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Identification and Functional Analysis of Novel Bioactive Peptides Regulating Salt and Water Homeostasis

Hideki Yamaguchi and Masamitsu Nakazato

Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki

Summary

Here, we report the identification of two novel peptides derived from the neurosecretory protein VGF, designated neuroendocrine regulatory peptide (NERP)-1 and -2, by focusing on carboxy-terminal amidation, a post-translational modification most frequently found in bioactive peptides (J Biol Chem, 282, 26354-60, 2007). Rat NERP-1 and NERP-2, which are highly homologous to human NERPs, are expressed in the hypothalamic paraventricular nucleus (PVN) and the supraoptic nucleus (SON) and participate in the central control of water balance.

NERPs dose-dependently suppressed vasopressin release induced by icv injection of hypertonic NaCl/ angiotensin II (AII) *in vivo*. NERPs also suppressed basal and AII-induced vasopressin secretion from hypothalamic explants *in vitro*. Bioactivity of NERPs required carboxy-terminal amidation. To elucidate the mechanism by which NERPs suppress vasopressin secretion, we used an *in vivo* microdialysis technique to measure glutamate (Glu) release. The Glu release induced by icv administration of AII was significantly suppressed by icv injection of NERP-1 and NERP-2. Whole-cell patch-clamp recordings of SON slice preparations showed that NERP-1 reversibly reduced the frequency of AII-evoked excitatory postsynaptic currents (EPSCs) without altering their amplitude. This effect was not influenced by the Na^+-channel blocker tetrodotoxin (TTX), applied to block all action potential-driven inhibitory postsynaptic currents. NERP-1 thus appears to act on the presynaptic terminal of glutamatergic neurons connected to vasopressin neurons. NERP-2 also reduced the frequency of AII-evoked EPSCs without altering their amplitude. This suppression was abolished by TTX and bicuculline, a $GABA_A$ receptor antagonist, suggesting that NERP-2 modulates the excitability of GABAergic interneurons connected to the terminals of the glutamatergic neurons. Two NERPs of different sequences, derived from one precursor, serve as novel peptidergic retrograde modulators to suppress vasopressin neuron activity via different mechanisms.

Six normal subjects were subjected to salt-loading test to examined the effect of plasma l NERPs on the response of an osmotic stimulus (5% NaCl, 0.05 mL/kg/min, 120 min). Plasma osmolality and plasma level of AVP increased during the osmotic stimulus, but the plasma levels of NERP-1 and -2 did not. The localization of NERPs in the hypothalamus and no change of plasma NERPs after the osmotic stimulus suggested that NERPs might work as a central neuromodulator in body fluid homeostasis.