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Analysis of TRPV1-Dependent Osmolarity Sensing Mechanisms of Neurons in the Central Nervous System

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Summary

Sodium ion has the largest contribution to the determination of the body fluid osmolarity, and its homeostasis largely depends on sodium ion concentrations. The osmolarity of the body fluid is monitored by the central nervous system (CNS) in addition to peripheral osmo-sensors. Supra optic nucleus (SON) neurons in the hypothalamus is considered to function as a sensor of the plasma osmolarity. It is reported that SON neurons of mice expressed the N-terminal-truncated variant of transient receptor potential vanilloid 1 (mΔN-TRPV1) and it is proposed that mΔN-TRPV1 functions as an osmo-sensor in the CNS. On the other hand, we found that rat TRPV1 with the full length (rTRPV1full) but not the N-terminal truncated variant (rTRPV1SON) functions as an osmo-sensing molecule when heterologously expressed in human embryonic kidney (HEK) 293 cells. To examine the molecular basis of the osmo-sensation by TRPV1, we constructed several N-terminal mutant molecules of rTRPV1 and analyzed cellular and molecular physiology of these molecules.

We constructed mutant rTRPV1 DNAs, in which 5'-terminal residues coding N-terminal amino acids at positions 1-109 (ΔN109) and 1-110 (ΔN110) are deleted. Constructed DNAs were inserted into a plasmid vector and were transfected into HEK293 cells. Changes in intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) were measured by the $[Ca^{2+}]i$ imaging technique using the fluorescent Ca^{2+} indicator, Fura-2.

HEK293 cells transfected with no DNA showed no $[Ca^{2+}]i$ response to stimulations with capsaicin (1 μM) and a hypertonic solution (+50 mOsm/kg). Among cells expressing rTRPV1full and ΔN109, a part of the cells showed $[Ca^{2+}]i$ rises in response to stimulations with both capsaicin and +50 mOsm/kg. ΔN110-expressing cells responded to capsaicin but not to +50 mOsm/kg at all. These results indicate that arginine positioned at 110 of the N-terminal of rTRPV1 is essential for the osmo-sensation. The first ankyrin-like domain containing arginine positioned at 110 may interact with some structure connected to the cell membrane and detected changes in the membrane tension caused by the changes in the extracellular osmolarity. At present, we conclude that TRPV1 with the full length but not the N-terminal truncated variant has a functional role as an osmo-sensing molecule in the CNS.