Regulation of NHE3-Dependent Proton Secretion in Epididymal Principal Cells: pH, cAMP, and Adenosine Signaling

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The epididymis, a crucial male reproductive organ, consists of a highly convoluted, single tubule that connects the testis to the vas deferens. Structurally, it is divided into four distinct regions in rodents: the initial segment, caput, corpus, and cauda, each serving unique anatomical and functional roles. Spermatozoa acquire the ability to fertilize an oocyte as they transit in the epididymis, a process called epididymal maturation. The epididymal lumen is lined by a pseudostratified epithelium that plays a pivotal role in the establishment and maintenance of a unique acidic luminal environment (pH 6.6), which is essential for the maturation and storage of sperm cells. Epididymal principal cells (PC) contribute to luminal acidification via the sodium / hydrogen exchanger type 3 (NHE3). NHE3-dependent proton secretion is regulated via recycling mechanisms that control its localization in sub-apical endosomes and apical stereocilia. PC also secrete ATP, which is rapidly hydrolyzed into adenosine by ectonucleotidases. Adenosine exhibits opposing effects based on the receptors it activates. A1 and A3 receptor activation reduces intracellular cAMP (cAMPi), while A2A and A2B receptor activation increases cAMPi. In other epithelia, cAMPi triggers NHE3 internalization from the apical membrane. We showed high expression of A2B and A3 receptors in the apical membrane of PC by immunofluorescence. We then investigated the roles of pH, cAMP, and adenosine (via A3 and A2B receptors) in the subcellular localization of NHE3 in PC. Adult C57Bl/6Ncrl (aged 10-12 weeks) male mice were used for *in vivo* luminal perfusion of the cauda epididymidis. All experiments were performed in at least 6 mice for each group. Immunofluorescence NHE3 labeling and phase contrast images (to visualize PC stereocilia) were obtained using a Zeiss LSM 900 confocal microscope. The localization of NHE3 in stereocilia was quantified using Fiji (ImageJ) software. When the cauda epididymidis was perfused at an alkaline luminal pH of 7.8, NHE3 was mainly localized in PC stereocilia. In contrast, an acidic luminal pH of 6.0 induced NHE3 internalization. cpt-cAMP, a permeant analog of cAMP, and the A2B receptor agonist BAY-60-6583 prevented NHE3 stereocilia accumulation induced at pH 7.8. Adenosine or the A3 agonist 2-Cl-IB-MECA induced NHE3 stereocilia accumulation at pH 6.0. The A3 antagonist MRS1523 prevented the accumulation of NHE3 in stereocilia induced by adenosine at pH 6.0, while the A2B antagonist PSB1115 had no effect. Our findings suggest that NHE3 subcellular localization in epididymal PC is modulated by luminal pH and adenosine. We propose that activation of A3 by luminal adenosine, leading to a decrease in cAMPi, maintains NHE3 on the cell surface. Conversely, A2B activation by adenosine, followed by an increase in cAMPi, induces NHE3 internalization. This intricate interplay of pH and adenosine highlights some of the regulatory mechanisms

influencing the establishment of an optimal acidic environment for sperm maturation and storage in the epididymis.