Biochemical and Biophysical Research Communications 458 (2015) 168–173

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hippocampal neuronal maturation triggers post-synaptic clustering of brain temperature-sensor TRPV4

Koji Shibasaki [a, *], Makoto Tominaga [b, c, d], Yasuki Ishizaki [a]

[a] Department of Molecular and Cellular Neurobiology, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan
[b] Division of Cell Signaling, National Institute for Physiological Sciences, Okazaki 444-8787, Japan
[c] Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Okazaki 444-8787, Japan
[d] Department of Physiological Sciences, The Graduate University for Advanced Studies, Okazaki 444-8585, Japan

ARTICLE INFO

Article history:
Received 5 January 2015
Available online 28 January 2015

Keywords:
TRPV4
Hippocampus
Development
Synapse
Cerebellum
PSD-95

ABSTRACT

Compartmentalization of neuronal function is achieved via specifically localized clustering of ion channels in discrete subcellular membrane domains. Transient receptor potential (TRP) channels exhibit highly variable cellular and subcellular patterns of expression. We previously revealed that the thermo-sensor TRPV4 (activated above 34 °C) is gated by physiological brain temperatures in hippocampal neurons and thereby controls their excitability. Here, we examined synaptic clustering of TRPV4 in developing hippocampal neurons. We found that TRPV4 accumulated in the soma of immature hippocampal neurons, and did not localize to post-synaptic locations although PSD-95-labeled post-synaptic structures were evident. During the maturation of neurons, TRPV4 was targeted to dendrites and also clustered at post-synaptic locations. Taken together, we reveal that TRPV4 localizes to post-synaptic sites and the post-synaptic targeting is strictly regulated in a neuronal maturation-dependent manner.

1. Introduction

The hippocampus contains neural circuitry that is crucial for higher brain functions, such as learning and memory [1]. The neurons that form circuits in the hippocampus require transmembrane cation influx for depolarization and action potential generation [2,3]. Many kinds of ion channels, such as voltage-gated Na^+ channels and Ca^{2+} channels, contribute to membrane depolarization [2,4].

TRPV4 is a nonselective cation channel, first described as an osmosensor detecting hypotonic stimuli, that shares 40% amino acid identity with TRPV1 [5–8]. TRPV4 can also be activated by heat (i.e., temperatures >27–34 °C), the phorbol ester derivative 4α-PDD (4α-phorbol 12,13 didecanoate), and lipid metabolites [9–12]. TRPV4 was reported to be necessary for the response to changes in osmotic pressure and functions as an osmotic sensor in the CNS [13,14]. We found that TRPV4 was strongly expressed in hippocampal neurons and constitutively activated by physiological brain temperature to control neuronal excitability [15]. Furthermore, we reported that TRPV4 is also expressed in microglia and specific subtypes of astrocytes in the brain, where it regulates synaptic activity through gliotransmitter release [16,17]. These results strongly indicate that TRPV4 is a key molecule regulating neuronal excitability.

It is well known that small changes in brain temperature affect brain function [18–21]. Clinical evidence suggests that brain temperature is particularly a critical determinant of cognitive function. For example, it has been reported that therapeutic hypothermia leads to cognitive dysfunction in heart disease patients [22], while cooling of the prefrontal cortex causes an impairment in the performance of a delayed matching-to-sample task [23]. In rodents, brain cooling significantly impairs spatial learning [24]. These reports indicate that the maintenance of brain temperature is important for healthy brain function. We have already revealed an involvement of the thermo-sensor function of TRPV4 in brain function following its activation by brain temperature changes [15–17]. In this study, we examined the synaptic targeting of TRPV4 in developing hippocampal neurons.

2. Materials and methods

2.1. Animals

TRPV4-deficient (TRPV4KO) mice were generated by crossing heterozygous mice, and the genotypes were determined by PCR as

* Corresponding author. Department of Molecular and Cellular Neurology, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan. Fax: +81 27 220 7955.
E-mail address: shibasaki@gunma-u.ac.jp (K. Shibasaki).

http://dx.doi.org/10.1016/j.bbrc.2015.01.087
0006-291X/

previously described [13]. We utilized the C57BL/6Cr strain of mice as the wild-type (WT) control. All animal care and procedures were performed according to Gunma University guidelines.

2.2. In situ hybridization and immunohistochemical analysis

Digoxigenin-labeled antisense/sense probes were used for *in situ* hybridization as reported [15,25]. Detection of mRNA on cryosectioned tissues (14 μm) was performed by NBT/BCIP through an alkaline phosphatase-conjugated anti-DIG antibody (Roche). Immunohistochemistry was performed as previously described [15,26]. The following antibodies were used: rabbit polyclonal anti-TRPV4 antibody (1:500, a generous gift from Dr. B. Nilius [27]), mouse anti-PSD-95 (1:400, UC Davis NeuroMab) antibody, and mouse anti-MAP2 (1:500, Sigma) antibody.

