Journal Club

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The Expression Pattern of TRPV1 in Brain

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Review of Cavanaugh et al.

TRPV1 is a nonselective cation channel with high calcium permeability. It is the archetypal member of the vanilloid TRP family and was first identified as the receptor for capsaicin, the pungent ingredient of chili pepper (Caterina et al., 1997). TRPV1 is a polymodal TRP channel that can be activated by noxious heat, pH changes, fatty acid amides, and endogenous lipid ligands (Ross, 2003). It is well established that TRPV1 acts as a molecular detector of noxious stimuli in the peripheral nervous system (Caterina and Julius, 2001).

First cloned from rat dorsal root ganglia (DRGs) (Caterina et al., 1997), TRPV1 expression has also been established in trigeminal (TG) and nodose ganglia (Tominaga et al., 1998). Now accumulated evidence from diverse experimental approaches suggests that TRPV1 has a wide distribution. Using immunohistochemistry, *in situ* hybridization, RT-PCR, and capsaicin response, TRPV1 has been identified in several brain structures, spinal cord, and bladder. Although this expression pattern is well described, the function of TRPV1 in these structures is poorly understood and remains a controversial subject. Indeed, Benninger et al. (2008) and Everaerts et al. (2010) did not detect capsaicin-induced calcium response

respectively in brain and urothelium. Because of the therapeutic potential of TRPV1 pharmacology as pain killers, the expression pattern of TRPV1 should be determined more precisely to minimize possible side effects of such therapies on brain.

Cavanaugh et al. (2011) examined TRPV1 expression using a new genetic tool: a TRPV1 reporter mouse in which two reporter genes, nuclear LacZ and the placental alkaline phosphatase (PLAP), were inserted after an IRES sequence (site for internal entrance of ribosomes). This system allows the expression of separate genes under the control of the same regulatory elements. Therefore, the expression of the reporter gene does not disrupt TRPV1 expression, avoiding side effects (compensatory process or ectopic expression) due to reporter gene insertion. The authors observed LacZ and PLAP staining in DRG and TG neurons [Cavanaugh et al. (2011), their Fig. 1B,C]. To confirm the specificity of the reporter staining, they tested the capsaicin response of the LacZ⁺ cells in cultured DRG neurons by calcium imaging. Capsaicin is the most potent and specific known agonist of TRPV1 (Caterina et al., 1997); therefore, intracellular calcium should increase in the LacZ⁺ neurons if it faithfully identifies TRPV1-expressing cells. Indeed, 99% of the stained neurons positively responded to capsaicin [Cavanaugh et al. (2011), their Fig. 1J]. Cavanaugh et al. (2011) corroborated these functional data by comparing the reporter expression pattern to that of TRPV1 using immunohis-

tochemistry on DRG slices. More than 95% of the TRPV1 antibody-positive neurons were LacZ positive. These two experiments demonstrated the expression of the TRPV1 reporter is well correlated with functional expression of TRPV1 in DRGs, validating their strategy.

Nonetheless, some neurons responded to capsaicin (~10%) or were immunolabeled for TRPV1 (5%), but did not express the reporter gene. The authors did not speculate about those neurons. Perhaps the response is mediated by other members of the TRPV family, but so far capsaicin has been described to be specific to TRPV1. Alternatively the discrepancy could indicate their reporter system is not sensitive enough to visualize very low expression of TRPV1. Indeed, it is known that gene expression under IRES sequence control is often inferior to the expression level of the gene upstream (Licursi et al., 2011). Therefore, it is possible that the reporter is not expressed or not detectable when TRPV1 expression is low.

Because the controversy about TRPV1 expression is mainly focused on brain expression, Cavanaugh et al. (2011) investigated reporter (PLAP and nLacZ) expression in brain slices. They could detect only limited reporter expression in the hypothalamus and in discrete nonpyramidal hippocampal neurons [Cavanaugh et al. (2011), their Fig. 2]. Because these results were inconsistent with previous reports, they used complementary approaches (immunohistochemistry, RT-PCR, and *in situ* hybridization) to verify the expression. All these methods failed to detect any TRPV1

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expression in brain areas except caudal hypothalamus, where TRPV1 expression was previously reported (Cristino et al., 2006). Reasoning that the discrepancy might result from transient expression of TRPV1 during development, the authors created a second reporter line in which Cre recombinase is expressed under the control of TRPV1 promoter. This reporter allows persistent visualization of transient expression of TRPV1. Consistent with there being transient expression, the Cre expression pattern was widespread compared to that of LacZ staining. Nevertheless, the authors were still unable to detect TRPV1 hippocampal expression with this method.

