Structure/Function Analysis of Na,K-ATPase α1 and α2 Central Isoform-Specific Regions Reveals Their Involvement in Regulation by Protein Kinase C

S. V. PIERRE, M-J. DURAN, D. L. CARR, AND T. A. PRESSLEY

Department of Physiology, Texas Tech University Health Sciences Center, Lubbock, Texas, USA

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INTRODUCTION

The four isoforms of the Na,K-ATPase α-subunit are nearly identical, with the exception of the amino-terminus and a 10-residue region near the center of the molecule, the central isoform-specific region (ISR, K^{489}–L^{499}, FIG. 1). The specific functions served by the various isoforms may originate within these regions of structural divergence. The amino-terminus is clearly a source of isoform functional diversity, but the variability in kinetics and regulation of ion transport properties among species and isoforms suggests that additional regions may be involved.[1,2] We hypothesized that the central ISR is such a region. To examine its role, we constructed chimeric molecules in which the central ISRs of rat α1 and α2 isoforms were exchanged, and we characterized them for two properties known to differ dramatically among the isoforms—their K^+ deocclusion pattern and their response to protein kinase C (PKC) activation.

METHODS

Complementary DNA of rat ouabain-resistant α1, as well as α2 isoform modified to display α1-like ouabain-resistance, were used to obtain chimeras in which the central ISRs were exchanged. After stable transfection into ouabain-sensitive opossum kidney (OK) cells, recipient colonies were selected by exposure to ouabain concentrations sufficient to kill nontransfected cells. The expression and structure of the introduced forms were confirmed by direct detection of the exogenous polypeptides and mRNAs with specific probes (Western blotting and RT-PCR[3]). The rate

Address for correspondence: S.V. Pierre, Department of Physiology, Texas Tech University Health Sciences Center, 3601, 4th Street, Lubbock, TX 79430. Voice: 806-743-4056; fax: 806-743-1512.
Sandrine.Pierre@ttuhsc.edu.

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of K^+ deocclusion was assessed at low ATP concentration (1 μM) in membranes isolated from transfected cells. In this condition, the response of Na^+-dependent ATP hydrolysis to varying concentrations of K^+ is a convenient and sensitive indication of the E2(K)→E1 pathway of the Na^+,K^+-ATPase reaction.[1] Indeed, this part of the reaction becomes rate-limiting at low ATP concentration, and K^+ inhibits Na^+-ATPase activity of the α1 enzyme, whereas α2 is stimulated. Finally, the response to PKC was assessed by measuring Na,K-ATPase-mediated Rb^+ uptake in transfectants treated or not with the PKC activator, phorbol 12-myristate 13-acetate (PMA).

RESULTS/DISCUSSION

Comparisons of the chimeras with rat wild-type α1 and α2 isoforms expressed under the same conditions showed no difference in K^+ deocclusion kinetics. However, substitution of the α2 ISR into α1 doubled the increase in pump-mediated

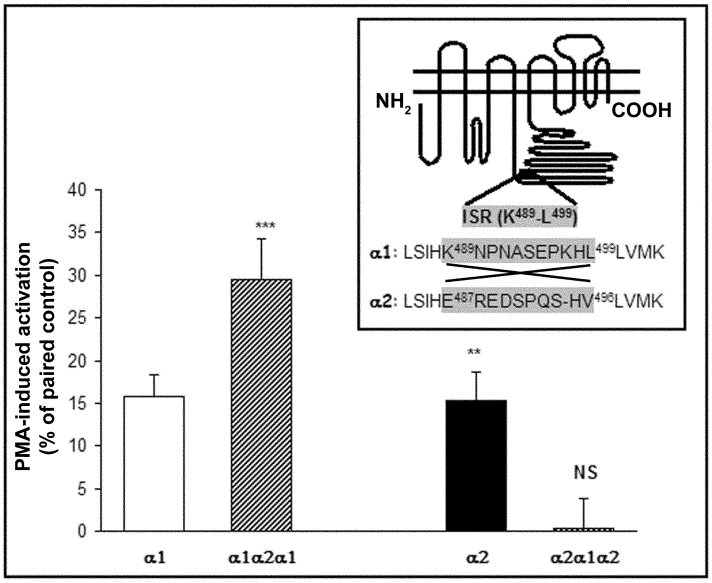

FIGURE 1. Effect of α1/α2 ISR exchange on PMA-dependent activation of cellular Na^+,K^+-ATPase-mediated Rb^+ transport. Na^+,K^+-ATPase-mediated transport was assayed in attached cells by measuring the ouabain-sensitive uptake of the K^+ congener, $^{86}Rb^+$. PKC activation was induced by a 5-min exposure of the cells to 10 μM PMA prior to the addition of Rb^+, and compared to paired control plates of cells exposed for 5 min to the same amount of vehicle alone (DMSO). Values are means ± SEM (n = 6–11) of flux activations induced by PMA exposure, expressed in percent of their paired controls (same transfection group, same passage, same day). Values were compared using two-tailed Student's paired t test—NS: nonsignificant; $^{**}P < 0.01$; $^{***}P < 0.001$. The inset shows the location of the central ISR. The aligned amino acid sequences of the α1 and α2 ISR are shadowed.

transport elicited by exposure to the PKC agonist, PMA. In contrast, substitution of the α1 ISR into α2 eliminated the normal response seen with α2 (FIG. 1). These results suggest that the structure of the ISR may influence the overall response of the Na,K-ATPase to PKC, perhaps by altering the efficiency of recycling between the plasmalemma and intracellular membranes.

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