2.3. Reverse transcription PCR

TRPV4 channel expression was examined by reverse transcription PCR (RT-PCR). Total RNA was prepared from the hippocampus of WT mice using TRIzol reagent (Invitrogen). Total RNA (1 μg) was converted to cDNA using SuperScriptII RNaseH (-) Reverse Transcriptase (Invitrogen). *TRPV4* and *beta-actin* were PCR-amplified from cDNA with reported *TRPV4* and *beta-actin* PCR primer sets [15].

2.4. Cultivation of dissociated astrocytes and neurons

Mouse hippocampal neurons were prepared as reported [15]. In brief, hippocampi were dissected from P0 pups and dissociated using trypsin (0.25%) and trituration. Neurons were plated at a final density of $1–5 \times 10^5$ cells/coverslip on poly-L-lysine-coated coverslips (glass coverslip 13 mm round, Assistant) in Neurobasal Medium (Invitrogen) with B27 supplement (Invitrogen, Carlsbad, CA). After 12 h, coverslips were filled with astrocyte-conditioned medium (Neurobasal Medium with B27 supplement). To prevent overgrowth of glia, neuron cultures were treated with cytosine arabinoside (5 μM; Calbiochem) at 3 days *in vitro* (DIV). Cortical astrocytes were prepared from P0–P2 mice as previously described [15]. The astrocytes were cultured in MEM + 10% FBS + 0.6% D-glucose + 100 U/mL penicillin + 100 μg/mL streptomycin. For ectopic expression of TRPV4-EGFP fusion protein, we transfected the plasmid (TRPV4-EGFP/pEGFPC3, which contains the full coding sequence of mouse TRPV4 in the vector with XhoI and EcoRI sites) into developing hippocampal neurons using Lipofectamine2000 reagent (Invitrogen). Two days after transfection, we fixed and stained the transfected neurons.

3. Results and discussion

We first determined whether *Trpv4* expression was observed in developing brain via *in situ* hybridization analysis utilizing a reportedly specific anti-Trpv4 cRNA probe [15]. Specificity of the probe was confirmed as described by our previous work [15]. In the hippocampus, very weak *Trpv4* expression was observed at embryonic day 18 (E18) in the CA3 region (Fig. 1A), where neurons are reported to express *Trpv4* [15]. Compared with E18 hippocampus, the *Trpv4* signal was increased at P3 (Fig. 1B), and *Trpv4* was detected in the entire hippocampus (Fig. 1B). At P3, the *Trpv4* signal in Ammon's horn was stronger than that in dentate gyrus (DG). The *Trpv4* signal was further increased at P7 (Fig. 1C). At P7, the *Trpv4* expression pattern was very similar to that at P3 (The *Trpv4* signal in Ammon's horn was stronger than that in DG). Finally, *Trpv4* was strongly expressed in hippocampal neurons, and a specific subtype of astrocytes and microglia in adult (8 week old) mice (Fig. 1D) as we reported [15–17]. In the adult, *Trpv4* signal in the CA3 and DG were strongest in the hippocampus (Fig. 1D). These results strongly indicate that neuronal *Trpv4* expression begins in the embryonic stage, and expression levels increase during progression to the adult stage. To further confirm increases in *Trpv4* expression levels during development, we isolated the hippocampus from WT mice of various ages and performed semi-quantitative RT-PCR. RT-PCR

