

Fig. 4. Immunostaining with anti-GAD67 antibody in the hippocampus of control, hypothyroid and T4-replacement groups at PND28. Hippocampal sections (8 μm thick) from control, hypothyroid and T4-replacement groups at PND28 were stained with anti-GAD67 antibody. (A) Representative photomicrographs of the hippocampal subregions (CA1, CA3 and DG) of control, hypothyroid and T4-replacement groups at PND28. (B) The number of GAD67-positive cells in the whole hippocampus and the hippocampal subregions (CA1, CA2, CA3 and DG) of control (open bar), hypothyroid (solid bar) and T4-replacement groups (shaded bar) were counted in each section obtained at PND28 and expressed as means + SEM. $n = 5-6$ (control, hypothyroid, T4-replacement groups). o: stratum oriens, py: stratum pyramidale, r: stratum radiatum, luc: stratum lucidum, m: stratum moleculare, g: stratum granulare, h: hilus. No significant difference in GAD67-positive cell number was observed between control and hypothyroid or hypothyroid and T4-replacement groups in any subregion.

were also observed in the postsynaptic components of the GABAergic system. Some of the GABA(A) receptor subunits ($\alpha 1$, $\gamma 2$) showed up-regulation in the hypothyroid group and were normalized by T4-replacement at PND28 (Fig. 7A–C). Similar TH-responsive mRNA expression was observed with gephyrin, a scaffold protein localized to inhibitory synapses (Fig. 7D), but not in the guanine nucleotide exchange factor for CDC42, collybistin (*arhgef9*; Fig. 7E).

Another postsynaptic component strongly affected by TH status was the neuronal K^+/Cl^- co-transporter, KCC2, which is essential for

the excitatory to inhibitory switching of the GABAergic neurotransmission (Fig. 8). In the control group, there was a strong transient up-regulation of *kcc2* at PND10, which was absent in the hypothyroid and the T4-replacement groups (Fig. 8A). Accordingly, KCC2 protein was reduced to 26% and 22% of control in the hypothyroid group at PND10 and PND15, respectively. Between PND15 and 28, however, there was a robust increase in KCC2 protein in the hippocampus of all 3 experimental groups including the hypothyroid group, so that it was comparable among these groups at PND28 (Fig. 8B).

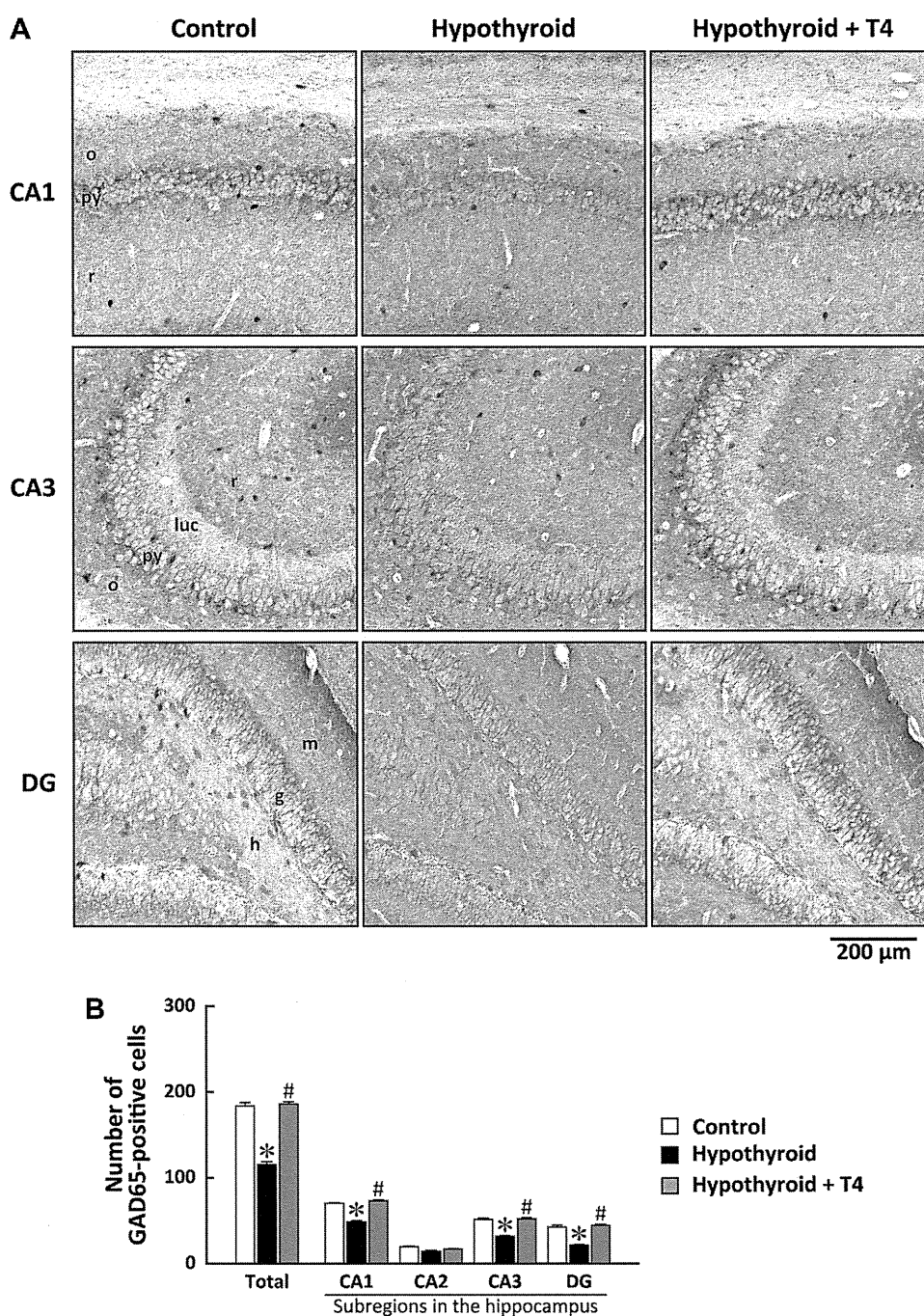


Fig. 5. Immunostaining with anti-GAD65 antibody in the hippocampus of control, hypothyroid and T4-replacement groups at PND28.

