, emotion, behavior, learning, and memory.

Ⅰ. Mechanisms for regional specification in the developing retina

Topographic maps are a fundamental feature of neural networks in the nervous system. The retinotectal projection of lower vertebrates including birds has been used as a readily accessible model system of the topographic projection. We have been studying the mechanisms for regional specification in the developing retina as the basis of the topographic retinotectal projection.

In the past ten years, we have devoted our efforts to searching for molecules with asymmetrical distribution in the embryonic chick retina, and to the characterization of their roles in the topographic retinotectal projection. We performed a large-scale screening using differential hybridization and restriction landmark cDNA scanning (RLCS) on the embryonic day 8 (E8) chick retina, and we successfully identified 33 asymmetric molecules along the nasotemporal (N-T) axis and 20 along the dorsoventral (D-V) axis.

We subsequently conducted misexpression and knockdown experiments on the embryonic chick retina by *in ovo* electroporation using retroviral vectors to elucidate the molecular functions of these asymmetric molecules and the hierarchy among them. We have revealed gene cascades of topographic molecules for the retinal patterning and for the topographic retinotectal projection (see Annual Report 2006).

Although it is known that different neuronal subtypes are organized asymmetrically with respect to the two axes in the retina, the mechanisms of the final differentiation of neuronal subtypes still remain unclear. The region-specific distribution and development of these cells in the retina are also attributable to the retinal patterning which should be determined by morphogens and transcription factors expressed asymmetrically in the developing retina. Among the RLCS clones, we have already identified some molecules that are expressed in a specific subtype of the retinal ganglion cells (Figure 1). Studies to explore functional roles of these genes are currently underway.

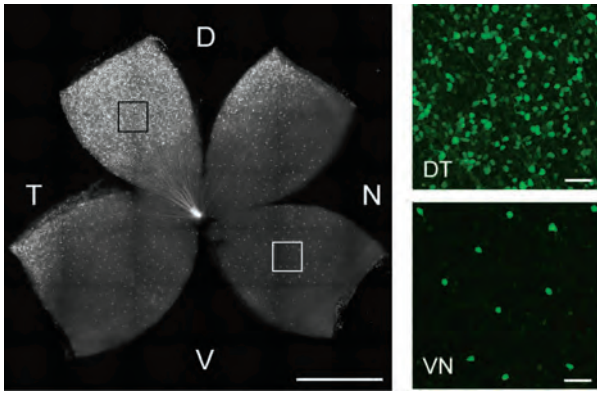

Figure 1. SPIG1 expression in the mouse retina at P5. SPIG1-positive retinal ganglion cells are densely distributed in the dorsotemporal retina. In the remaining region of the retina, only a subtype of the retinal ganglion cell appears to express SPIG1, showing a mosaic distribution. Enlargements of the boxed regions are shown on the right. N, T, D, and V indicate nasal, temporal, dorsal, and ventral, respectively. Scale bars: 1 mm (left panel), 50 μm (right panels).

Ⅱ. Mechanisms for the topographic retinotectal projection

At the onset of the retinotectal projection at E12, the axons of retinal ganglion cells exhibit a crude topographic order both anteroposteriorly and mediolaterally on the tectum/superior colliculus. Next, axonal sprouting begins predominantly in the vicinity and towards the site of the normal terminal zone (TZ). Further axon branching and arborization lead to the formation of mature TZs, while aberrant axon segments and branches are eliminated in an activity-dependent manner. These processes are completed at around E16 in chick (Figure 2) and P8 in mouse, respectively.

General attention is now devoted to the molecular mechanisms for the axon branching and arborization and their selective elimination. Among the region-specific

molecules in the developing retina, we have already identified several molecules which induce abnormal branching and arborization when their expression was experimentally altered *in vivo*. We expect that our research will shed light on these issues.

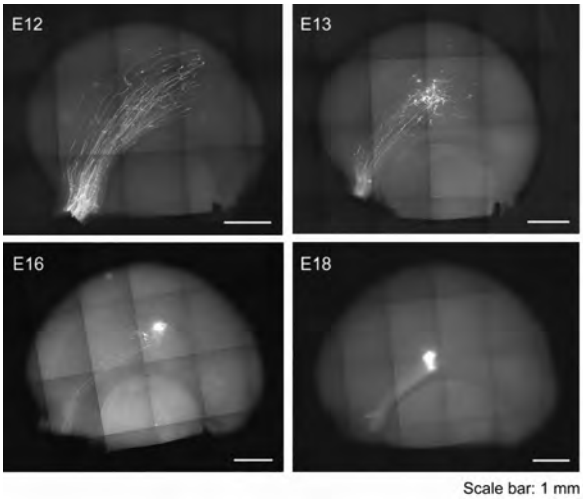

Figure 2. Development of retinotectal projection in chicks. Dorsal axons were labeled with DiI and the feature of axon terminals on the tectum was developmentally explored.

