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Role of Enhanced Na^+ Entry in the Control of Na,K-ATPase Gene Expression by Serum

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Abstract. The role of enhanced Na^+ entry in the induction of Na,K-ATPase subunit mRNAs by serum was investigated in a "nontransformed" rat liver cell line, Clone 9. Exposure of cells to 10% calf serum resulted in a 1.5-fold increase in the rate of Na^+ entry associated with a transient rise in cell Na^+ content (twofold at 15 min) and a sustained 1.15-fold rise in cell K^+ content. After 6 hr of exposure to serum $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ content increased by 1.8- and 2.6-fold, respectively. In nuclear run-on assays, serum stimulated the transcription of the $\alpha 1$ gene ~1.9-fold while the transcription rate of the $\beta 1$ gene remained unchanged. In cells incubated in Na^+-free medium where NaCl was replaced by choline chloride, the induction of $mRNA_{\alpha 1}$ by serum was fully preserved, whereas the increase in $mRNA_{\beta 1}$ was prevented. An unexpected finding was that incubation of cells in Na^+-free medium alone for 6 hr increased $mRNA_{\alpha 1}$ but not $mRNA_{\beta 1}$ content. These results indicate that Na,K-ATPase subunit mRNAs are differentially induced by serum, and that the induction of $mRNA_{\alpha 1}$, in contrast to that of $mRNA_{\beta 1}$, is transcriptionally mediated and does not require the presence of Na^+ in the extracellular medium.

Key words: Na,K-ATPase gene transcription—Cell Na^+ and K^+—Na^+-free medium—Na^+ entry

Introduction

Addition of serum to growth-inhibited quiescent cells results in an early (seconds to minutes) and striking stimulation of passive Na^+ influx that is accompanied by an increase in the rate of active Na,K transport [18, 28]. This early stimulation of active Na,K transport reflects a stimulation of pre-existing Na,K-pump sites in the plasma membrane and, in some instances, a recruitment of Na,K-ATPase to the plasma membrane [1, 5, 21]. In recent experiments on a "nontransformed" rat-liver cell line, Clone 9, we found that addition of fresh serum to contact-inhibited cells results in an increase in Na,K-ATPase $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ content at 3 hr by 2- and 2.7-fold, respectively, and is followed by a stimulation of Na,K-ATPase activity and increases in DNA, RNA, and protein per plate of cells [2]. To gain a better understanding of the regulation of Na,K-ATPase subunit mRNA expression by serum, we examine in the present study the role of transcriptional events in the above induction. This question is of importance because the induction mechanism of the subunit mRNAs by serum is unknown and because of increasing evidence that the expression of the two subunit mRNAs may not be coordinately regulated under certain circumstances [4, 10, 11, 27, 33, 34]. In addition, we investigated whether the early stimulations of Na^+ influx and active Na,K transport are required steps in the induction of Na,K-ATPase subunit mRNAs by serum. Such a possibility is strengthened by the observation that elevations of intracellular Na^+ concentration brought about by other means—including use of Na^+ ionophores, exposure to veratridine, and inhibition of Na,K-pump activity by ouabain or by reduced external K^+ concentration—lead to an induction of Na,K-ATPase mRNAs and Na,K-ATPase enzymatic activity in a wide variety of cell systems [6, 7, 16, 23–26, 33, 34], raising the possibility that the observed induction of the subunit mRNAs may be caused by the early increase in Na^+ influx and intracellular Na^+ concentration by serum. If this were the case, then the induction of the subunit mRNAs by serum should be abolished in cells incubat-

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ed in medium devoid of Na^+. The results indicate that: (i) addition of serum to quiescent contact-inhibited Clone 9 cells results in a rapid rise in the rate of Na^+ entry and in intracellular Na^+ content; (ii) the induction of $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ by serum in cells incubated in Na^+-containing medium is associated with enhanced transcription of Na,K-ATPase $\alpha 1$, but not the $\beta 1$, gene; and (iii) that in cells incubated in Na^+-free medium the induction of $mRNA_{\alpha 1}$ by serum is fully preserved while, in contrast, the increase in $mRNA_{\beta 1}$ content is abolished. A preliminary report of some of the findings has been presented [17].