Hippocampal sections (8 μ m thick) from control, hypothyroid and T4-replacement groups at PND28 were stained with anti-GAD65 antibody. (A) Representative photomicrographs of the hippocampal subregions (CA1, CA3 and DG) of control, hypothyroid and T4-replacement groups at PND28. These sections were adjacent to the sections shown in Fig. 4. (B) The number of GAD65-positive cells in the whole hippocampus and the hippocampal subregions (CA1, CA2, CA3 and DG) of control (open bar), hypothyroid (solid bar) and T4-replacement groups (shaded bar) were counted in each section obtained at PND28 and expressed as means + SEM. $n = 5-6$ (control, hypothyroid, T4-replacement groups). o: stratum oriens, py: stratum pyramidale, r: stratum radiatum, luc: stratum lucidum, m: stratum moleculare, g: stratum granulare, h: hilus. * $P < 0.05$ between control and hypothyroid groups. # $P < 0.05$ between hypothyroid and T4-replacement groups.

4. Discussion

4.1. Sensitivity of the hippocampus toward TH status during development

By comparing the expression profiles of three TH-dependent genes in the three experimental groups, we confirmed that the developing hippocampus was one of the highly TH-sensitive brain regions (Fig. 2). Hippocampal neurons exhibit high expression

levels of both TH receptors (Bradley et al., 1992) and the mono-carboxylate transporter 8 (MCT8; *slc16a2*) (Heuer et al., 2005) identified as a specific TH transporter in the brain (Friesema et al., 2003). In addition, type 2 deiodinase responsible for converting T4 into active T3 is highly expressed in astrocytes in the hippocampus, especially in the dentate gyrus (Guadaño-Ferraz et al., 1997). High sensitivity of the hippocampus toward TH thus results from the abundant expression of its receptors as well as molecules regulating T3 availability in this region during the perinatal period.

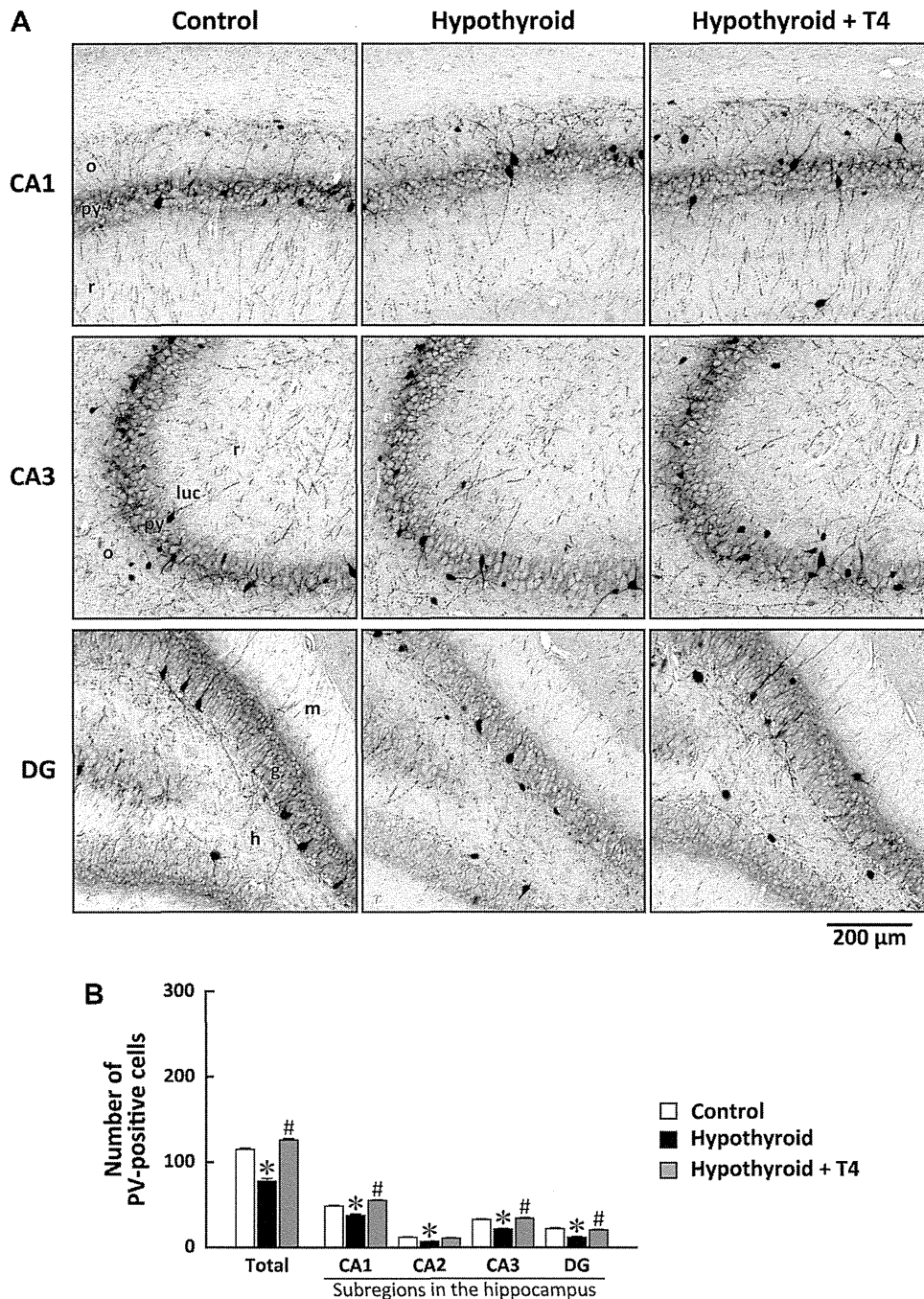


Fig. 6. Immunostaining with anti-PV antibody in the hippocampus of control, hypothyroid and T4-replacement groups at PND28. Hippocampal sections (8 μ m thick) from control, hypothyroid and T4-replacement groups at PND28 were stained with anti-PV antibody. (A) Representative photomicrographs of the hippocampal subregions (CA1, CA3 and DG) of control, hypothyroid and T4-replacement groups at PND28. These sections were adjacent to the sections shown in Fig. 4. (B) The number of PV-positive cells in the whole hippocampus and the hippocampal subregions (CA1, CA2, CA3 and DG) of control (open bar), hypothyroid (solid bar) and T4-replacement groups (shaded bar) were counted in each section obtained at PND28 and expressed as means \pm SEM. $n=5-6$ (control, hypothyroid, T4-replacement groups). o: stratum oriens, py: stratum pyramidale, r: stratum radiatum, luc: stratum lucidum, m: stratum moleculare, g: stratum granulare, h: hilus. * $P<0.05$ between control and hypothyroid groups. # $P<0.05$ between hypothyroid and T4-replacement groups.