Ⅲ. Physiological roles of protein tyrosine phosphatase receptor type Z (Ptprz)

Ptprz (also called PTPζ /RPTPβ) is a receptor-type protein tyrosine phosphatase (RPTP) predominantly expressed in the brain as a chondroitin sulfate proteoglycan. Among the twenty-one RPTPs expressed in mammals, only the R5 subfamily, Ptprz and Ptprg (PTPγ), have a canonical PDZ-binding motif (-S-L-V) at their carboxyl-termini. ErbB4 is a member of the ErbB-family tyrosine kinases known as a neuregulin (NRG) receptor. Ptprz and ErbB4 are reported to bind to postsynaptic density-95 (PSD95) on the second and the first/second PDZ (PSD95/Disc large/zona occludens1) domains, respectively, through the PDZ-binding motif of their carboxyl termini. We found a functional interaction between Ptprz and ErbB4.

An intracellular carboxyl-terminal region of Ptprz pulled-down PSD95 and ErbB4 from an adult rat synaptosomal preparation. ErbB4 and Ptprz showed co-localization in cell bodies and apical dendrites of neurons in the prefrontal cortex. *In vitro* experiments using the whole intracellular region (ICR) of ErbB4 also showed that PSD95 stimulates the autophosphorylation of ErbB4, and that the ICR of Ptprz dephosphorylates ErbB4 independent of the presence of PSD95 (Figure 3A, B). In HEK293T cells, phosphorylation of ErbB4 was raised by co-expression of PSD95, which was repressed by additional expression of Ptprz (Figure 3C). Taken together with the finding that the tyrosine phosphorylation level of ErbB4 was increased in *Ptprz*-knockout mice, these results suggest that Ptprz has a role in suppressing the autoactivation of ErbB4 by PSD95 at the postsynaptic density in the adult brain.

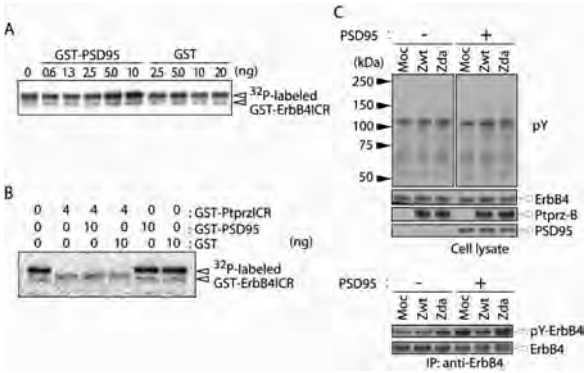

Figure 3. Tyrosine phosphorylation of ErbB4 is enhanced by PSD95 and repressed by Ptprz. (A) *In vitro* phosphorylation assay of ErbB4. GST-ErbB4ICR pre-incubated with indicated amounts of GST-PSD95 or GST was subjected to autophosphorylation with [γ-^{32}P]ATP, and then analyzed by SDS–PAGE followed by autoradiography. (B) *In vitro* dephosphorylation assay of ErbB4. After indicated amounts of GST-PtprzICR, GST-PSD95 and GST were mixed and preincubated, the ^{32}P-labelled GST-ErbB4ICR was added and incubated, and then analyzed as above. (C) Tyrosine phosphorylation of ErbB4 and its dephosphorylation by Ptprz in HEK293T cells. The tyrosine phosphorylation of cellular proteins and ErbB4 was analyzed by Western blotting, along with the protein expression.

Ⅳ. Mechanisms of Na-level sensing in the brain for the body-fluid homeostasis

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluids. For Na homeostasis of the body, controls of Na and water intake and excretion are of prime importance. Although it was suggested that the circumventricular organs (CVOs) are involved in body-fluid homeostasis, the system for sensing Na levels within the brain, which is responsible for the control of Na- and water-intake behavior, has long been an enigma. Na_x is an atypical sodium channel that is assumed to be a descendant of the voltage-gated sodium channel family. Our studies on the *Na$_x$*-knockout mice revealed that Na_x channels are localized to the CVOs and serve as a sodium-level sensor of body fluids. *Na$_x$*-knockout mice do not stop ingesting salt when dehydrated, while wild-type mice avoid salt.

As the first step toward understanding the cellular mechanism by which the information sensed by Na_x channels is reflected in the activity of the organs, we dissected the subcellular distribution of Na_x. Double-immunostaining and immuno-electron microscopic analyses revealed that Na_x is exclusively localized to perineuronal lamellate processes extending from ependymal cells and astrocytes in the organs. In addition, glial cells isolated from the subfornical organ (SFO), a member of the CVOs, were sensitive to an increase in the extracellular sodium level, as analyzed by an ion-imaging method. These results suggest that glial cells bearing Na_x channels are the first to sense a physiological increase in the level of sodium in body fluids, and regulate the neural activity of the CVOs by enveloping neurons. Thus, close communication between inexcitable glial cells and excitable neural cells is supposedly the basis

of the central control of salt homeostasis.