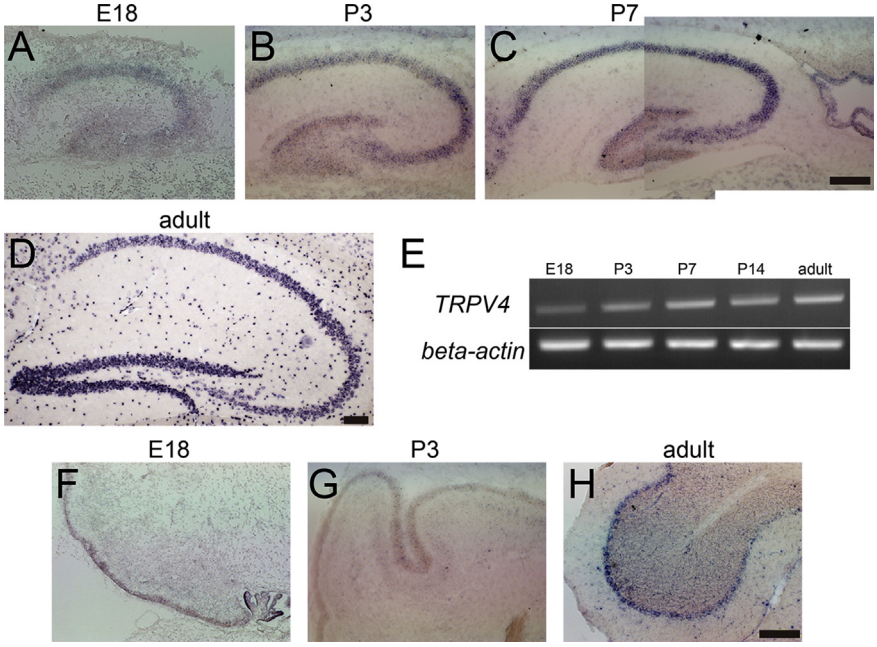

Fig. 1. Developmental expression of *Trpv4* in hippocampus and cerebellum A–D; *In situ* hybridization of *Trpv4* in developing mouse hippocampus. Scale bar; 100 μm. E; RT-PCR was performed from total RNA of isolated developing hippocampus by *Trpv4* and *beta-actin* primer sets. F–H; *In situ* hybridization of *Trpv4* in developing mouse cerebellum. Scale bar; 200 μm.

revealed that *Trpv4* expression levels in the hippocampus increased during development (Fig. 1E), consistent with the *in situ* hybridization results (Fig. 1A–D).

We also examined *Trpv4* expression in the developing cerebellum. In contrast to hippocampal expression (Fig. 1A and B), we failed to detect a *Trpv4* signal at E18 or P3 (Fig. 1F and G). In the adult cerebellum, strong *Trpv4* expression was observed in the Purkinje cell layer (Fig. 1H). These results strongly indicate that *Trpv4* expression in immature neurons is very different depending on neuronal subtypes, and is likely determined by regional and cellular characteristics.

The above results on the expression of TRPV4 during development prompted us to conduct studies to determine the developmental cellular localization of TRPV4. Hence, we performed immunocytochemical experiments utilizing cultured hippocampal neurons. For the analysis, we used the reportedly specific anti-TRPV4 antibody [27]. Specificity of the antibody was confirmed on adult brain sections (Fig. 2A). The antibody detected TRPV4 signal only in WT choroid plexus but not in TRPV4KO tissue (Fig. 2A), consistent with our previous report [28]. At 7 DIV, neurons properly extended dendrites, which were labeled by MAP2 (Fig. 2B), and the majority of TRPV4 was observed in the soma and tips of dendrites (Fig. 2B). At 14 DIV, the dendrites were further extended, and TRPV4 signal was increased (Fig. 2C) compared with 7 DIV immature neurons (Fig. 2B). The majority of TRPV4 signals were observed in dendrites (which were distinguished by MAP2 staining) and soma at 14 DIV (Fig. 2C). At 21 DIV, hippocampal neurons were fully matured, and displayed long dendrites (Fig. 2D). TRPV4 was highly localized in dendrites, and weakly clustered in soma at 21 DIV (Fig. 2D). These results indicate that dendritic targeting of TRPV4 is strictly regulated during neuronal maturation (Fig. 2B–D), and that the final targeting might correspond with final neuronal maturation. TRPV4 is an important molecule controlling brain activity through its activation by brain temperature (*submitted*). Hence, synaptic transmission might be required for dendritic TRPV4 to modulate neuronal information through

