DIVISION OF MOLECULAR NEUROBIOLOGY

Professor
NODA, Masaharu

Associate Professor
SHINTANI, Takafumi

Assistant Professors:	*SHINTANI, Takafumi**
	SAKUTA, Hiraki
	HIYAMA, Takeshi
Technical Staff:	*TAKEUCHI, Yasushi*
NIBB Research Fellow:	*YOSHIDA, Masahide*
Postdoctoral Fellows:	*FUJIKAWA, Akihiro*
	SUZUKI, Ryoko
	NISHIHARA, Eri
	MATSUMOTO, Masahito
Graduate Students:	*NAGAKURA, Ayano*
	TANI, Sachiko
	SAKURABA, Jyuichi
	MIYAZAKI, Yuriko
Technical Assistants:	*MIZOGUCHI, Masae*
	HATTORI, Nobuko
	TOMITA, Nao
	ISOSHIMA, Yoshiko
	MIURA, Seiko
	NAKANISHI, Norie
	DOKYO, Yumi
Secretary:	*KODAMA, Akiko*

We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system (CNS), mainly using the visual systems of chicks and mice. This research covers many developmental events including the patterning of the retina, neuronal terminal differentiation, 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 body-fluid regulation, behavior control, learning, and memory.

I. Mechanisms for neural circuit formation

Topographic maps are a fundamental feature of neural networks in the nervous system. We have long studied the molecular mechanisms for regional specification in the developing retina as the basis of the topographic retinotectal projection. Our special attention is now devoted to the molecular mechanisms underlying axon branching and arborization for synapse formation, along with elimination of mistargeted axons and branches. Among the region-specific molecules in the developing retina, we have already found several molecules that induce abnormal branching or arborization when their expression was experimentally manipulated *in vivo*. One is adenomatous polyposis coli 2 (APC2), which is preferentially expressed in the nervous system from early developmental stages through to adulthood.

APC2 is distributed along microtubules in growth cones as well as axon shafts of retinal axons. The knockdown of *APC2* in chick retinas reduced the stability of microtubules in retinal axons and yielded their abnormal behaviors including a reduced response to ephrin-A2 and misprojection in the tectum without making clear target zones. Recently, we have generated *APC2*-deficient mice by a gene-targeting technique. We are now analyzing the defects in them to clarify the role of APC2 in the development of the CNS.

II. Development of direction-selective retinal ganglion cell subtypes

Visual information is transmitted to the brain by roughly a dozen distinct types of retinal ganglion cells (RGCs) defined by characteristic morphology, physiology, and central projections. However, because few molecular markers corresponding to individual RGC types are available, our understanding of how these parallel pathways develop is still in its infancy.

The direction of image motion is coded by direction-selective (DS) ganglion cells in the retina. Particularly, the ON DS ganglion cells project their axons specifically to terminal nuclei of the accessory optic system (AOS) responsible for optokinetic reflex. We recently generated a knock-in mouse in which SPIG1 (SPARC-related protein containing immunoglobulin domains 1)-expressing cells are visualized with GFP, and found that SPIG1-positive RGCs project to the medial terminal nucleus (MTN), the principal nucleus of the AOS. Combination of genetic labeling and conventional retrograde labeling revealed that MTN-projecting ganglion cells are comprised of SPIG1^{+} and SPIG1^{-} ganglion cells distributed in distinct mosaic patterns in the retina. Furthermore, we revealed that SPIG1^{+} and SPIG1^{-} ganglion cells respond preferentially to upward motion and downward motion, respectively, by targeted electrophysiological recordings.

A key circuit module of DS ganglion cells is a spatially asymmetric inhibitory input from starburst amacrine cells. However, it was not known how and when this circuit asymmetry is established during development. We recently found that random or symmetric synaptic connections from starburst amacrine cells are established as early as postnatal day 6, and that inhibitory synaptic inputs are selectively reorganized over a 2-day period.

