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## Sodium Pump and Leaks in Cultured Vascular Endothelial Cells

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expression. The native *Xenopus* oocyte has essentially no endogenous inward rectification, allowing a clear qualitative difference between injected and uninjected oocytes to serve in the functional assay. The injection of total RNA or poly A selected mRNA rapidly prepared from 18-d chick embryonic ventricles produced a characteristic inwardly rectifying current in the oocytes after a 3-d incubation for expression. The current was barium blockable, passed little or no outward current, and reversed near  $E_{K^+}$ , all as expected for the cardiac  $I_{K1}$  current. Size fractionation of the mRNA revealed that the active fraction resides between 2 and 4 kb. cDNA prepared from active mRNA fractions has been produced and directionally cloned to produce a cDNA library suitable for the isolation of the  $I_{K1}$  cDNA sequence through either subtractive sib selection or direct expression of in vitro transcribed message. [Supported by NIH grant PO1-HL-27385.]

103. Presence of an Inward Background Current Sensitive to Sodium in the Isolated Rabbit Sinoatrial Node Cell N. HAGIWARA,\* H. IRISAWA,\* H. KASANUKI,\* and S. HOSODA,\* *The Heart Institute, Tokyo Women's Medical College, Tokyo, Japan* (Sponsor: W. Giles, University of Calgary)

Deactivation of  $K^+$  conductance in the presence of an inward background current has been considered as one of the mechanisms of the pacemaker depolarization in the mammalian sinoatrial node cells. The inward background current has been assumed to exist in the simulational study as the necessary inward current for the spontaneous rhythm. The background inward current, however, has not been investigated experimentally. Using the whole-cell patch clamp mode, we measured the time-independent inward background current sensitive to  $Na^+$  (IbNa) in the pacemaker cell. Patch pipette contained 150 mM Cs aspartate and the external fluid contained 2 mM Ni, 2 mM Ba, 2 mM Cs, and 10  $\mu$ M ouabain to block all possible time-dependent ionic currents as well as  $Na/K$  pump and  $Na/Ca$  exchange current. IbNa was obtained by subtracting the  $I-V$  curves obtained in  $Na$ -free Tris solution from that obtained in 150 mM  $Na$  solution. The background current sensitive to  $Na$  had a slope conductance of  $0.4 \pm 0.2$  nS and a current density of  $0.6 \pm 0.2$  pA/pF in 14 different cells. That IbNa was insensitive to TTX thus excluded the possibility of the noninactivating fast  $Na$  current. IbNa was permeable to Li, Na, K, Cs, and Rb nonselectively, although the conductances vary in the order of  $Li < Na < K < Cs < Rb$ . Since the current is inward at the entire phase of the diastolic depolarization, the contribution of IbNa to the pacemaker potential could play an essential role for the decaying K hypothesis of the pacemaker mechanism.

104. Sodium Pump and Leaks in Cultured Vascular Endothelial Cells NORMA C. ADRAGNA, *Department of Pharmacology & Toxicology, Wright State University School of Medicine, Dayton, Ohio*

To understand the generation and maintenance of Na and K gradients in cultured vascular endothelial cells (CVEC), net active ( $Na/K$  pump) and passive ("leak") Na and K movements were determined under the following conditions: (a) the  $Na/K$  pump as the ouabain-induced (OI) flux in the presence and absence of furosemide; (b) the furosemide-sensitive (FS) flux in the presence and absence of ouabain; and (c) the ouabain- and furosemide-resistant (OFR) flux in the presence of ouabain and furosemide. The main findings were: (a) Replacement of bicarbonate and phosphate buffers in the normal culture medium (CM) by HEPES (balanced salt solution, BSS) decreased OI and OFR fluxes by >50% with no effect on the FS components except for the K flux in the absence of ouabain. In contrast, the K/Na ratio rose by 50% in BSS but only in the absence of furosemide, whereas the  $IC_{50}$  for ouabain increased by more than threefold for Na fluxes. (b) In BSS, Ba (1–10 mM) reduced the K/Na ratio by 50%. (c) In BSS but not in CM, furosemide inhibited the OI K and Na fluxes by 66 and 36%, respectively. (d) Ouabain significantly decreased the total cation content, an effect abolished by addition of furosemide, both in CM and BSS. (e) The behavior of the FS fluxes was complex and dependent on the incubation medium and the presence of ouabain, suggesting a tight coupling between the pump and FS fluxes. In conclusion, these results show that substitution of bicarbonate and phosphate by HEPES produces significant changes in active and passive net Na

and K fluxes in CVEC. In addition, the data are consistent with the hypothesis that ouabain inhibition of the pump induces a furosemide-sensitive net outward K leak, which together with the inhibitory effect of Ba may explain a K/Na ratio larger than 1.0. [Supported by grants from AHA/MV, AHA/Dayton, and WSU.]