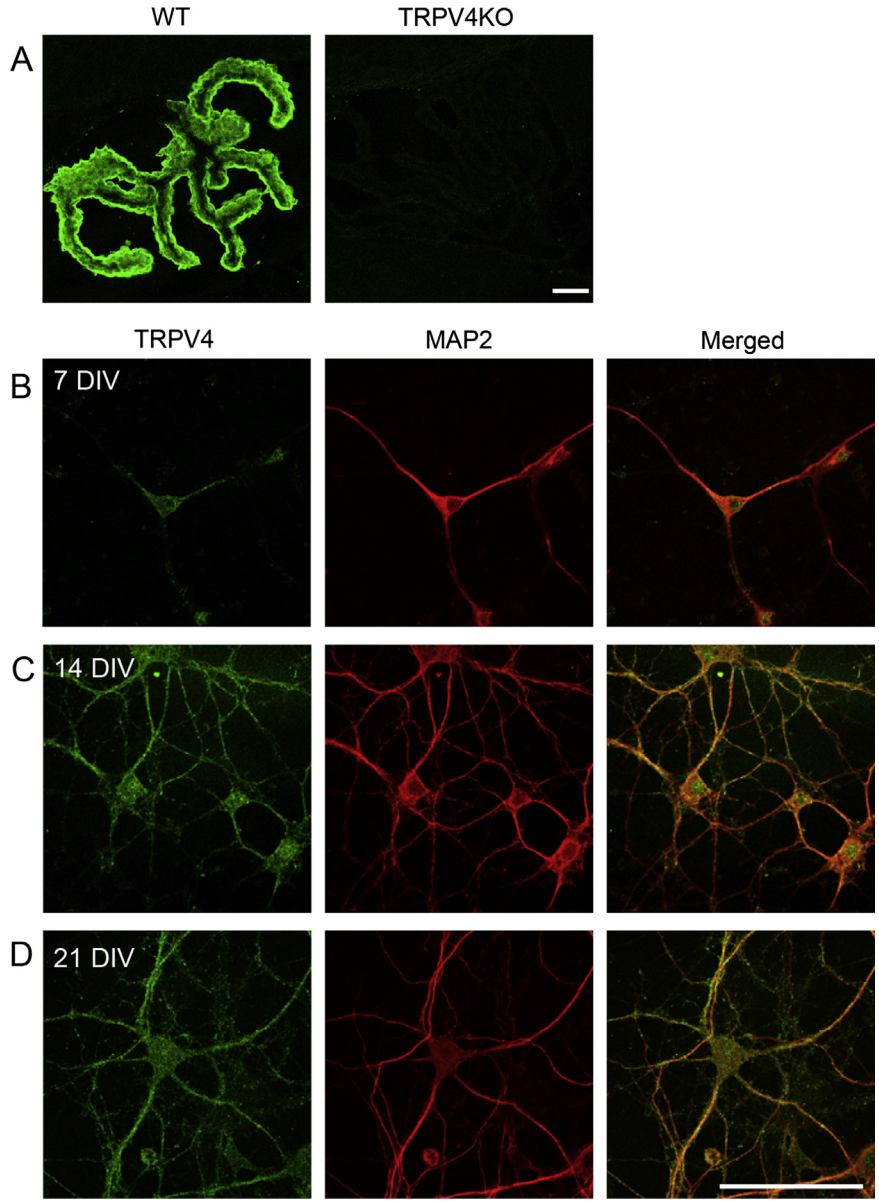

Fig. 2. Cellular distribution of TRPV4 in cultured hippocampal neurons during development A; Immunostaining of TRPV4 in WT or TRPV4KO choroid plexus. Scale bar; 100 μm. B–D; Immunostaining of TRPV4 (green) and MAP2 (red) in developing cultured hippocampal neurons. Scale bar; 50 μm.

intrinsic brain temperature. If so, why do immature neurons also express TRPV4? In our observation, immature neurons express TRPV4 in the tips of dendrites (Fig. 2B). Since activation of TRPV4 enhances neurite extension [29], TRPV4 in immature hippocampal neurons is involved in the formation of neuronal networks. However, the contribution might be very small, since we have already revealed that TRPV4KO hippocampus did not display any morphological defects [15]. This raised another question as to whether dendritic targeting of TRPV4 during neuronal maturation also caused post-synaptic clustering. To examine this, we co-labeled cultured hippocampal neurons with anti-TRPV4 and anti-PSD-95 antibodies. In immature neurons (at 7 DIV), the majority of dendritic TRPV4 was not co-localized with PSD-95, although proximal dendrites expressed TRPV4 (Fig. 3A). In contrast to this observation, the majority of TRPV4 was associated with formation of punctate clusters (Fig. 3B and C as high magnification of Fig. 3B) on dendrites in mature neurons (at 21 DIV), and most of these TRPV4-clusters were co-localized with PSD-95 (Fig. 3B and C). These results indicate that post-synaptic targeting of TRPV4 occurred at critical time periods after 7 DIV. To further confirm this, we ectopically transfected the TRPV4-EGFP fusion expression-vector into cultured hippocampal neurons. In this assay system, over-expressed TRPV4-EGFP caused spillover from soma to dendritic shafts (Fig. 4A) different from endogenous TRPV4 localization at P7 (Fig. 3A), since over-expression produced very large amount of TRPV4-EGFP proteins. Although TRPV4-EGFP targeted to dendritic shafts as the spillover (Fig. 3A), the majority of spiny PSD-95 clusters, which were in dendritic spines, were not co-localized with TRPV4-EGFP in the immature neurons at 7 DIV (Fig. 4A, arrowheads). These results indicate that the spillover of TRPV4 is not sufficient to induce the post-synaptic targeting. Thus, it was considered that the post-synaptic targeting of TRPV4 required specific triggering signals. Indeed, PSD-95 signals on dendritic shafts were well co-localized with TRPV4-EGFP signals at 7 DIV (Fig. 4A). These results suggest that post-synaptic targeting of TRPV4 is defined by waiting periods until neuronal maturation is completed. In fact, PSD-95 clusters in dendritic spines were well co-localized with TRPV4-EGFP signals at 21 DIV (Fig. 4B, arrowheads). Taken together, the post-synaptic clustering of TRPV4 is strictly regulated depending on the state of neuronal maturation.