Analysis of gene-expression profiles in the two types of ON DC ganglion cells is now under way. This will shed light on molecular mechanisms for the differentiation and distinct circuit formation of the two ganglion cell types.

III. Physiological roles of protein tyrosine phosphatase receptor type Z

Protein-tyrosine phosphatase receptor type Z (Ptprz, also known as PTPζ/RPTPβ) is a member of R5 receptor-like protein tyrosine phosphatase (RPTP) subfamily. Ptprz is predominantly expressed in the brain and its physiological importance has been demonstrated through studies with *Ptprz*-deficient mice. Ptprz modulates hippocampal synaptic plasticity: adult *Ptprz*-deficient mice display impairments in spatial and contextual learning. Ptprz is expressed also in the stomach, where it functions as a receptor of VacA, a cytotoxin secreted by *Helicobacter pylori*: *Ptprz*-deficient mice are resistant to gastric ulcer induction by VacA. Although our understanding about physiological functions of Ptprz is thus progressing, our knowledge about its

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

biochemical properties such as substrate specificity is still limited. We previously identified Git1, p190RhoGAP, and Magi1, as substrates for Ptprz by developing a new genetic method named "yeast substrate-trapping system". Recently, we found that Ptprz selectively dephosphorylates specific phospho-tyrosine residues in these substrate molecules. Alignment of the primary sequences surrounding the target phospho-tyrosine residues revealed considerable similarity. We performed kinetic analyses using various fluorescent substrate peptides with replacement at each position in series to determine the consensus substrate motif for Ptprz. The motif sequence thus obtained predicted paxillin as a novel substrate candidate. We verified that the site in paxillin is efficiently dephosphorylated by Ptprz in a cell-based assay.

IV. Brain systems for body-fluid homeostasis

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluids. For Na homeostasis of the body, control of Na and water intake and excretion are of prime importance. It was suggested that the circumventricular organs (CVOs), where the blood-brain barrier is missing, are involved in monitoring body-fluid conditions. However, molecular and cellular mechanisms for sensing Na levels and osmolality of body fluids within the brain have long been an enigma. Our studies with *Na$_x$*-deficient mice revealed that Na$_x$, atypical sodium channel, is localized to the CVOs and serves as a sodium-level sensor of body fluids. *Na$_x$*-deficient mice do not stop ingesting salt when dehydrated, while wild-type mice avoid it.

Recently, we revealed that autoimmunity to Na$_x$ causes essential hypernatremia. Essential hypernatremia is clinically characterized by upward resetting of both the osmotic set point for vasopressin release and the threshold for thirst perception, resulting in persistent hypernatremia with a euvolemic state. Usually, structural abnormalities in the hypothalamic-pituitary area are detected. However, several cases of essential hypernatremia without demonstrable hypothalamic structural lesions have been reported.

We studied a case with clinical features of essential hypernatremia without demonstrable hypothalamic structural lesions. The patient was a 6.5-year-old Asian girl complaining of general fatigue and fluctuating drowsiness persisting for a week at the time of admission. Clinical tests revealed that she had marked hypernatremia with a serum Na concentration as high as 199 mM. Of note, she did not complain of any sensation of thirst despite her extreme hypernatremia. Intravenous infusion therapy with a series of hypotonic fluids resulted in a gradual decrease in her serum Na concentration. Further clinical tests revealed a solid tumor adjacent to the right adrenal gland. The tumor was surgically removed, on the assumption that the tumor was somehow related to her extreme hypernatremia; however, the patient's hypernatremia was not cured by the removal of the tumor. We therefore instructed her to drink 1500–2000 ml of water/day, however, her serum Na level has continued to fluctuate until now, 4 years after removal of the tumor.