Materials and Methods

Materials

Calf serum and powdered culture medium were purchased from GIBCO (Grand Island, NY), and plastic culture dishes were obtained from Corning Glass Works (Medfield, MA). Nitrocellulose paper (Optibind) was obtained from Schleicher and Schuell (Keene, NH). Choline chloride, N-methyl glucamine, and standard chemical compounds were purchased from Sigma Chemical (St. Louis, MO). Dialysis membrane (12,000 to 14,000 cut-off) was obtained from Spectrum Medical Industries (Los Angeles, CA). RNAase inhibitor and DNAase were purchased from Promega (Madison, WI). [^{32}P]α-dCTP (3,000 Ci/mmol) and [^{32}P]α-UTP (3,000 Ci/mmol) were obtained from Amersham (Arlington Heights, IL).

Cell Culture

Clone 9 cells, a "nontransformed" rat-liver cell line, were maintained in 100-mm plastic culture plates in Dulbecco's modified Eagle's medium (DME medium) containing 10% calf serum, as described previously [2]. In all experiments, confluent cells were preincubated in DME medium devoid of serum for 24 hr prior to study. In certain experiments (as indicated) cells were rinsed twice with either 8 ml DME medium or Na^+-free medium prior to initiation of experiments.

Na^+-free Medium and Preparation of Na^+-free Serum

Na^+-free medium had the following composition (mM): choline chloride 120, KCl 5.0, $CaCl_2$ 1.0, $MgCl_2$ 2.0, KH_2PO_4 2.0, HEPES 10, glucose 5.0; pH 7.45. Na^+-free serum was prepared by repeated dialysis of calf serum against the above medium. The concentrations of thyroid hormones in serum, measured by radioimmunoassay in a commercial laboratory (MetPath, Teterboro, NJ), were 6.5 T_4 μg/dl and 150 ng T_3/dl (both within the normal range for calf serum), and did not change significantly after dialysis. The concentrations of Na^+ in the "Na^+-free" medium and in the dialyzed serum determined by flame photometry was less than 0.1 mM. Na^+-free media containing higher concentrations of K^+ were prepared by isomolar substitution of KCl for choline chloride.

Cell Na^+ and K^+ Content

Cell Na^+ and K^+ content was measured using flame photometry according to previously described procedures [12].

Isolation and Analysis of Cytoplasmic RNA

Cytoplasmic RNA was isolated by methods utilizing an NP-40-containing lysis buffer [2, 22]. Cell lysates from two 100-mm plates were pooled for each sample, yielding 80–100 μg cytoplasmic RNA. Analysis of $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ content was performed by Northern blotting as previously described [2]. In all instances RNA isolated from both control and experimental cells was analyzed on the same blot. Ethidium bromide staining of ribosomal 28 and 18 S bands on gels and on nitrocellulose paper was monitored throughout to ensure equal RNA loading of the lanes and to control for completeness of RNA transfer [2, 3]. Northern blots were hybridized with ~30 × 106 cpm of full-length rat Na,K-ATPase $cDNA_{\alpha 1}$ or with $cDNA_{\beta 1}$ that were ^{32}P-labeled by nick-translation to ~5 × 108 cpm/μg DNA [29, 30]. Hybridization, washing, autoradiography, and densitometry were performed as previously described [2]. The relative abundances of the subunit mRNAs were calculated on the basis of densitometric readings per μg RNA, and the data were expressed as the ratio of relative abundance in experimental cells over that in control cells.

Measurement of Na,K-ATPase Gene Transcription

Na,K-ATPase $\alpha 1$ and $\beta 1$ subunit transcription was measured by nuclear run-on assays performed according to previously described procedures [4, 9, 10]. Briefly, cells were lysed in a Nonidet P-40-containing buffer, and the nuclei were then washed in a buffer containing Mn^{2+}, frozen, and assayed within a week [4]. Samples containing 100 μg DNA were incubated for 20 min at 30°C in the presence of 5 μM UTP and 200 μCi [α-^{32}P]UTP [4, 10]. After completion of the reaction, 1 unit of DNAase and 1 μg of proteinase K was added per μg DNA. Subsequent steps were as described previously [10]. The isolated RNA was hybridized to nitrocellulose discs containing either 10 μg of immobilized pSPT18 plasmid including full-length rat $\alpha 1$ or $\beta 1$ cDNA or 5 μg of the plasmid alone as control; RNA isolation, hybridization and washing were carried out in accordance with the procedures described by Gick et al. [10]. Following autoradiography, the intensities of the resulting spots were determined by densitometry by means of a laser scanner (Molecular Dynamics Model 300A computing densitometer [Sunnyvale, CA]).

