Role of the Isoform-Specific Region of the Na,K-ATPase Catalytic Subunit

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KEYWORDS: Na,K-ATPase; isoform-specific region (ISR); PKC activation

INTRODUCTION

We have identified a region, the isoform-specific region (ISR), located in the major cytoplasmic loop of the Na,K-ATPase α catalytic subunit that greatly differs between the isoforms (FIG. 1). To evaluate the importance of this region, we constructed chimeras of the rodent α1 isoform in which the ISR was replaced.

METHODS

The α1HKα1 chimera was constructed by exchanging the rat α1 ISR with the corresponding sequence (TLEDPRDPRHL) from the rat gastric H,K-ATPase catalytic subunit. After transfection of the rat wild-type α1 and the α1HKα1 chimera into opossum kidney (OK) cells, selection of transfected cells was achieved using 3 μM ouabain, a concentration sufficient to kill nontransfected cells. All transfections produced viable colonies. Expression of the introduced sequences in OK cells was assessed by RT-PCR (data not shown). Enzymatic function was verified using ouabain-sensitive $^{86}Rb^{+}$ uptake assays. As Na,K-ATPase transport in OK cells is known to be increased by protein kinase C (PKC) stimulation,[1] we checked whether the ISR was involved in this process by treating the cells with phorbol myristate acetate (PMA).

RESULTS/DISCUSSION

Transfection of OK cells with the α1HKα1 chimera produced viable colonies, indicating that the α1 ISR is not essential for the overall enzymatic function. Replacement of the α1 ISR by the H,K-ATPase sequence abolished the PMA-induced increase in Na,K-ATPase transport observed with the wild-type α1 (FIG. 2). These results suggest that, although the ISR is not critical for overall enzymatic

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Ann. N.Y. Acad. Sci. 986: 258–259 (2003).

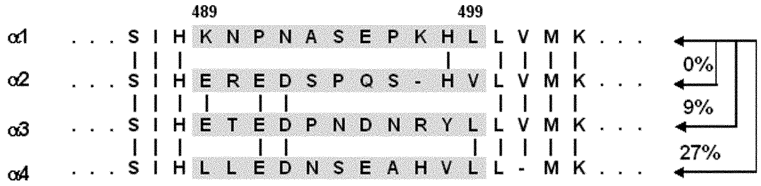

FIGURE 1. Alignment of the rat ISR (shaded) amino acid sequences. The percentage of homology between the α1 ISR and the other α isoform ISRs is also shown.

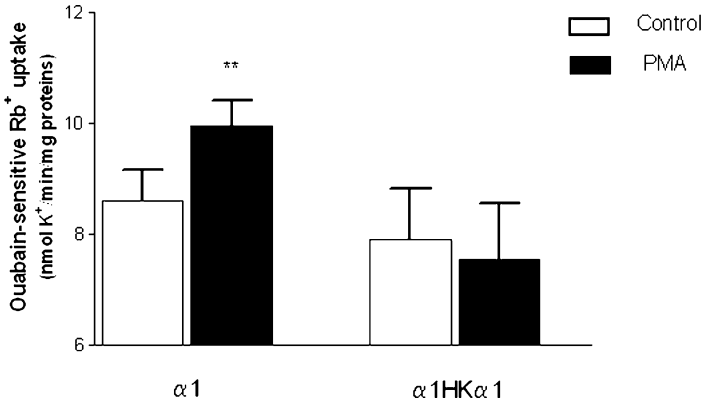

FIGURE 2. Ouabain-sensitive Rb^+ uptakes. $^{86}Rb^+$ uptakes were performed as previously described.[2] $^{**}p < 0.01$ vs. control.

function, the α1 ISR is implicated in PKC activation in OK cells. Our current hypothesis is that there is either a direct or indirect interaction between the phosphorylated serines (Ser 11 and 18) located in the N-terminus and the ISR of the Na,K-ATPase α1 isoform.

ACKNOWLEDGMENTS

Wild-type α1 cDNA was a gift from J. B. Lingrel (University of Cincinnati, Cincinnati, OH). This project was supported in part by a grant from the National Center for Research Resources [No. RR-19799].

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