S 4-4

FUNCTIONAL ROLE OF TWO-PORE POTASSIUM CHANNELS IN THE GASTROINTESTINAL TRACT

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Gastrointestinal motility is affected by the resting membrane potential (RMP) of smooth muscle cell (SMC) which is dominantly regulated by background K^+ conductance (s). Two-pore potassium channels have recently been reported as a background K^+ conductance in SMC. However, the role of these channels in gastrointestinal motility has not yet been fully investigated. In this study, we provide functional evidence using conventional microelectrode recordings, patch clamp, molecular studies and immunohistochemistry for the role of TASK and TREK channels in setting RMP in the murine ileum and colon. Intracellular microelectrode recordings from ileal smooth muscle revealed that acidic pH produced membrane depolarization. The pH-induced depolarization was abolished by pre-incubation with lidocaine and zinc which are known to inhibit TASK channels. Whole cell voltage clamp experiments performed on myocytes from murine ileum revealed that exposure to a bathing solution with a low pH (pH 6.5) decreased mean peak outward currents. The pretreatment of tetraethyl ammonium and/or 4-aminopyridine did not inhibit the pH effects on outward currents. However, the pretreatment of Zn^{2+} and lidocaine completely abolished the observed pH effects on outward currents. RT-PCR revealed the expression of TASK-1 and TASK-2 channels in both murine ileal tissues and isolated SMC's. Immunohistochemical labeling demonstrated TASK-1 and TASK-2 immunoreactivity within ileum both on isolated cells and in tissues. To evaluate the role of stretch-dependent potassium (SDK) channels in stretch dependent responses in GI muscles, we performed a series of experiments where length ramps were applied to colonic muscles. Application of length ramps at a rate of 6 or 31 μm s-1 until an isometric force of 5 mN was reached produced transient hyperpolarizations and loss or attenuation of spike complexes. These responses were reproducible when ramps were applied every 30 seconds. This hyperpolarization was also sensitive to blocked by L-NNA and by inhibition of SDK channels by L-methionine. Negative pressure applied to on-cell membrane patches activated K^+ channels that were voltage independent and had a slope conductance of 95 pS in symmetrical K^+ gradients. Cell elongation activated K^+ channels with the same properties as those activated by negative pressure. Nitric oxide donors and cell-permeable cGMP analogues activated SDK channels. SDK channels were inhibited by L-methionine. In summary, these data provide the first evidence that TASK channels are major contributors to the background K^+ conductance, and SDK channels may stabilize membrane potential during dynamic changes in cell length and mediate responses to enteric neurotransmitters (Supported by DK41315).

S 5-1

A ROLE OF A NOVEL NEURAL TISSUE-SPECIFIC PROTEIN, NELL2 IN THE REGULATION OF NEUROENDOCRINE BRAIN

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We have recently identified, an EGF-like repeat domain-containing protein, NELL2 as a novel estrogen-dependent, and a neural tissue-specific protein during sexual maturation of neuroendocrine hypothalamus. We cloned 5' flanking region of mouse NELL2 gene, and studied further about estrogen-dependent NELL2 function. NELL2 promoter contains several conserved estrogen binding domains, which was proven to be functionally active by electrophoretic mobility shift assays and promoter analysis. Its transcription was activated in a dose-dependent manner by estrogen in the HiB5 hippocampal neuroprogenitor cells. Further studies have shown that NELL2 plays a role in the estrogen-dependent protection of cell death in an *in vitro* cultured HiB5 cell and in the sexually dimorphic nucleus of preoptic area of male rats. Histochemical studies revealed that NELL2 is expressed in the most subsets of nuclei and gray matters in the developing and adult rat brains. Detailed immunohistochemical and in situ hybridization studies have shown that NELL2 is expressed in glutamatergic neurons in the forebrain regions and in the GABAnergic neurons of cerebellar Purkinje cell layers. However, no detectable signal was found in the GFAP-containing glial cell populations. Studies aimed to determine NELL2 function in the hypothalamic glutamatergic neurons revealed that NELL2 is involved in the cellular mechanisms for the regulation of vesicular exocytosis and glutamate release. Knock down of NELL2 synthesis by an intracerebroventricular administration of an antisense (AS) oligodeoxynucleotide (ODN) induced a significant delay of the first ovulation and a decreased release of LHRH from the median eminence nerve terminals. These physiological changes are likely to be mediated by a NELL2-induced change in glutamate release, because we found that NELL2 is specifically expressed in the glutamatergic neurons but not in the LHRH neurons of the hypothalamus, and activates glutamate release from the in vitro cultured HiB5 cells. Immunoblots and fluorescence resonance transfer (FRET) assays showed that NELL2 physically interacts with some intracellular proteins such as protein kinase C, and synaptophysin and synaptobrevin, components of vSNARE complex that is essential for membrane fusion of secretary vesicles. Exocytosis assays using Synapto-pHluorin showed that NELL2 enhances vesicular exocytosis. Biochemical and molecular analyses revealed that NELL2 contains more than 5 splice variants including a variant with signal sequence deleted. Therefore, a splice variant without a signal sequence may be involved in the intracellular events of neural apparatus via interacting with proteins involved in the glutamate release and other regulatory proteins, while secreted NELL2 may play a different role probably via ligand-receptor interaction.