4.2. TH affects the phenotype of GABAergic neurons in the developing hippocampus

In the present study, the two GAD isotypes, GAD67 and GAD65, were shown to be differentially affected by hypothyroidism. Reduction of GAD65 protein to 50% or less of the control level was observed at PND4, 15 and 28 (Fig. 3B) which was correlated immunohistochemically with a 37% reduction of GAD65-positive cells as well as reduction of GAD65-positive processes and

terminals at PND28 (Fig. 5). In contrast, the amount of GAD67 protein or the number of GAD67-positive cells was not affected by hypothyroidism (Figs. 3A and 4). The two isotypes originating from two distinct genes differ in their intracellular distribution and regulation. GAD67 exists as an active form with bound coenzyme and is localized throughout the cytosol of GABAergic neurons, whereas GAD65 is predominantly in an inactive form without the coenzyme and is enriched in nerve terminals (Erlander et al., 1991; Esclapez et al., 1994; Fukuda et al., 1997). In the rodent

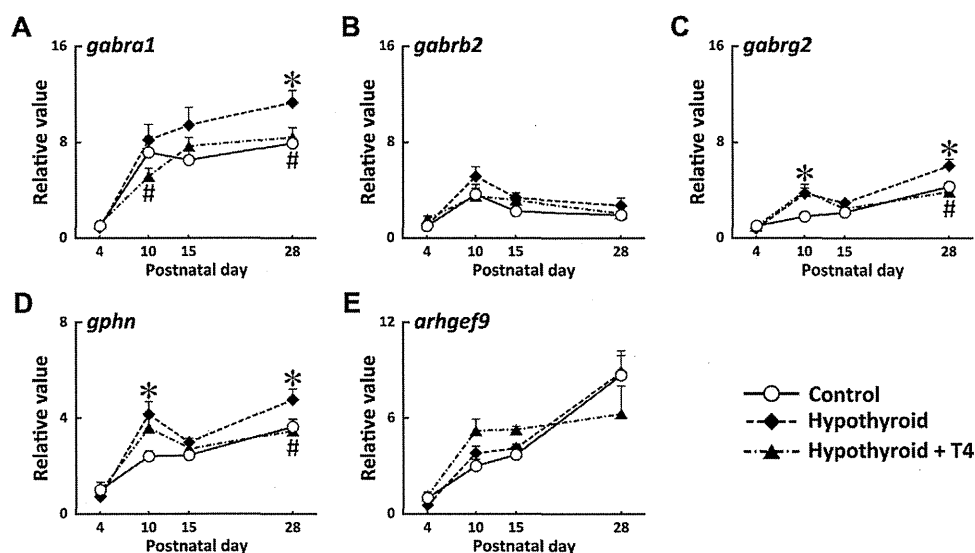


Fig. 7. Comparison of expression profiles of the inhibitory post-synaptic component genes in the hippocampus of control, hypothyroid and T4-replacement groups during development. The mRNA expression of GABA(A) receptor subunits (A: *gabra1*, B: *gabrb2*, C: *gabrg2*) and the inhibitory scaffolding protein (D: *gphn*, E: *arhgef9*) in the hippocampus of pups in the control (open circles), hypothyroid (solid squares) and T4-replacement groups (solid triangles) was quantified by real-time PCR at PND4, 10, 15 and 28 using *ppia* as internal standard. Each mRNA level is expressed relative to that of the control group at PND4 (means + SEM of 5 rats/group/age). * $P < 0.05$ between control and hypothyroid groups. # $P < 0.05$ between hypothyroid and T4-replacement groups.