The relationship between serum osmolality and the plasma vasopressin level obtained during a 4-year follow-up clearly showed that the normal increase of vasopressin secretion in response to serum hyperosmolality is lacking in this patient (Figure 1A). Western blotting with the patient's serum revealed that the patient developed autoantibodies, which detect both human and mouse Na$_x$ (Figure 1B and C). The histological diagnosis of the removed tumor was ganglioneuroma, predominantly composed of Na$_x$-positive Schwann-like cells, along with some ganglion cells (Figure 1D). As such, this neoplasia likely evoked an antitumor immune response, suggesting a paraneoplastic neurologic disorder characterized by neurologic dysfunction in the setting of a remote cancer.

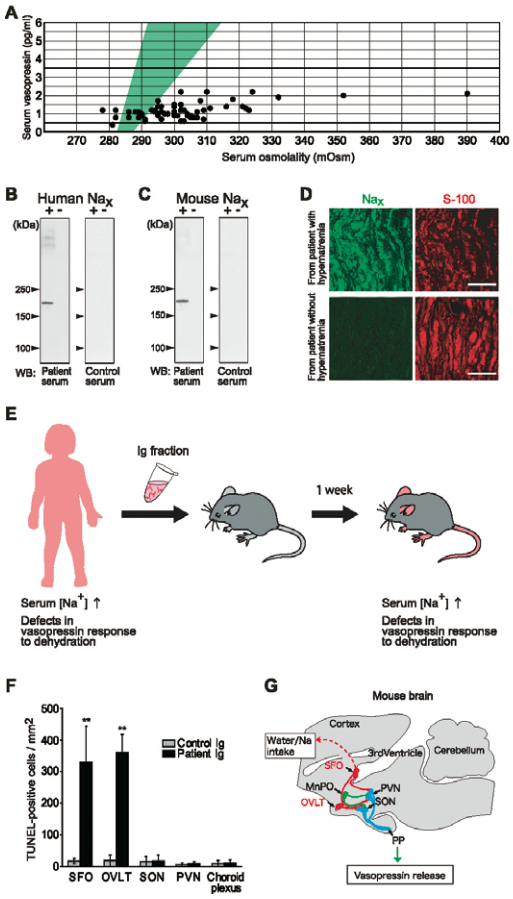

Figure 1. Autoimmunity to the sodium-level sensor in the brain causes essential hypernatremia. (A) Relationship between the serum osmolality and plasma vasopressin level obtained during a 4-year follow-up of the patient. The green area indicates the normal range. (B) Western blot analysis of the membrane extracts of C6 glioblastoma cells with (+) or without (–) expression of human Na$_x$ using serum from either the patient with hypernatremia (Patient serum) or a healthy human subject (Control serum). (C) Western blot analysis of the membrane extracts of C6 glioblastoma cells with (+) or without (–) expression of mouse Na$_x$. (D) Expression of Na$_x$ (green) and S-100 (red) in the ganglioneuroma removed from a patient with hypernatremia (upper). Na$_x$ expression was negative in a ganglioneuroma from a patient without hypernatremia (lower). S-100, a marker of Schwann cells. Scale bars, 100 μm. (E) Generation of animal model of the disease. Passive transfer of the immunoglobulin (Ig) fraction of the patient's serum reproduced her symptoms with abnormal reductions in water intake and vasopressin-release in wild-type mice. (F) Summary of terminal deoxyribonucleotidyl transferase-mediated dUTP-nick end labeling (TUNEL) assays in tissue sections of the SFO, OVLT, SON, PVN, and choroid plexus from mice 3 days after the injection of control Ig or the patient's Ig. **p < 0.01 (compared with Control Ig in the same tissue region), by two-tailed t test; data are mean and SE of three independent experiments. (G) Neural pathways from Na$_x$-positive SFO and OVLT. MnPO, median preoptic nucleus; PP, posterior pituitary.

We therefore examined the pathophysiological effects of the autoantibody to Na_x by intravenously injecting wild-type mice with the patient's immunoglobulin (Ig) fraction (Figure 1E). After 1 week, we found that the mice took in significantly less water than those given the vehicle or control Ig under non-feeding conditions. After 24 hr of dehydration, the vasopressin level of the mice injected with the patient's Ig was elevated but significantly lower than that of the control mice given the vehicle or control Ig. Consistent with this impaired response, the amount of urine of the patient-Ig-injected mice during 24 hr of dehydration was significantly larger than that of the control mice. Of note, after absorption of the autoantibody with Na_x-peptide beads, the depleted patient's Ig preparation was almost ineffective.