To determine the effect of exposure to serum on RNA polymerase II activity, 100-μl samples of nuclei containing 50 μg of DNA isolated from control cells and cells pre-exposed to serum for 1 hr were preincubated on ice for 5 min with either 2 μg/ml α-amanitin or diluent (H_2O) prior to incubation in the above reaction mixture containing 5 μM UTP and 2–5 μCi of [α-^{32}P]UTP for 10 min at 30°C; all subsequent steps were performed as described previously [10]. RNA polymerase II activity was measured as the α-amanitin-inhibitable component of the incorporated radioactivity [10].

Statistical Analysis

All values are expressed as means ± SEM. In comparisons between two groups, P values were calculated by Student's unpaired two-tailed t-test [32]. In experiments involving comparisons between multiple groups, the probability that differences existed between the means of the groups was determined by the analysis of variance using the least significant difference for multiple comparisons. P values less than 0.05 were considered significant.

Results and Discussion

In previous studies we have shown that the addition of fresh serum to Clone 9 cells, incubated either in un-

changed "conditioned" medium or pre-incubated for 24 hr in culture medium devoid of serum, results in an increase in the abundance of both Na,K-ATPase $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ [2, 3]. Because serum that had been extensively dialyzed to become devoid of Na^+ was to be used in the experiments described in the present study, we first tested the efficacy of the dialyzed Na^+-free serum in increasing subunit mRNA content. Similar to our previous results using nondialyzed calf serum [2, 3], it was found that a 4-hr exposure of cells to 10% Na^+-free serum resulted in 1.9- and 2.5-fold increments in $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$, respectively, indicating that the dialyzed Na^+-free serum was fully active in increasing subunit mRNA content (*data not shown*). In previous studies we have shown that these cells express only the $\alpha 1$ and $\beta 1$ isoforms of Na,K-ATPase under control and stimulated conditions [2–4, 29]. It should be noted that serum contains a number of hormones and growth factors and that the induction of Na,K-ATPase subunit mRNAs cannot be attributed to a single agent present in serum. Indeed, we have previously noted that Na,K-ATPase subunit mRNA content in Clone 9 cells is augmented by epidermal growth factor, platelet-derived growth factor, basic fibroblast growth factor, insulin, dexamethasone, and T_3, with the fractional increment in $mRNA_{\beta 1}$ exceeding that of $mRNA_{\alpha 1}$ [3].

We next determined the role of transcriptional regulation of the $\alpha 1$ and $\beta 1$ gene in the serum-induced increase in Na,K-ATPase subunit mRNA expression. Exposure of confluent cells preincubated for 24 hr in serum-free medium to 10% serum for 45 min resulted in a stimulation of $\alpha 1$-gene transcription, while transcription of the $\beta 1$ gene was only minimally increased (Fig. 1). In time-course studies it was shown that transcription of the $\alpha 1$ gene was stimulated ~1.9-fold after 45 min of exposure to serum and remained elevated at 3 hr (Fig. 2). In contrast to the $\alpha 1$-gene transcription rate, the rate of $\beta 1$-gene transcription remained at the control level during the 3-hr interval despite the larger serum-induced increment in $mRNA_{\beta 1}$ content, implying that the molecular mechanisms involved in the induction of the two subunit mRNAs by serum are distinct. The lack of stimulation of $\beta 1$-gene transcription in nuclei isolated from serum-treated cells that exhibit augmented $\alpha 1$-gene transcription serves as an important negative control and suggests that the observed enhancement of $\alpha 1$-gene transcription does not merely reflect a presumed generalized stimulation of mRNA synthesis in serum-treated cells. In keeping with this premise is the finding that α-amanitin-inhibitable transcription (reflecting RNA polymerase II activity [10]) was augmented by only 10% in nuclei isolated from cells exposed to serum for 1 hr (*data not shown*).