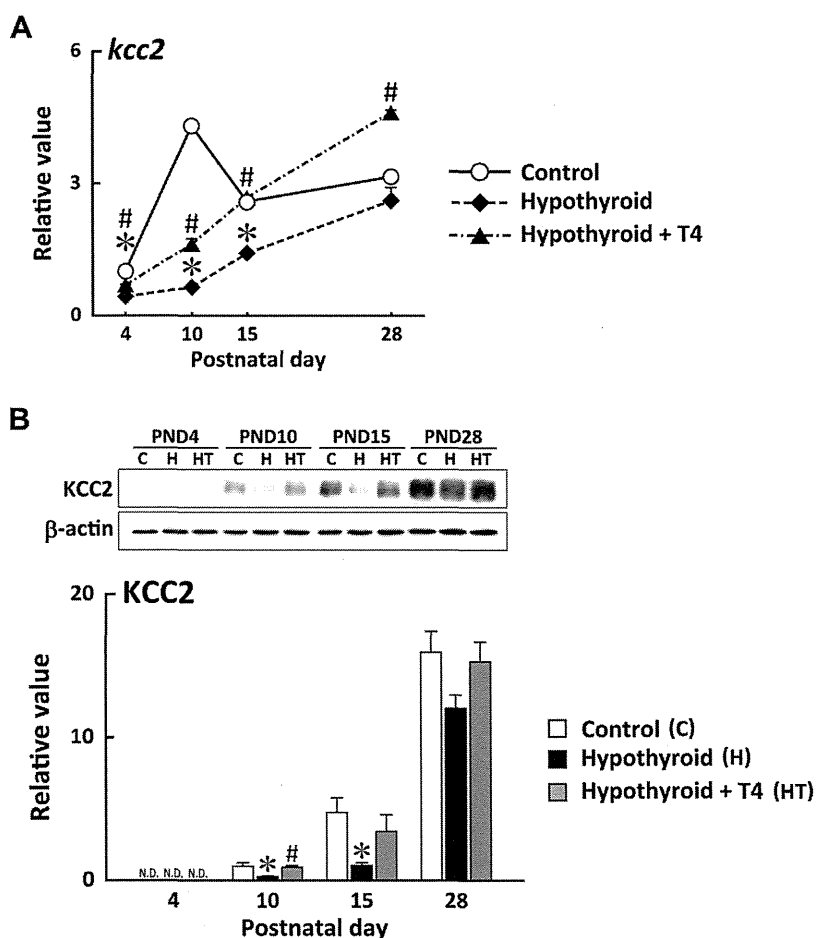


Fig. 8. Comparison of mRNA and protein expressions of *kcc2* gene in the hippocampus of control, hypothyroid and T4-replacement groups during development. (A) The mRNA expression of *kcc2* in the hippocampus of control (open circles), hypothyroid (solid squares) and T4-replacement groups (solid triangles) were quantified by real-time PCR at PND4, 10, 15 and 28 using *ppia* as internal standard. Each mRNA level is expressed relative to that of the control group at PND4 (means + SEM of 5 rats/group/age). (B) KCC2 protein level quantified by Western blotting with anti-KCC2 antibody. Immunoreactive band of KCC2 (examples shown in upper panels) in the hippocampus of control (open bar), hypothyroid (solid bar), T4-replacement groups (shaded bar) at PND4, 10, 15 and 28 were quantified using β -actin as standard and expressed relative to that of the control group at PND10 (means + SEM of 5 rats/group/age). N.D.: not detected. * $P < 0.05$ between control and hypothyroid groups. # $P < 0.05$ between hypothyroid and T4-replacement groups.

hippocampus, most GABAergic neurons exhibit moderate to intense GAD67 immunoreactivity in somata, but some of them show markedly low immunoreactivity toward GAD65 (Fukuda et al., 1997). Furthermore, GABAergic terminals located in the perisomatic domain of principal neurons show intense immunoreactivity toward GAD67 whereas those in the dendritic domain show stronger immunoreactivity toward GAD65 (Fukuda et al., 1998). Our results thus show that the total number of GABAergic neurons was not affected by TH status but the expression of GAD65 both in somata and processes was TH-dependent.

Parvalbumin (PV) is an intracellular Ca^{2+} buffering protein which is considered as a marker for a subpopulation of hippocampal GABAergic neurons (Chard et al., 1993; Fukuda et al., 1997). PV-positive cells and their processes were dramatically reduced in the hypothyroid hippocampus (Fig. 6) as reported previously in models of hypothyroidism induced chemically with PTU or MMI as well as in TR α knockout mice and mice possessing mutated TR α with low affinity toward T3 (Gilbert et al., 2007; Guadaño-Ferraz et al., 2003; Venero et al., 2005). PV-positive cells are mainly located in the pyramidal cell layer and contribute to fast-spiking, perisomatic input onto pyramidal cells (Fukuda et al., 1997). In a previous study on animals developmentally exposed to lower doses of PTU, no change in GAD67-immunoreactivity was detected while PV-positive cells were reduced in number (Gilbert et al., 2007).

The results of the present study thus confirm that the total number of GABAergic neurons in the hippocampus was not affected but the phenotype of GABAergic neurons was significantly altered and GABAergic processes and terminals were reduced by developmental hypothyroidism. Such alterations lead to impairments in circuit formation and inhibitory function in the hippocampus as detected by electrophysiological methods (Guadaño-Ferraz et al., 2003; Gilbert et al., 2007).

4.3. TH-dependent expression of KCC2 and maturation of the GABAergic system

The postsynaptic components of the GABAergic system including GABA(A) receptor subunits ($\alpha 1$, $\gamma 2$) and gephyrin (a scaffold protein localized to inhibitory synapses) showed TH-responsive expression changes (up-regulated in hypothyroid and normalized by T4-replacement) only at a later stage of PND28 (Fig. 7), possibly reflecting a decrease in GABA release in the hypothyroid state.

In contrast, expression of the neuron-specific K^+/Cl^- co-transporter, *kcc2*, was positively regulated by TH from PND4, prior to increases in mRNA expression of GABARs (Fig. 8). A sharp rise of *kcc2* mRNA expression observed at PND10 in the control animal was TH-dependent and resulted in a 4.8-fold increase in KCC2 protein between PND10 and 15, which corresponds to the switching of GABA action from excitatory to inhibitory (Payne et al., 2003; Ben-Ari et al., 2007; Farrant and Kaila, 2007). Absence of this rise in *kcc2* expression led to a marked reduction in KCC2 protein at PND15 (Fig. 8B), suggesting a significant delay in the excitatory to inhibitory switching of GABA action as detected electrophysiologically in hippocampal slices from hypothyroid animals by Friauf et al. (2008b). In contrast to our observation, these authors detected no difference in the intensity or localization of KCC2 immunoreactivity in the auditory brain stem of euthyroid and hypothyroid rats at PND12, but the situation in the hippocampus was not examined. Such differences in TH-dependency of KCC2 expression may reflect the highly heterogeneous nature of the timing and strength of TH signaling in the developing brain (Quignodon et al., 2004).

Another characteristic of KCC2 protein expression in the developing hippocampus was the presence of a second phase of robust increase between PND15 and 28 which was not dependent on TH (Fig. 8B). Since KCC2 expression is shown to be up-regulated by brain-derived neurotrophic factor (BDNF) during development

(Aguado et al., 2003; Blaesse et al., 2009), this latter phase of increase may be regulated by the activity-dependent up-regulation of BDNF. In this context, recent studies on the expression of KCC2 in the adult after brain injury have revealed an interesting interplay between TH and BDNF on GABAergic neurotransmission (Shulga et al., 2009; Shulga and Rivera, 2013). In contrast to the situation during development, BDNF down-regulates KCC2 in mature neurons under both physiological and pathological conditions, resulting in reversal of GABA(A) responses toward depolarization (Payne et al., 2003; Rivera et al., 2004; Shulga et al., 2009). A recent study on lesioned hippocampal slices, however, has demonstrated that thyroxine up-regulates the expression of KCC2 in injured neurons independent of BDNF. The level of TH which is high during development and becomes lower after maturation may thus be a critical factor in setting the direction of BDNF action on KCC2 expression.