Cavanaugh et al. (2011) also investigated TRPV1 functional expression in hypothalamus. On slices and dissociated neurons, 97% of cells that responded to capsaicin were also LacZ⁺, but only 53% of the reporter-expressing cells responded to capsaicin. The observation that the LacZ⁺ population is larger than the capsaicin-responsive population is consistent with there being a developmental regulation of TRPV1 expression. Capsaicin response could not be detected in TRPV1 knock-out mice or after Ruthenium Red application. Despite this somewhat indirect method (i.e., use of Ruthenium Red instead of the specific TRPV1 antagonist capsazepine) to test the specificity of the response, the TRPV1 expression reported by the LacZ expression seems to be correlated to a functional expression of TRPV1 in hypothalamus.

Previous studies from Cristino et al. (2006) and Zschenderlein et al. (2011) showed TRPV1 expression in hippocampal and amygdala neurons with two different antibodies. Moreover, Marsch et al. (2007) showed that TRPV1 was involved in hippocampal synaptic plasticity using TRPV1 knock-out, and Gibson et al. (2008) confirmed this finding by showing that capsaicin and capsazepine, respectively, induced and repressed hippocampal long-term depression (LTD). Nevertheless, Cavanaugh et al. (2011) could not detect any TRPV1 expression in these two structures. This discrepancy could reflect a very low expression of TRPV1 in these brain structures, below the detection threshold of the reporter. To test this possibility, the authors looked for TRPV1 responses in hippocampal neurons. It has been shown that capsaicin application on granule cells from dentate gyrus depresses excitatory transmission in the medial perforant path (Chávez et al., 2010). This effect is dose dependent and cannot be observed in the presence of capsazepine or in TRPV1 knock-out

mice. These observations were not confirmed by Cavanaugh et al. (2011). Indeed, they could not record by calcium imaging any capsaicin response in hippocampus slices or in hippocampal pyramidal neuron cultures.

These findings are, nonetheless, exciting. Previous studies suggested TRPV1 is involved in hippocampal synaptic plasticity based on capsaicin application, but did not directly demonstrate TRPV1 activation by calcium imaging or whole-cell recording. These discrepancies could have several explanations. First, Chávez et al. (2010) and Grueter et al. (2010) suggested a role of TRPV1 in the synaptic plasticity in response to endocannabinoids. This endocannabinoid-induced plasticity could also be triggered by other TRP channels. Indeed Wanabe et al. (2003) showed that anandamine could activate TRPV4 via epoxycicosatrienoic acid and TRPV4 is known to be expressed in hippocampus (Shibasaki et al., 2007). Another possibility is that TRPV1 triggers LTD via a nonconducting function (Kaczmarek, 2006) or via a capsaicin-resistant TRPV1. For example, the alternatively spliced variant TRPV1b has been described as a functional ion channel although it does not respond to capsaicin (Lu et al., 2005). Indeed, the authors reported TRPV1 expression [Cavanaugh et al. (2011), their Fig. 2F] in hippocampal interneurons, but did not detect any capsaicin response by calcium imaging. Together, these data suggest that a non-capsaicin-activated form of TRPV1 is expressed. It would be informative to study induction of LTD in the reporter⁺ interneurons, to see if capsaicin could modulate it as reported by Gibson et al. (2008).

This newly established TRPV1 expression pattern in mouse brain is totally unexpected. However, the expression pattern of TRP channels could vary across species. For instance, TRPV4 has been reported to be expressed in neurons and astroglial, in mouse (Mezey et al., 2000) and rat (Tóth et al., 2005) hippocampi, respectively. Given that TRPV1 might be expressed in a different pattern in rat or human brain, Cavanaugh et al. (2011) investigated TRPV1 mRNA expression in brain slices from rat, monkey, and human by *in situ* hybridization. They found that the expression pattern in rat was highly similar to that in mouse. TRPV1 was absent from hippocampus in monkey and human, and the hypothalamus could not be investigated because this tissue was not available. If the restricted localization of TRPV1 in human brain is confirmed, it is a breakthrough discovery for TRPV1-based therapies.