The differential regulation of transcription could reflect differences in the *cis*-acting "response-elements" present in the promoter regions of the two subunit genes.

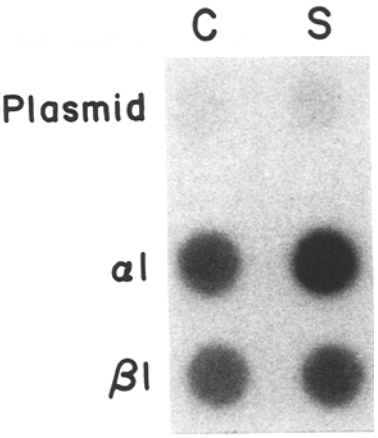

Fig. 1. Effect of serum on the transcription of Na,K-ATPase $\alpha 1$ and $\beta 1$ genes in Clone 9 cells. Confluent cells preincubated in DME medium devoid of serum were exposed to 10% serum (*S*) or diluent (*C*) for 45 min prior to isolation of nuclei for analysis by nuclear run-on assay (*see* Materials and Methods for further details). The input of radiolabeled RNA products for hybridization in control and serum-treated cells was 1.8×10^6 and 2.5×10^6 cpm, respectively. Hybridization to the plasmid (pSPT18) served as the blank.

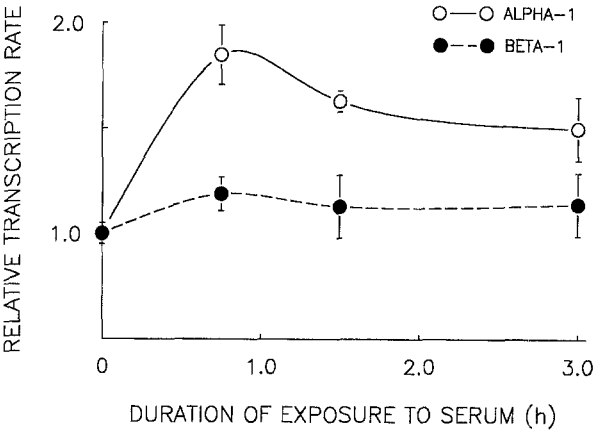

Fig. 2. Time course of effect of serum on transcription of $\alpha 1$ and $\beta 1$ genes. The experimental protocol was the same as that in Fig. 1 and nuclei were isolated at various intervals after the addition of 10% serum. In each experiment (performed in triplicate at each time point and for each condition), the intensities of the spots containing Na,K-ATPase $\alpha 1$ and $\beta 1$ cDNA were corrected for the blank (pSPT18), and the resulting value for nuclei isolated from serum-treated cells was normalized against the average value observed for diluent-treated control cells (means ± SEM).

For example, DNA sequences upstream to human and rat Na,K-ATPase $\alpha 1$ genes contain several AP-1 (and AP-1-like) sites that potentially could mediate the enhanced transcription of these genes by serum [8, 31, 36]; in contrast, such sites are not present upstream to the human or rat $\beta 1$ gene [19, 20]. These results additionally suggest that the induction of $mRNA_{\beta 1}$ by serum is mediated by post-transcriptional mechanisms [13]. We

have previously shown that in Clone 9 cells under basal conditions $mRNA_{\beta 1}$ present in the cytoplasmic RNA pool exhibits a half-life of ~10 hr [2]. This observation implies that decreased degradation of $mRNA_{\beta 1}$ in the cytoplasmic RNA pool cannot quantitatively account for the greater than 2.5-fold increase in $mRNA_{\beta 1}$ content observed after 4 hr of exposure to serum. (For example, complete stabilization of cytoplasmic $mRNA_{\beta 1}$ in the absence of any change in its rate of synthesis would lead to only a 1.3-fold increase in $mRNA_{\beta 1}$ content at 4 hr [13].) The above analysis in conjunction with the absence of β1-gene transcription leads to the postulate that much of the serum-induced increase in cytoplasmic $mRNA_{\beta 1}$ content is mediated by stabilization of β1 primary RNA transcripts in the nucleus. Further studies are required to test this possibility directly.