Previously, we reported that targeting of voltage-gated potassium channel, Kv4.2 from the soma to the dendrites and synapses of developing cerebellar granule neurons is induced by synapse formation, and can be dynamically controlled by synaptic activity [30–32]. However, the post-synaptic clustering of TRPV4 was triggered by other mechanisms compared with that of Kv4.2, since formation of post-synaptic structures was not sufficient for the induction of the post-synaptic clustering of TRPV4 different from that of Kv4.2 (Figs. 3A and 4A) [30]. It is reported that TRPV4 can bind to alpha or beta-catenin, and modulates the function of adherence junctions through its binding with E-cadherin and actin-filament networks [33,34]. Maturation of synapses requires similar molecular components such as beta-catenin and N-cadherin [35,36]. Hence, the binding of TRPV4 with these proteins might be involved in the post-synaptic clustering of TRPV4 in the developing neurons after post-synaptic formation.

During neonatal development, mice cannot maintain constant body temperature without their mother's care. Usually, mothers

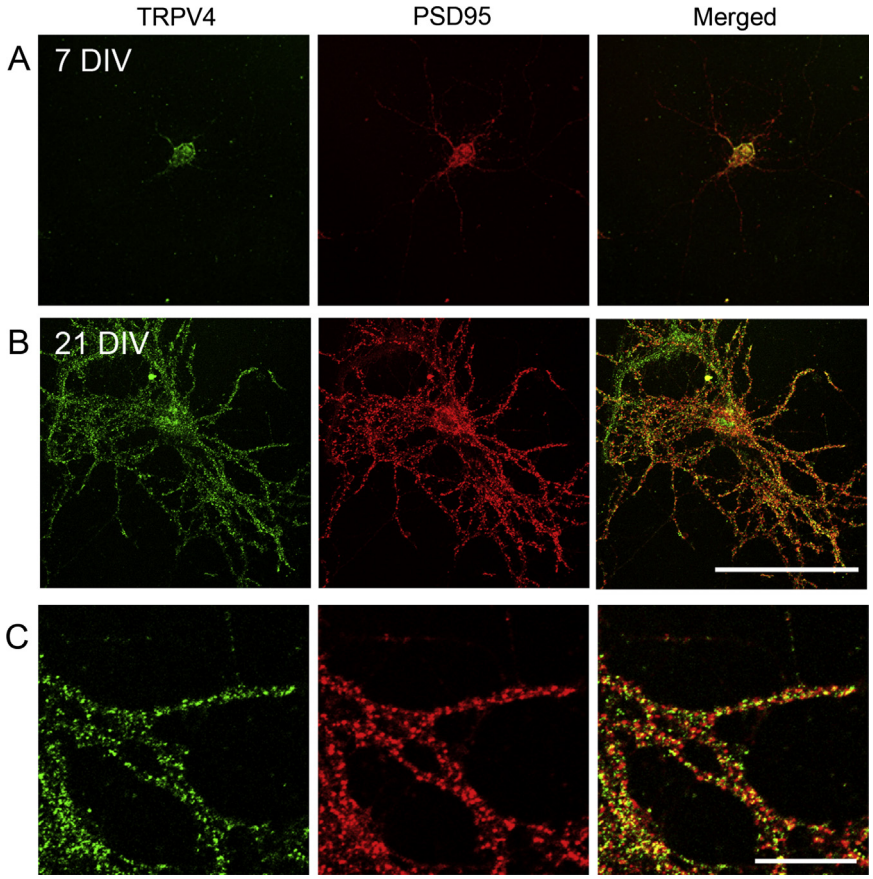

Fig. 3. Post-synaptic clustering of TRPV4 is observed in mature hippocampal neurons during development A–C; Immunostaining of TRPV4 (green) and PSD-95 (red) in developing cultured hippocampal neurons. Scale bar; 50 μm.