TH is thus found to be essential not only for the development of the presynaptic GABAergic neurons and their terminals but also for the maturation of the postsynaptic GABAergic system, and one of the primary targets of TH seems to be the K^+/Cl^- co-transporter, KCC2. Apart from its function as a transporter, recent studies revealed a critical role of KCC2 in both the formation (Li et al., 2007) and functional maintenance (Gauvain et al., 2011) of glutamatergic synapses through interaction with the submembranous actin cytoskeleton. Reduction of KCC2 in the hypothyroid hippocampus thus influences also synaptic efficacy at glutamatergic synapses.

A variety of behavioral deficits have been described in hypothyroid animals, some of which implicate impairment of hippocampal function such as memory impairment and learning disorder (Akaike et al., 1991; Smith et al., 2002; Venero et al., 2005) and a reduction in seizure susceptibility (Hadjab-Lallemend et al., 2010). Impairments in the development and maturation of the GABAergic system may thus be essential factors involved in such disorders caused by hypothyroidism.

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References

- Aguado, F., Carmona, M.A., Pozas, E., Aguiló, A., Martínez-Guijarro, F.J., Alcantara, S., Borrell, V., Yuste, R., Ibañez, C.F., Soriano, E., 2003. BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K^+/Cl^- co-transporter KCC2. *Development* 130, 1267–1280.
- Akaike, M., Kato, N., Ohno, H., Kobayashi, T., 1991. Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. *Neurotoxicol. Teratol.* 13, 317–322.
- Ben-Ari, Y., 2002. Excitatory actions of GABA during development: the nature of the nurture. *Nat. Rev. Neurosci.* 3, 728–739.
- Ben-Ari, Y., Gaiarsa, J.L., Tyzio, R., Khazipov, R., 2007. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol. Rev.* 87, 1215–1284.
- Bernal, J., Nunez, J., 1995. Thyroid hormones and brain development. *Eur. J. Endocrinol.* 133, 390–398.
- Blaesse, P., Airaksinen, M.S., Rivera, C., Kaila, K., 2009. Cation-chloride cotransporters and neuronal function. *Neuron* 61, 820–838.
- Bradley, D.J., Towle, H.C., Young 3rd, W.S., 1992. Spatial and temporal expression of alpha- and beta-thyroid hormone receptor mRNAs, including the beta 2-subtype, in the developing mammalian nervous system. *J. Neurosci.* 12 (6), 2288–2302.
- Chard, P.S., Bleakman, D., Christakos, S., Fullmer, C.S., Miller, R.J., 1993. Calcium buffering properties of calbindin D28k and parvalbumin in rat sensory neurones. *J. Physiol.* 472, 341–357.