Next, we examined the effect of the patient's Ig on salt intake behavior: the mice treated were allowed free access to both normal (Na-repleted) and Na-depleted food. During water restriction, the control mice progressively showed a preference for Na-depleted food. In contrast, those that received the patient's Ig did not show this normal behavioral response. After water restriction for 3 days, the plasma $[Na^+]$ of the mice given the patient's Ig reached over 160 mM, while it remained at a physiological level in the controls. On repletion with enough water, the preference for Na-depleted food of the control mice recovered to the normal level within 1 day, and importantly, their plasma $[Na^+]$ remained at the basal level during the test. In contrast, the mice given patient's Ig showed slightly but significantly higher levels of $[Na^+]$ in plasma even 1 week after water repletion. Thus, injection of the patient's Ig reproduced the persistent hypernatremia in mice as observed in the patient.

Immunohistochemical studies of the brain from the mice injected with the patient's Ig indicated that binding of patient's Ig lead to complement deposition and infiltration of inflammatory cells in Na_x-positive regions, suggesting that the complement-mediated cell death occurred in the CVOs where Na_x is expressed. Indeed, both apoptosis (Figure 1F) and necrosis were evident in the CVOs of the mice injected with the patient's Ig.

The subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) have projections to the supraoptic nucleus (SON) and paraventricular nucleus (PVN), which are responsible for the regulation of vasopressin production (Figure 1G). Na-level sensors and osmosensors are thought to be involved in the regulation of the activity of these projection neurons in the SFO and/or OVLT. Histological damage to the SFO and OVLT would be a reason for the dysregulation of vasopressin production/release. In addition, damage to the posterior pituitary, the site where vasopressin is released into the blood circulatory system, would also affect the release. This defect in the regulation of vasopressin appears to cause serious symptoms for the patient.

This study shows, for the first time, that a ganglioneuroma formed in the peripheral nervous system triggered an autoimmune channelopathy targeting Na_x, the Na-level sensor of body fluids in the brain, causing essential hypernatremia to develop. Pathogenetically, autoantibodies to Na_x likely induce persistent tissue damage within the Na_x-positive CVOs essential for systemic water/salt homeostasis through the activation of complement and infiltration of inflammatory cells.

Publication List

〔Original papers〕

- Chagnon, M.J., Wu, C.-L., Nakazawa, T., Yamamoto, T., Noda, M., Blanchetot, C., and Tremblay, M.L. (2010). Receptor tyrosine phosphatase sigma (RPTPσ) regulates, p250GAP, a novel substrate that attenuates Rac signaling. Cell. Signal. *22*, 1626-1633.
- Hiyama, T.Y., Matsuda, S., Fujikawa, A., Matsumoto, M., Watanabe, E., Kajiwara, H., Niimura, F., and Noda, M. (2010). Autoimmunity to the sodium-level sensor in the brain causes essential hypernatremia. Neuron *66*, 508-522.
- Nagakura, A., Hiyama, T.Y., and Noda, M. (2010). Na_x-deficient mice show normal vasopressin response to dehydration. Neurosci. Lett. *472*, 161-165.

〔Original papers (E-publication ahead of print)〕

- Nayak, G., Goodyear, R.J., Legan, P.K., Noda, M., and Richardson, G.P. Evidence for multiple, developmentally regulated isoforms of PTPRQ on hair cells of the inner ear. Dev. Neurobiol., 2010 Aug 16.
- Yonehara, K., Balint, K., Noda, M., Nagel, G., Bamberg, E., and Roska, B. Spatially asymmetric reorganization of inhibition establishes a motion-sensitive circuit. Nature, 2010 Dec 19.