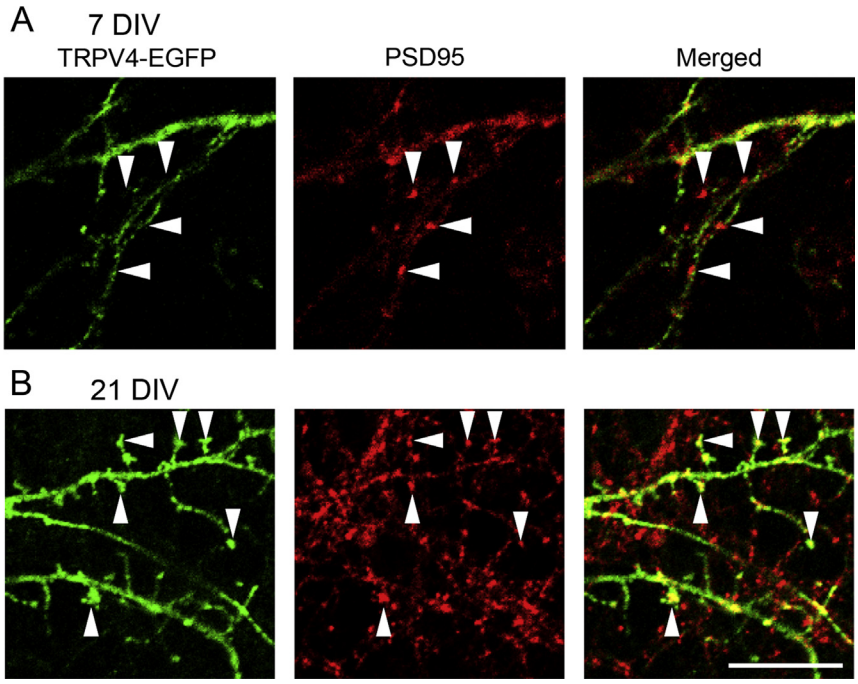

Fig. 4. Post-synaptic clustering of TRPV4 exhibits a strict waiting period during development A and B; We transfected the TRPV4-EGFP plasmid into developing hippocampal neurons using Lipofectamine2000 reagent. Two days after transfection, we fixed and stained the transfected neurons. Immunostaining of EGFP (green) and PSD-95 (red) in developing cultured hippocampal neurons is shown. Arrowheads represent PSD-95 positive dendritic spines. Scale bar; 50 μm.

heat their babies under their body until the babies reach a critical age (~10 days old). If mother mice leave in search of food, the body temperature of babies is drastically decreased. Under such situations, the brain thermo-sensor TRPV4 might perturb neuronal excitation, since TRPV4 activation and inactivation sequentially occur in neonatal brain. To attenuate these disturbances, post-synaptic clustering of TRPV4 (Figs. 3 and 4) might be strictly regulated, as occurs in matured hippocampal neurons. Hence, our results in immature neurons might involve temperature-independent activation of TRPV4.

Thermosensitive TRP channels exhibit two different features, including activation by temperature and activation by other chemical ligands [37]. We recently reported that TRPV2 in embryos promotes axon outgrowth and is involved in the regulation of intestinal movements as a mechanosensor [38,39], although it is also well known that TRPV2 is a noxious heat sensor [40]. Furthermore, we reported that TRPV4 in urinary bladder detects urinary volume increases as a mechanosensor [41] while TRPV4 acts as a thermo-sensor in skin or esophageal keratinocytes [9,42,43] and brain [15]. These results indicate that multiple ligands exist for thermosensitive TRP channels. Indeed, very recently we reported that arachidonic acid activates astrocytic TRPV4 in the hippocampus and that excited astrocytes release ATP as a gliotransmitter [17]. Therefore, dendritic TRPV4 in immature neurons (Figs. 2–4) might be activated primarily by endogenous chemical ligands to promote the formation of neural networks as reported [29]. Since the post-synaptic clustering of TRPV4 is strictly regulated, this phenomenon implies a very important signaling mechanism whereby regulation of neuronal excitability through TRPV4 activation via brain temperature is required following critical development periods after which the maintenance of constant body temperature is acquired.

Conflict of interest

None.

Acknowledgments

We thank Mrs. S. Mizuno and M. Fukuda (Gunma Univ.) for technical assistance. TRPV4KO mice were kindly provided by Dr. A. Mizuno (Jichi Medical University). This research was supported by Grants-in-Aid for Scientific Research (Project No. 21200012, 20399554, 24111507+26111702 <Brain Environment>, 26117502 <Glial Assembly> to K.S.; 23650159 to Y.I. and 18077012 to M.T.); from the Ministry of Education, Culture, Sports, Science and Technology, Japan; by a grant from Uehara Memorial Foundation (to K.S.); by a grant from Takeda Science Foundation, Tokyo, Japan (to K.S.); by a grant from the Sumitomo Foundation (to K.S.); by a grant from the Brain Science Foundation (to K.S.); by a grant from Narishige Neuroscience Research Foundation (to K.S.); by a grant from Salt Science Research Foundation No.14C2 (to K.S.); and by a grant from the Ichiro Kanehara Foundation (to K.S.).

References

[1] R.C. Malenka, R.A. Nicoll, Long-term potentiation—a decade of progress? Science 285 (1999) 1870–1874.
[2] J.G. Nicholls, A.R. Martin, B.G. Wallace, P.A. Fuchs, From Neuron to Brain, Fourth ed., 2001, pp. 25–225.
[3] R.A. Nicoll, Expression mechanisms underlying long-term potentiation: a postsynaptic view, Philos. Trans. R. Soc. London B Biol. Sci. 358 (2003) 721–726.
[4] J.S. Trimmer, Regulation of ion channel expression by cytoplasmic subunits, Curr. Opin. Neurobiol. 8 (1998) 370–374.
[5] W. Liedtke, Y. Choe, M.A. Marti-Renom, A.M. Bell, C.S. Denis, A. Sali, A.J. Hudspeth, J.M. Friedman, S. Heller, Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor, Cell 103 (2000) 525–535.
[6] R. Stortmann, C. Harteneck, K. Nunnenmacher, G. Schultz, T.D. Plant, OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity, Nat. Cell. Biol. 2 (2000) 695–702.
[7] U. Wissenbach, M. Bodding, M. Freichel, V. Flockerzi, Trp12, a novel Trp related protein from kidney, FEBS Lett. 485 (2000) 127–134.
[8] B. Nilius, J. Prenen, U. Wissenbach, M. Bodding, G. Droogmans, Differential activation of the volume-sensitive cation channel TRP12 (OTRPC4) and volume-regulated anion currents in HEK-293 cells, Pflugers Arch. 443 (2001) 227–233.