To determine whether the observed increase in Na,K-ATPase subunit mRNA content in serum-treated cells requires an earlier stimulation of Na^+ entry and a rise in cell Na^+ content, we first tested the ability of the dialyzed serum to elicit the described ionic changes in Clone 9 cells. Cells incubated in DME medium were exposed to 10^{-3} M ouabain in the presence or absence of 10% dialyzed serum. It was found that when active Na^+ efflux was inhibited by ouabain the rate of rise in cell Na^+ was ~1.5-fold greater in cells treated with serum (Fig. 3), indicating that dialyzed Na^+-free serum increases the rate of Na^+ entry in Clone 9 cells. We additionally determined the effect of exposure to serum on intracellular Na^+ and K^+ content of cells in the absence of ouabain. Upon addition of serum, cell Na^+ content became significantly elevated (~2-fold) after 15 min and returned to near-control levels at 30 and 60 min of exposure to serum (Fig. 4). Cell K^+ content increased at 15 min and remained significantly elevated (1.15-fold control levels) throughout the 60-min exposure. Expression of cell Na^+ as the ratio $[Na^+/(Na^+ + K^+)]$, a value that is proportional to intracellular Na^+ concentration under conditions of unchanging osmolality of the external medium [12, 15], also revealed that the ratio increased from the control value of 0.046 ± 0.03 to 0.071 ± 0.04 after 15 min of exposure to serum ($P < 0.05$). Taken together, and in keeping with previous reports in other cell systems [18, 28], the above findings indicate that addition of serum results in a stimulation of the rate of Na^+ entry in Clone 9 cells that is associated with a significant and transient increase in Na^+ content, raising the possibility that the observed changes in intracellular Na^+ and K^+ concentrations are causally related to the induction of Na,K-ATPase subunit mRNAs.

We next tested the hypothesis that serum-induced increases in Na^+ entry and cell Na^+ content are *necessary* for the enhanced expression of the subunit mRNAs by comparing the efficacy of serum in increasing $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ content of cells incubated in

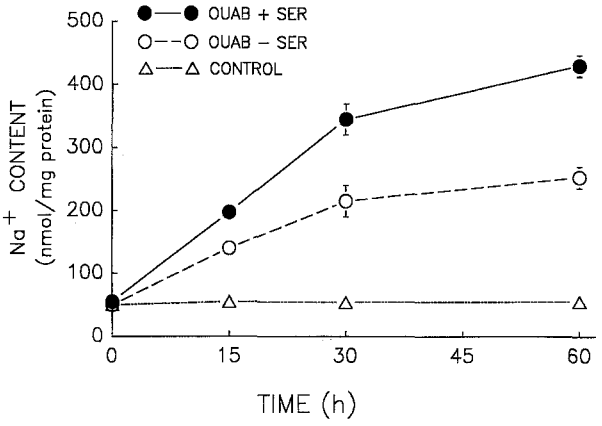

Fig. 3. Effect of serum on the rate of Na^+ entry. Confluent cells pre-incubated for 24 hr in DME medium devoid of serum were treated with 10-3 M ouabain and either with 10% dialyzed calf serum (*OUAB + SER*) or without added serum (*OUAB − SER*). At various intervals, cell Na^+ content was determined in triplicate sets of plates. An additional set of plates received DME diluent alone (*CONTROL*). The experiment was repeated two times and the results averaged (means ± SEM).