- Chatonnet, F., Guyot, R., Picou, F., Bondesson, M., Flamant, F., 2012. Genome-wide search reveals the existence of a limited number of thyroid hormone receptor alpha target genes in cerebellar neurons. *PLoS One* 7 (5), e30703.
- Dong, H., Yauk, C.L., Rowan-Carroll, A., You, S.H., Zoeller, R.T., Lambert, I., Wade, M.G., 2009. Identification of thyroid hormone receptor binding sites and target genes using ChIP-on-chip in developing mouse cerebellum. *PLoS One* 4 (2), e4610.
- Erlander, M.G., Tillakaratne, N.J., Feldblum, S., Patel, N., Tobin, A.J., 1991. Two genes encode distinct glutamate decarboxylases. *Neuron* 7, 91–100.
- Esclapez, M., Tillakaratne, N.J., Kaufman, D.L., Tobin, A.J., Houser, C.R., 1994. Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. *J. Neurosci.* 14, 1834–1855.
- Farrant, M., Kaila, K., 2007. The cellular, molecular and ionic basis of GABA(A) receptor signalling. *Prog. Brain Res.* 160, 59–87.
- Farsetti, A., Mitsuhashi, T., Desvergne, B., Robbins, J., Nikodem, V.M., 1991. Molecular basis of thyroid hormone regulation of myelin basic protein gene expression in rodent brain. *J. Biol. Chem.* 266, 23226–23232.
- Fiumelli, H., Woodin, M.A., 2007. Role of activity-dependent regulation of neuronal chloride homeostasis in development. *Curr. Opin. Neurobiol.* 17, 81–86.
- Friauf, E., Wenz, M., Oberhofer, M., Nothwang, H.G., Balakrishnan, V., Knipper, M., Löhrke, S., 2008a. Hypothyroidism impairs chloride homeostasis and onset of inhibitory neurotransmission in developing auditory brainstem and hippocampal neurons. *Eur. J. Neurosci.* 28 (12), 2371–2380.
- Friauf, E., Wenz, M., Oberhofer, M., Nothwang, H.G., Balakrishnan, V., Knipper, M., Löhrke, S., 2008b. Hypothyroidism impairs chloride homeostasis and onset of inhibitory neurotransmission in developing auditory brainstem and hippocampal neurons. *Eur. J. Neurosci.* 28, 2371–2380.
- Friesema, E.C., Ganguly, S., Abdalla, A., Manning Fox, J.E., Halestrap, A.P., Visser, T.J., 2003. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J. Biol. Chem.* 278, 40128–40135.
- Fukuda, T., Aika, Y., Heizmann, C.W., Kosaka, T., 1998. GABAergic axon terminals at perisomatic and dendritic inhibitory sites show different immunoreactivities against two GAD isoforms, GAD67 and GAD65, in the mouse hippocampus: a digitized quantitative analysis. *J. Comp. Neurol.* 395, 177–194.
- Fukuda, T., Heizmann, C.W., Kosaka, T., 1997. Quantitative analysis of GAD65 and GAD67 immunoreactivities in somata of GABAergic neurons in the mouse hippocampus proper (CA1 and CA3 regions), with special reference to parvalbumin-containing neurons. *Brain Res.* 764, 237–243.
- Gauvain, G., Chamma, I., Chevy, Q., Cabezas, C., Irinopoulou, T., Bodrug, N., Carnaud, M., Lévi, S., Poncer, J.C., 2011. The neuronal K-Cl cotransporter KCC2 influences postsynaptic AMPA receptor content and lateral diffusion in dendritic spines. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15474–15479.
- Gilbert, M.E., Sui, L., Walker, M.J., Anderson, W., Thomas, S., Smoller, S.N., Schon, J.P., Phani, S., Goodman, J.H., 2007. Thyroid hormone insufficiency during brain development reduces parvalbumin immunoreactivity and inhibitory function in the hippocampus. *Endocrinology* 148, 92–102.
- Guadaño-Ferraz, A., Benavides-Piccone, R., Venero, C., Lancha, C., Vennström, B., Sandi, C., DeFelipe, J., Bernal, J., 2003. Lack of thyroid hormone receptor alpha1 is associated with selective alterations in behavior and hippocampal circuits. *Mol. Psychiatry* 8, 30–38.
- Guadaño-Ferraz, A., Obregón, M.J., St Germain, D.L., Bernal, J., 1997. The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 94, 10391–10396.
- Gutiérrez, R., Romo-Parra, H., Maqueda, J., Vivar, C., Ramírez, M., Morales, M.A., Lamas, M., 2003. Plasticity of the GABAergic phenotype of the “glutamatergic” granule cells of the rat dentate gyrus. *J. Neurosci.* 23 (13), 5594–5598.
- Hadjab-Lallemend, S., Wallis, K., van Hogerlinden, M., Dudazy, S., Nordström, K., Vennström, B., Fisahn, A., 2010. A mutant thyroid hormone receptor alpha1 alters hippocampal circuitry and reduces seizure susceptibility in mice. *Neuropharmacology* 58 (7), 1130–1139.
- Heuer, H., Maier, M.K., Iden, S., Mittag, J., Friesema, E.C., Visser, T.J., Bauer, K., 2005. The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. *Endocrinology* 146, 1701–1706.
- Koibuchi, N., Chin, W.W., 2000. Thyroid hormone action and brain development. *Trends Endocrinol. Metab.* 11, 123–128.
- Li, H., Khirug, S., Cai, C., Ludwig, A., Blaesse, P., Kolikova, J., Afzalov, R., Coleman, S.K., Lauri, S., Airaksinen, M.S., Keinänen, K., Khiroug, L., Saarma, M., Kaila, K., Rivera, C., 2007. KCC2 interacts with the dendritic cytoskeleton to promote spine development. *Neuron* 56, 1019–1033.
- Manzano, J., Cuadrado, M., Morte, B., Bernal, J., 2007. Influence of thyroid hormone and thyroid hormone receptors in the generation of cerebellar GABAergic interneurons from precursor cells. *Endocrinology* 148, 5746–5751.
- Martínez de Arrieta, C., Morte, B., Coloma, A., Bernal, J., 1999. The human RC3 gene homolog, NRGN contains a thyroid hormone-responsive element located in the first intron. *Endocrinology* 140, 335–343.
- Muñoz, A., Rodríguez-Peña, A., Pérez-Castillo, A., Ferreira, B., Sutcliffe, J.G., Bernal, J., 1991. Effects of neonatal hypothyroidism on rat brain gene expression. *Mol. Endocrinol.* 5, 273–280.
- Oppenheimer, J.H., Schwartz, H.L., 1997. Molecular basis of thyroid hormone-dependent brain development. *Endocr. Rev.* 18, 462–475.
- Payne, J.A., Rivera, C., Voipio, J., Kaila, K., 2003. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.* 26, 199–206.
- Quignodon, L., Grijota-Martinez, C., Compe, E., Guyot, R., Allioli, N., Laperrière, D., Walker, R., Meltzer, P., Mader, S., Samarut, J., Flamant, F., 2007. A combined approach identifies a limited number of new thyroid hormone target genes in post-natal mouse cerebellum. *J. Mol. Endocrinol.* 39 (1), 17–28.
- Quignodon, L., Legrand, C., Allioli, N., Guadano-Ferraz, A., Bernal, J., Samarut, J., Flamant, F., 2004. Thyroid hormone signaling is highly heterogeneous during pre- and postnatal brain development. *J. Mol. Endocrinol.* 33, 467–476.
- Rivera, C., Voipio, J., Payne, J.A., Ruusuvoori, E., Lahtinen, H., Lamsa, K., Pirvola, U., Saarma, M., Kaila, K., 1999. The K⁺/Cl⁻ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397, 251–255.
- Rivera, C., Voipio, J., Thomas-Crusells, J., Li, H., Emri, Z., Sipilä, S., Payne, J.A., Minichiello, L., Saarma, M., Kaila, K., 2004. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. *J. Neurosci.* 24, 4683–4691.
- Shulga, A., Blaesse, A., Kysenius, K., Huttunen, H.J., Tanhuanpää, K., Saarma, M., Rivera, C., 2009. Thyroxine regulates BDNF expression to promote survival of injured neurons. *Mol. Cell Neurosci.* 42, 408–418.
- Shulga, A., Rivera, C., 2013. Interplay between thyroxine, BDNF and GABA in injured neurons. *Neuroscience* 3 (239), 241–252.
- Sloviter, R.S., Dichter, M.A., Rachinsky, T.L., Dean, E., Goodman, J.H., Sollas, A.L., Martin, D.L., 1996. Basal expression and induction of glutamate decarboxylase and GABA in excitatory granule cells of the rat and monkey hippocampal dentate gyrus. *J. Comp. Neurol.* 373 (4), 593–618.
- Smith, J.W., Evans, A.T., Costall, B., Smythe, J.W., 2002. Thyroid hormones, brain function and cognition: a brief review. *Neurosci. Biobehav. Rev.* 26, 45–60.
- Takahashi, M., Kondoh, Y., Tashiro, H., Koibuchi, N., Kuroda, Y., Tashiro, T., 2005. Monitoring synaptogenesis in the developing mouse cerebellum with an original oligonucleotide microarray. *J. Neurosci. Res.* 80 (6), 777–788.
- Takahashi, M., Negishi, T., Tashiro, T., 2008. Identification of genes mediating thyroid hormone action in the developing mouse cerebellum. *J. Neurochem.* 104 (3), 640–652.
- Thompson, C.C., Botcher, M.C., 1997. The product of a thyroid hormone-responsive gene interacts with thyroid hormone receptors. *Proc. Natl. Acad. Sci. U.S.A.* 94 (16), 8527–8532.
- Thompson, C.C., Potter, G.B., 2000. Thyroid hormone action in neural development. *Cereb. Cortex* 10, 939–945.
- Venero, C., Guadaño-Ferraz, A., Herrero, A.L., Nordström, K., Manzano, J., de Escobar, G.M., Bernal, J., Vennström, B., 2005. Anxiety, memory impairment, and locomotor dysfunction caused by a mutant thyroid hormone receptor alpha1 can be ameliorated by T3 treatment. *Genes Dev.* 19, 2152–2163.
- Wallis, K., Sjogren, M., von Hogerlinden, M., Silberberg, G., Fisahn, A., Nordstrom, K., Larsson, L., Westerblad, H., de Escobar, G.M., Shupliakov, O., Vennström, B., 2008. Locomotor deficiencies and aberrant development of subtype-specific GABAergic interneurons caused by an unliganded thyroid hormone receptor alpha1. *J. Neurosci.* 28, 1904–1915.
- Westerholz, S., de Lima, A.D., Voigt, T., 2010. Regulation of early spontaneous network activity and GABAergic neurons development by thyroid hormone. *Neuroscience* 168 (2), 573–589.
- Wiens, S.C., Trudeau, V.L., 2006. Thyroid hormone and g-aminobutyric acid (GABA) interactions in neuroendocrine systems. *Comp. Biochem. Physiol. A* 144, 332–344.
- Williams, G.R., 2008. Neurodevelopmental and neurophysiological actions of thyroid hormone. *J. Neuroendocrinol.* 20, 784–794.