[9] A.D. Guler, H. Lee, T. Iida, I. Shimizu, M. Tominaga, M. Caterina, Heat-evoked activation of the ion channel, TRPV4, J. Neurosci. 22 (2002) 6408–6414.
[10] H. Watanabe, J.B. Davis, D. Smart, J.C. Jerman, G.D. Smith, P. Hayes, J. Vriens, W. Cairns, U. Wissenbach, J. Prenen, V. Flockerzi, G. Droogmans, C.D. Benham, B. Nillius, Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives, J. Biol. Chem. 277 (2002a) 13569–13577.
[11] H. Watanabe, J. Vriens, S.H. Suh, C.D. Benham, G. Droogmans, B. Nillius, Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells, J. Biol. Chem. 277 (2002b) 47044–47051.
[12] H. Watanabe, J. Vriens, J. Prenen, G. Droogmans, T. Voets, B. Nillius, Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels, Nature 424 (2003) 434–438.
[13] A. Mizuno, N. Matsumoto, M. Imai, M. Suzuki, Impaired osmotic sensation in mice lacking TRPV4, Am. J. Physiol. Cell. Physiol. 285 (2003) C96–C101.
[14] W. Liedtke, J.M. Friedman, Abnormal osmotic regulation in trpv4-/- mice, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13698–13703.
[15] K. Shibasaki, M. Suzuki, A. Mizuno, M. Tominaga, Effects of body temperature on neural activity in the hippocampus: regulation of resting membrane potentials by transient receptor potential vanilloid 4, J. Neurosci. Offic. J. Soc. Neurosci. 27 (2007) 1566–1575.
[16] M. Konno, H. Shirakawa, S. Iida, S. Sakimoto, I. Matsutani, T. Miyake, K. Kageyama, T. Nakagawa, K. Shibasaki, S. Kaneko, Stimulation of transient receptor potential vanilloid 4 channel suppresses abnormal activation of microglia induced by lipopolysaccharide, Glia 60 (2012) 761–770.
[17] K. Shibasaki, K. Ikenaka, F. Tamalu, M. Tominaga, Y. Ishizaki, A novel subtype of astrocytes expressing TRPV4 (transient receptor potential vanilloid 4) regulates neuronal excitability via release of gliotransmitters, J. Biol. Chem. 289 (2014) 14470–14480.
[18] A.I. Hodgkin, B. Katz, The effect of temperature on the electrical activity of the giant axon of the squid, J. Physiol. 109 (1949) 240–249.
[19] J.M. Ritchie, R.W. Straub, The effect of cooling on the size of the action potential of mammalian non-medullated fibers, J. Physiol. 134 (1956) 712–717.
[20] S.J. Schiff, G.G. Somjen, The effects of temperature on synaptic transmission in hippocampal tissue slices, Brain Res. 345 (1985) 279–284.
[21] E. Moser, I. Mathiesen, P. Andersen, Association between brain temperature and dentate field potentials in exploring and swimming rats, Science 259 (1993) 1324–1326.
[22] J. Torgersen, K. Strand, T.W. Bjelland, P. Klepstad, R. Kvale, E. Soreide, T. Wentzel-Larsen, H. Flaatten, Cognitive dysfunction and health-related quality of life after a cardiac arrest and therapeutic hypothermia, Acta Anaesthesiol. Scand. 54 (2010) 721–728.
[23] J.M. Fuster, R.H. Bauer, Visual short-term memory deficit from hypothermia of frontal cortex, Brain Res. 81 (1974) 393–400.
[24] E.I. Moser, P. Andersen, Conserved spatial learning in cooled rats in spite of slowing of dentate field potentials, J. Neurosci. 14 (1994) 4458–4466.
[25] K. Shibasaki, N. Murayama, K. Ono, Y. Ishizaki, M. Tominaga, TRPV2 enhances axon outgrowth through its activation by membrane stretch in developing sensory and motor neurons, J. Neurosci. 30 (2010) 4601–4612.
[26] K. Shibasaki, H. Takebayashi, K. Ikenaka, L. Feng, L. Gan, Expression of the basic helix-loop-factor Olig2 in the developing retina: Olig2 as a new marker for retinal progenitors and late-born cells, Gene Expr. Patterns 7 (2007) 57–65.
