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Regulation of Body Fluid: Identification of Molecular Machineries for Cellular Na^+ Transport in Kidney Macula Densa

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Summary

A newly established mouse macula densa cell line (NE-MD) shows up-regulation of the neuronal nitric oxide synthase (nNOS) protein and generates L-arginine (Arg)-induced NO when NE-MD cells were incubated with either low [NaCl] solution or furosemide (12 μM), an inhibitor of Na^+-K^+-$2Cl^-$ transporter (Yasuoka *et al*, 2005; Kawada *et al*, 2006). Molecular weight of furosemide-induced nNOS protein (65 kD) by Western blotting was smaller than brain and cardiac types of nNOSs (160 kD), suggesting that the nNOS protein of NE-MD cells may be largely truncated. To further investigate a molecular structure of the nNOS protein, we have examined the furosemide-induced proteomes of NE-MD by two-dimensional gel electrophoresis (2-DE) combined with mass spectrometry (MALDI-TOF-MS). A total of 46 selected protein spots, corresponding to 18 unique proteins, have been identified by 2-DE method. Although 6 protein spots, including nucleolin, pyruvate kinase, and nNOS, were increased by 1.5 times in NE-MD cells treated with furosemide (5 hrs), only one unique protein spot, later determined as nNOS, increased by more than 5 times. Although an N-terminal heme-binding domain was conserved, a C-terminal reductase domain, being essential for transfer of electrons to the catalytic heme center, was surprisingly missing. In conclusion, nNOS protein identified from NE-MD cells may function without the C-terminal or require an undetermined protein for a functional complex. C-terminal truncated nNOS of mouse NE-MD cells may explain unique regulation of gene expression and activity in the macula densa.