105. Anion and Cation Pathways Identified in the Inner Mitochondrial Membrane by Patch Clamping Y. N. ANTONENKO,\* H. TEDESCHI, and K. W. KINNALLY,\* A. N. Belozersky Laboratory, Moscow State University, Moscow, USSR; Department of Biological Sciences, State University of New York, Albany, New York; and Department of Biology, Siena College, Loudonville, New York

Patch clamping of mice liver mitoplast was used to study ion fluxes, and the results correlate well with data obtained by others with suspensions of intact mitochondria. The current was blocked by several inhibitors, showing that it flows through the membrane and not the leak pathway. The membrane conductivity increased by  $80 \pm 25$  pS after pH shifts from 6.8 to 8.3, and the effect was reversible. The transitions varied from sharp single step (in 1 ms or less) to a gradual change. The addition of  $Mg^{2+}$  decreased the current at pH 8.3. *I-V* curves in the presence of a KCl gradient showed that the current was predominantly cationic and shifted to a more anionic current at pH 8.3.  $Mg^{2+}$  or suboptimal concentration of propranolol shifted the current to the more cationic side. Even under the conditions favoring the anion current (pH 8.3 without magnesium ions), the ratio of potassium to chloride permeability was 10. Amiodarone, propranolol, and quinine were found to block the current (in order of potency). Amiodarone ( $7 \mu M$ ) inhibited the swelling of mitochondria in the presence of potassium acetate and succinate, confirming the inhibition of potassium fluxes shown by patch clamping. The results are most simply interpreted by the presence of two channels, one cation selective (maximally  $\sim 100$ – $200$  pS) and the other anion selective ( $\sim 80$  pS). [Supported in part by NSF grant DCB-8818432 and USIA 1A-AEMP-G 193692.]

106. Schwann Cell-to-Axon Protein Transfer in Squid Axons Occurs Independently of Phosphatidylcholine and RNA DONGJIE LIU,\* MICHAEL TYTELL,\* MARK FRIEDMAN,\* and ROBERT M. GOULD, Institute for Basic Research in Developmental Disabilities, Staten Island, New York; Department of Neurobiology & Anatomy, Wake Forest University, Winston-Salem, North Carolina; and Olympus Corp., Columbia, Maryland

Squid giant axons, with attached small fibers, were incubated with radioactive precursor (tritiated leucine, choline, or uridine) in seawater for 45–60 min. After the labeling period, axons were transferred to seawater containing unlabeled precursor (10 mM) and incubated for periods up to 3 h. The giant axons were then embedded and prepared for autoradiographic analysis at the light microscopic level. The distributions of grains (representing protein, choline lipid, or RNA, respectively) were measured among the following compartments for both the giant axon and the smaller axons: axoplasm, Schwann cell layer, connective tissue layer, and extracellular space, using an Olympus image analysis system. Consistent with earlier studies (e.g., Lasek et al. 1977. *J. Cell Biol.* 74:501–523), there was a time-dependent transfer of labeled protein from Schwann cells to the giant axon during the chase. A similar time-dependent transfer was observed from Schwann cells to smaller axons with leucine-labeled protein. However, similar glial cell-to-axon transfers were not observed with choline-labeled lipid or uridine-labeled RNA. These results suggest that proteins are transferred selectively, i.e., without phospholipids or RNA. [Supported by grants NS-13980 (to R.M.G.) and BNS-881178 (to M.T.).]

107. Na-dependent Alanine Transport in Plasma Membrane Vesicles Isolated from Rat Skeletal Muscle PATRICIA A. KING, Department of Medicine, University of Vermont, Burlington, Vermont