[27] T. Gevaert, J. Vriens, A. Segal, W. Everaerts, T. Roskams, K. Talavera, G. Owsianik, W. Liedtke, D. Daelemans, I. Dewachter, F. Van Leuven, T. Voets, D. De Ridder, B. Nilius, Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding, J. Clin. Invest. 117 (2007) 3453–3462.
[28] Y. Takayama, K. Shibasaki, Y. Suzuki, A. Yamanaka, M. Tominaga, Modulation of water efflux through functional interaction between TRPV4 and TMEM16A/anoctamin 1, FASEB J. Offic. Publ. Fed. Am. Soc. Exp. Biol. 28 (2014) 2238–2248.
[29] Y. Jang, J. Jung, H. Kim, J. Oh, J.H. Jeon, S. Jung, K.T. Kim, H. Cho, D.J. Yang, S.M. Kim, I.B. Kim, M.R. Song, U. Oh, Axonal neuropathy-associated TRPV4 regulates neurotrophic factor-derived axonal growth, J. Biol. Chem. 287 (2012) 6014–6024.
[30] K. Shibasaki, K. Nakahira, J.S. Trimmer, R. Shibata, M. Akita, S. Watanabe, K. Ikenaka, Mossy fibre contact triggers the targeting of Kv4.2 potassium channels to dendrites and synapses in developing cerebellar granule neurons, J. Neurochem. 89 (2004) 897–907.
[31] R. Shibata, K. Nakahira, K. Shibasaki, Y. Wakazono, K. Imoto, K. Ikenaka, A-type K+ current mediated by the Kv4 channel regulates the generation of action potential in developing cerebellar granule cells, J. Neurosci. 20 (2000) 4145–4155.
[32] R. Shibata, Y. Wakazono, K. Nakahira, J.S. Trimmer, K. Ikenaka, Expression of Kv3.1 and Kv4.2 genes in developing cerebellar granule cells, Dev. Neurosci. 21 (1999) 87–93.
[33] T. Sokabe, T. Fukumi-Tominaga, S. Yonemura, A. Mizuno, M. Tominaga, The TRPV4 channel contributes to intercellular junction formation in keratinocytes, J. Biol. Chem. 285 (2010) 18749–18758.
[34] D.A. Janssen, J.G. Hoenderop, K.C. Jansen, A.W. Kemp, J.P. Heesakkers, J.A. Schalken, The mechanoreceptor TRPV4 is localized in adherence junctions of the human bladder urothelium: a morphological study, J. Urol 186 (2011) 1121–1127.
[35] B. Zhang, S. Luo, X.P. Dong, X. Zhang, C. Liu, Z. Luo, W.C. Xiong, L. Mei, Beta-catenin regulates acetylcholine receptor clustering in muscle cells through interaction with rapsyn, J. Neurosci. Offic. J. Soc. Neurosci. 27 (2007) 3968–3973.
[36] M. Nuriya, R.L. Huganir, Regulation of AMPA receptor trafficking by N-cadherin, J. Neurochem. 97 (2006) 652–661.
[37] M. Tominaga, M.J. Caterina, Thermosensation and pain, J. Neurobiol. 61 (2004) 3–12.
[38] K. Shibasaki, N. Murayama, K. Ono, Y. Ishizaki, M. Tominaga, TRPV2 enhances axon outgrowth through its activation by membrane stretch in developing sensory and motor neurons, J. Neurosci. Offic. J. Soc. Neurosci. 30 (2010) 4601–4612.
[39] H. Mihara, A. Boudaka, K. Shibasaki, A. Yamanaka, T. Sugiyama, M. Tominaga, Involvement of TRPV2 activation in intestinal movement through nitric oxide production in mice, J. Neurosci. Offic. J. Soc. Neurosci. 30 (2010) 16536–16544.
[40] M.J. Caterina, T.A. Rosen, M. Tominaga, A.J. Brake, D. Julius, A capsaicin-receptor homologue with a high threshold for noxious heat, Nature 398 (1999) 436–441.
[41] T. Mochizuki, T. Sokabe, I. Araki, K. Fujishita, K. Shibasaki, K. Uchida, K. Naruse, S. Koizumi, M. Takeda, M. Tominaga, The TRPV4 cation channel mediates stretch-evoked Ca2+ influx and ATP release in primary urothelial cell cultures, J. Biol. Chem. 284 (2009) 21257–21264.
[42] S. Mandadi, T. Sokabe, K. Shibasaki, K. Katanosaka, A. Mizuno, A. Moqrich, A. Patapoutian, T. Fukumi-Tominaga, K. Mizumura, M. Tominaga, TRPV3 in keratinocytes transmits temperature information to sensory neurons via ATP, Pflugers Arch. Eur. J. Physiol. 458 (2009) 1093–1102.
[43] H. Mihara, A. Boudaka, T. Sugiyama, Y. Moriyama, M. Tominaga, Transient receptor potential vanilloid 4 (TRPV4)-dependent calcium influx and ATP release in mouse oesophageal keratinocytes, J. Physiol. 589 (2011) 3471–3482.