IV. 研究成果の刊行物・別刷-1 (2011年のものを再掲)

3 総合12版 2011年(平成23年)9月17日(土曜日)

胎児の感情神経に影響

妊婦が魚を食べ体内に摂取することで胎児への影響が懸念される有機水銀が、胎児の脳神経のうち感情や行動をつかさどるセロトニン神経に発達異常を起す可能性のあることが、厚生労働省研究班(班長・成田正明三重大大学院医学系研究科教授)の研究で分かった。有機水銀による胎児内の反応メカニズムを解明したのは初めて。論文は米神経科学誌「ニューロサイエンス」レター電子版に掲載された。

三重大教授ら解明

妊婦の有機水銀摂取

魚介類に微量含まれ、食事ににより体内へ摂取される。大人に害はないが、厚労省は胎児への悪影響を考慮し妊婦に対し、食物連鎖をへて水銀濃度が高くなった一部大型魚を食べ過ぎないように注意を促している。

胎児は脳幹で発達中のセロトニン神経の量が二倍になり、本来は神経がない場所にも見つかった。セロトニン神経は大脳全体に指示を与え、感覚や行動、精神までコントロール。この神経の働きの不具合が、うつ病など関連する。

とも分かっていない。実験では魚食で摂取する有機水銀をはるかにしのぐ量を投与しており、実際には魚を食べた程度では影響はない。成田教授は「有機水銀がセロトニン神経に異常を起させば、生後の認知や行動に影響が出る恐れがあり、有機水銀の危険性が一層明らかになった」と指摘している。

研究班の江藤みちる同研究科助教らは、人間でいえば妊娠二カ月のラットに高濃度の有機水銀を注射し、六日後の胎児で発育の違いを調べた。有機水銀を与えたラットの脳神経に、胎児の脳幹で発達中のセロトニン神経の量が二倍になり、本来は神経がない場所にも見つかった。セロトニン神経は大脳全体に指示を与え、感覚や行動、精神までコントロール。この神経の働きの不具合が、うつ病など関連する。

意義深い基礎資料
日本周産期新生児医学
会理事を務める名古屋
市立大の戸蒔創(はじめ)
学長の話。有機水銀が
中枢神経に影響を及ぼ
すことは知られている
が、胎児への影響やど
の神経に異常を及ぼす
かなどは不明な点が多
く、今回の動物実験で
の証明は意義深い。衛
生行政の観点からも、
有機水銀の摂取を減ら
す基礎データになるは
ずだ。

IV. 研究成果の刊行物・別刷-2

子どもの発達障害

子どもがじっとしてられない。こだわりが強すぎる。これは個人差の範囲内なのか。近年、ニュースや本で話題にのぼる発達障害。三重大付属病院（津市）と紀南病院（御浜町）で小児発達外来を担当する三重大院医学系研究科の成田正明教授（51）に、障害の特徴や治療の最前線について聞いた。（小柳悠志）

発達障害とは、人との関わりが苦手な自閉症、落ち着かない注意欠陥多動性障害（ADHD）、読み書き計算など特定の分野が苦手な学習障害（LD）などの総称で、近年増加しているとされます。「障害」と名がつくものの、外見からうかがい知れない症状のため、日々の生活で苦労が多いといえます。

「気を行けるべき」とは、発達障害は、四らか

あの人に聞いてみよう

の原因による生まれながらの脳機能の障害です。生後の環境や育て方で生じるものではありません。早期発見と適切な支援が必要ですが、一方、子どもであればおかしくない程度で、こだわりが見られただけで、保護者や保育士、医師までもが「この子は発達障害では

三重大院医学系研究科教授

成田正明さん(51)