In conclusion, Cavanaugh et al. (2011) have created a new tool that could allow the study of TRPV1 inside and outside the nervous system. Their data support the idea that TRPV1 is not heavily expressed in brain, opening or reopening a large field in the understanding of TRP channel function. More and more TRPV1 antagonists are being developed to treat pain, and the exact TRPV1 expression pattern might lead to an understanding of side effects associated with these molecules. The described expression pattern should also lead to the reexamination of synaptic plasticity defects already reported in the TRPV1 KO mouse. Together, these data encourage further investigation of TRPV1-related therapeutic strategies to treat pain and might highlight the involvement of unexpected mechanisms in brain plasticity.

References

- Benninger F, Freund TF, Hajos N (2008) Control of excitatory synaptic transmission by capsaicin is unaltered in TRPV1 vanilloid receptor knockout mice. *Neurochem Int* 52:89–94.
- Caterina MJ, Julius D (2001) The vanilloid receptor: a molecular gateway to the pain pathway. *Annu Rev Neurosci* 24:487–517.
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389:816–824.
- Cavanaugh DJ, Chesler AT, Jackson AC, Sigal YM, Yamanaka H, Grant R, O'Donnell D, Nicoll RA, Shah NM, Julius D, Basbaum AI (2011) Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. *J Neurosci* 31:5067–5077.
- Chávez AE, Chiu CQ, Castillo PE (2010) TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. *Nat Neurosci* 13:1511–1518.
- Cristino L, de Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V (2006) Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience* 139:1405–1415.
- Everaerts W, Vriens J, Owsianik G, Appendino G, Voets T, De Ridder D, Nilius B (2010) Functional characterization of transient receptor potential channels in mouse urothelial cells. *Am J Physiol Renal Physiol* 298:F692–F701.
- Gibson HE, Edwards JG, Page RS, Van Hook MJ, Kauer JA (2008) TRPV1 channels mediate long-term depression at synapses on hippocampal interneurons. *Neuron* 57:746–759.
- Grueter BA, Brasnjic G, Malenka RC (2010) Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. *Nat Neurosci* 13:1519–1525.
- Kaczmarek LK (2006) Non-conducting functions of voltage-gated ion channels. *Nat Rev Neurosci* 7:761–771.
- Licursi M, Christian SL, Pongnopparat T, Hirasawa K (2011) In vitro and in vivo com-

- parison of viral and cellular internal ribosome entry sites for bicistronic vector expression. *Gene Ther* 18:631–636.
- Lu G, Henderson D, Liu L, Reinhart PH, Simon SA (2005) TRPV1b, a functional human vanilloid receptor splice variant. *Mol Pharmacol* 67:1119–1127.
- Marsch R, Foeller E, Rammes G, Bunck M, Kossel M, Holsboer F, Zieglgansberger W, Landgraf R, Lutz B, Wotjak CT (2007) Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. *J Neurosci* 27:832–839.
- Mezey E, Tóth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A (2000) Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc Natl Acad Sci U S A* 97:3655–3660.
- Ross RA (2003) Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol* 140:790–801.
- Shibasaki K, Suzuki M, Mizuno A, Tominaga M (2007) Effects of body temperature on neural activity in the hippocampus: regulation of resting membrane potentials by transient receptor potential vanilloid 4. *J Neurosci* 27:1566–1575.
- Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 21:531–543.
- Tóth A, Boczán J, Kedei N, Lizanecz E, Bagi Z, Papp Z, Edes I, Csiba L, Blumberg PM (2005) Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. *Brain Res Mol Brain Res* 135:162–168.
- Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B (2003) Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 424:434–438.
- Zschenderlein C, Gebhardt C, von Bohlen Und Halbach O, Kulisch C, Albrecht D (2011) Capsaicin-induced changes in LTP in the lateral amygdala are mediated by TRPV1. *PLoS One* 6:e16116.