This year, we revealed direct interaction between Na_x channels and α subunits of Na^+/K^+-ATPase, which brings about Na-dependent activation of the metabolic state of the glial cells. The metabolic enhancement leading to extensive lactate production was observed in the SFO of wild-type mice, but not of the *Na$_x$*-knockout mice. Furthermore, lactate, as well as Na, stimulated the activity of GABAergic neurons in the SFO (Figure 4). These results suggest that the information on a physiological increase of the Na level in body fluids sensed by Na_x in glial cells is transmitted to neurons by lactate as a mediator to regulate neural activities of the SFO. It is likely that this leads to the control of salt-intake behavior (Figure 5).

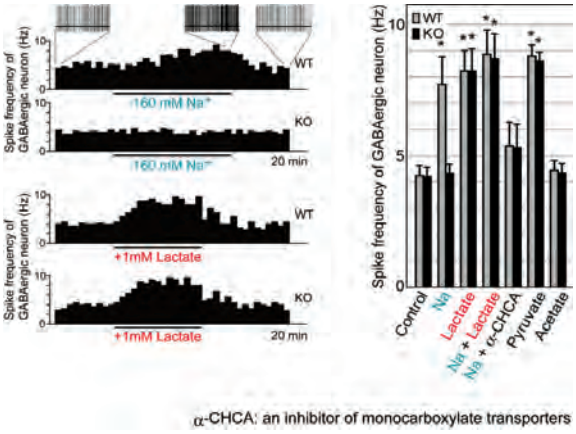

Figure 4. Properties of GABAergic neurons in the SFO of wild-type and *Na$_x$*-KO mice. There exist GABAergic neurons spontaneously firing in the SFO. Lactate, as well as Na, stimulated the activity of GABAergic neurons in the SFO. This activation by lactate is suppressed by α-CHCA, an inhibitor of lactate transporter.

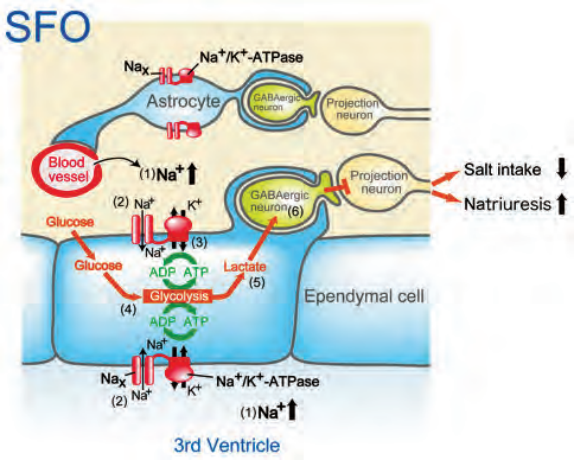

Figure 5. Schematic drawing of the Na-level sensing mechanism and Na-dependent regulation of neural activity in the SFO. When animals are dehydrated, Na concentration in plasma and cerebrospinal fluid increases above the usual level of ~145 mM (1). When the extracellular Na concentration exceeds ~150 mM, Na_x channels open, and the intracellular Na concentration in these glial cells is increased. This leads to activation of Na^+/K^+-ATPase in these cells (2). Activated Na^+/K^+-ATPase consumes ATP larger than the usual level to pump out Na ions (3). To fuel Na^+/K^+-ATPase with ATP, the glial cells enhance the glucose uptake to stimulate the anaerobic glycolysis (4). Lactate, the end product of the anaerobic glycolysis, is released from the glial cells and supplied to neurons, including GABAergic neurons, through the processes enveloping them (5). Lactate stimulates the activity of the GABAergic neurons through production of ATP, which presumably regulate hypothetic neurons involved in the control of salt-intake behavior (6).

Publication List

〔Original papers〕

- Fujikawa, A., Chow. J.P.H, Shimizu. H., Fukada. M., Suzuki. R., and Noda, M. (2007). Tyrosine phosphorylation of ErbB4 is enhanced by PSD95 and repressed by protein tyrosine phosphatase receptor type Z. J. Biochem. *142*, 343-350.
- Shimizu, H., Watanabe, E., Hiyama, T.Y., Nagakura, A., Fujikawa, A., Okado, H., Yanagawa, Y., Obata, K., and Noda, M. (2007). Glial Na_x channels control lactate signaling to neurons for brain [Na^+] sensing. Neuron *54*, 59-72.
- Yamamoto, H., Kamegaya, E., Hagino, Y., Imai, K., Fujikawa, A., Tamura, K., Enokita, T., Yamamoto, T., Takeshima, T., Koga, H., Uhl, G.R., Ikeda, K., and Sora, I. (2007). Genetic deletion of vesicular monoamine transpoter-2 (VMAT2) reduces dopamine transporter activity in mesencephalic neurons in primary culture. Neurochem. Int. *51*, 237-244.

〔Review article〕

- Noda, M. (2007). Hydromineral neuroendocrinology: Mechanism of sensing sodium levels in the brain. Exp. Physiol. *92*, 513-522.