「？」と思いつくことも多々あります。受診で何科に行けばよいか迷う人も少なくありません。児童精神科医と小児科医で対応が異なることもあります。

「成田教授の紀南病院での取り組みは、看護師や言語聴覚士らと、患者さんの場所の把握や社会への適応訓練を行っています。障害に対する周囲の理解があれば、訓練

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なりた・まさあき 1961年神戸市生まれ。87年に広島大医学部を卒業し、2006年三重大医学部教授。医学博士。日本小児科学会認定小児科専門医。日本小児神経学会認定小児神経専門医。専門は発生学、小児神経学。09年から厚生労働省の研究班班長として、三重大を拠点に発達障害研究に取り組む。

早期発見と支援必要

研究代表者は、本厚生労働科学研究を率いつつ、小児科の専門医として、子ども情動・認知行動異常についての診療にも従事している。記事は厚生労働科学研究費補助金研究の概要を一般のひとにわかりやすく紹介したもの。

(2013年1月6日、中日新聞)

IV. 研究成果の刊行物・別刷—3

意思の疎通が苦手で、人間関係をうまく築くことができないとされる発達障害。紀南地方の3市町(熊野市、御浜町、紀宝町)でつくる紀南地域母子保健医療推進協議会(紀南母子協)は、発達障害などが疑われる子どもを1歳半健診でピックアップし、就学までの継続支援を通じて早期療育につなげている。改善効果も確認されており、先進的な取り組みとして注目を集めている。(小柳悠志)

早期・継続療育で改善も

発達障害 1歳半からケア



子どもの発達状況を観察するため開かれている親子教室。紀南地域の福祉センターで

発達障害 人との関わりが苦手な自閉症、落ち着くことができない症状のたに発達障害の可能性がみられない注意欠陥多動性障害、周囲から理解されなかった。発達障害はクラス運営(ADHD)、読み書きケースも多い。近年増加傾向。営への影響のほか、家庭でや計算など特定の分野が苦向にあるとされ、文部科学の児童虐待のきつかけにちな手な学習障害(LD)など、省の昨年の調査では、普通(子ども)指摘されている。

「そろそろ、お片付け言葉の習得が遅いなど、ハウを共有することで、の時間かな」。保健師や保護者が気づいた言葉の発達の遅れや落ち込んでいた子どもが経過観察の。おもちやをしまい始め。紀南母子協が設立される。日常生活に近い状態。たのは一九九六年。医師からの脳機能の障害にもで、子どもの集中力アップ。不足に悩む同地方で医療。関わらず、「育て方や生ブや行動の切り替えを促と保健、福祉、教育を連携する」と。す「親子教室」の一コマ。携さるために始まった。一歳半や三歳児の健診。三市町の保健師が健診で、落ち着かない、診の内容を統一し、ノウハウを共有することで、



紀南3市町の取り組み注目

発達障害が疑われる子どもは親子教室と並行し、専門の医師らを含め、携し、継続して支援する。た広域二次健診を受けることが大切だ」と話している。症状を詳しく診ることで療育の方向を確認し、就学先を普通学級にするか特別支援学級にするかなど、医師や保育士から助言を受け。他地方で同様の健診は珍しく、子どもの就学後に保護者や教員が発達障害に気付くケースが多い。紀南母子協によると、近年の三市町の年間出生数は三百人弱で、二〇一一年度に二次健診を受け、た子どもは約七十人に上る。御浜町の保健師宮沢佳水さんは「生活リズムの改善や専門の施設に通うことで保育を受けること要だ」。

で、子どもの発達に成長が見られる場合もある。医師や保育士を含めて連携し、継続して支援すること大切だ」と話している。

三者一体で理想的な紀南地方の二次健診に加わる成田正明・三重大学医学系研究科教授(発生学・小児神経学)の話。発達障害の療育は、医師だけでは限界がある。紀南母子協が進める健診は地域と病院、保護者が一体となった理想的な取り組みだ。生活リズム改善などの早期療育は、効果が得られやすい。発達障害はネガティブな面が強調されがちだが、研究や芸術面で才能を開花させる児童も多い。子どもの個性や特徴を把握するの改善や専門の施設に通うことは、親にとっても重

視線

子の健やかな成長を願わない親はいない。そう思うと、取材する際も不思議なほど身構えなかった。一歳半で発達障害の症状すべてを判断できるわけではなく、親子教室に参加する子どもがその後、心配なく育つケースもある。それでも、わが子を毎日眺めながら、不安でいっぱいになる親も多かった。想像できた。子育て世代を精神面から支える取り組みは、紀南地方に限らずどこでも必要とされそうだった。

研究代表者が中心となって推進している三重県紀南3市町村(熊野市・御浜町・紀宝町)での取り組み；
本厚生労働科学研究の成果に基づき、情動・認知行動の異常の早期発見法、及び基礎研究に裏付けられた「療育法」について紹介されたもの
(2013年5月20日 中日新聞)

IV. 研究成果の刊行物・別刷-4

21 医療 ☆ 2013年(平成25年)5月14日(火曜日)

つなごう 医療 170 中部の最前線



三重大大学院の発生源生 前に三重大にきた。趣味の医学教室で、顕微鏡をのぞく毎日だ。成田正明教授の下で「モデル動物を用いた自閉症研究」に携わっていた。自閉症の特徴を持つラットの脳を解剖し、さまざまな化学物質を加えて、影

響を確かめたりする。元来の専門は化学。お茶の水女子大の大学院を経て、愛知県春日井市にある県心身障害者フロンティア研究所で、神経の精緻の機能を研究した。その後、中部大助教として神経の研究を続け、三年

三重大大学院医学系研究科 (津市)

えとう 助教 江藤 みちるさん (37)



「働きやすい環境で、ここにずっといたいです」と話す江藤みちるさん

研究の魅力を伝えたい

さを語り、「理系に関心を持ちたい」と呼び掛けたい。江藤さんが化学に興味を抱いたのは、高校時代の授業。炭素の結合の仕方の違い、ダイヤモンドになったり、

「堀口先生の影響で今の自分がある。教師としてこの仕事を続けたい」といいます。

「どれだけ教科書で知識を蓄積しても、実際に解剖させていただく、得られる情報はまったく違います。この目で見ることの大切さを痛感します。献体していただいた方に本当に感謝します」

余暇は、夫とともに地元の合唱団で活動すること。ライブにも一緒に出掛ける。

(編集委員・安藤明夫)

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