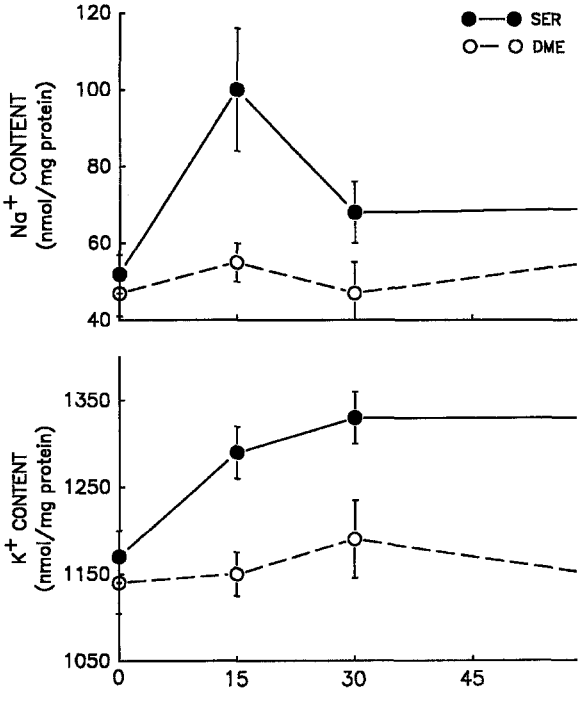

Fig. 4. Effect of serum on cell Na^+ and K^+ content. Confluent cells pre-incubated for 24 hr in DME medium devoid of serum were treated with 10% dialyzed calf serum, and at various intervals thereafter cell Na^+ and K^+ content was determined in triplicate sets of plates. The experiment was repeated three times and the results averaged (means ± SEM). (*Top panel*) Na^+ content. (*Bottom panel*), K^+ content.

Na^+-containing *vs.* Na^+-deficient culture medium. (In preliminary experiments it was shown that upon incubation in Na^+-deficient medium, cell Na^+ content decreases rapidly and reaches undetectable levels within 10 min.) Figure 5 shows the effect of dialyzed Na^+-free serum on $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ content of cells incubated in Na^+-containing and Na^+-deficient medium for 6 hr; averaged results of four such experiments are shown in Fig. 6. The content of both subunit mRNAs increased upon the addition of serum in cells incubated in Na^+-containing medium with the increments in $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ averaging 1.8- and 2.6-fold, respectively. In cells incubated in Na^+-deficient medium, $mRNA_{\alpha 1}$ content increased to levels comparable to those observed using Na^+-containing medium. In marked contrast, however, $mRNA_{\beta 1}$ content increased only minimally, if at all, in cells incubated in Na^+-deficient medium. These results strongly suggest that the regulation of $mRNA_{\beta 1}$ expression by serum differs significantly from that of $mRNA_{\alpha 1}$ and requires the presence of Na^+ in the external medium.

An unexpected result of the above experiments was the additional finding that $mRNA_{\alpha 1}$ content—in contrast to that of $mRNA_{\beta 1}$—is augmented by incubation of cells in Na^+-free medium in the absence of serum (Fig. 5). Indeed, as noted in the legend to Fig. 6, $mRNA_{\alpha 1}$ content of control cells incubated in Na^+-free medium for 6 hr averaged 2.0-times that of control cells incubated in DME medium. The possibility that the increase in $mRNA_{\alpha 1}$ content merely represents a nonspecific response to choline ions present in the Na^+-free medium was examined by incubation of cells in an alternative Na^+-free medium containing N-methyl glucamine in place of choline. $mRNA_{\alpha 1}$ content increased to comparable levels in cells incubated in either of the Na^+-free media (as compared to the $mRNA_{\alpha 1}$ content of cells incubated in DME medium), whereas $mRNA_{\beta 1}$ content remained unchanged (*data not shown*). Although the reasons for the increase in $mRNA_{\alpha 1}$ content under these conditions are not understood, we tested the possibility that the decrement in K^+ content of cells incubated in Na^+-free medium (a 60% decrease during the 6-hr incubation) might have led to the induction of $mRNA_{\alpha 1}$. Cells were accordingly incubated in Na^+-free choline chloride medium containing high concentrations of K^+ to prevent cell K^+ loss, and the effect of such incubations on cell K^+ and subunit mRNA content was determined (Fig. 7). Incubation in Na^+-free medium containing 50 mM K^+ instead of 5 mM K^+ decreased cell K^+ loss to 25% at 6 hr, and cell K^+ content was fully preserved upon incubation in Na^+-free medium containing 100 mM K^+. $mRNA_{\alpha 1}$ content, however, increased in stepwise fashion with increasing concentrations of external K^+ and reached more than four times the control value in cells incubated in the Na^+-free medium containing 100 mM K^+; in contrast, $mRNA_{\beta 1}$

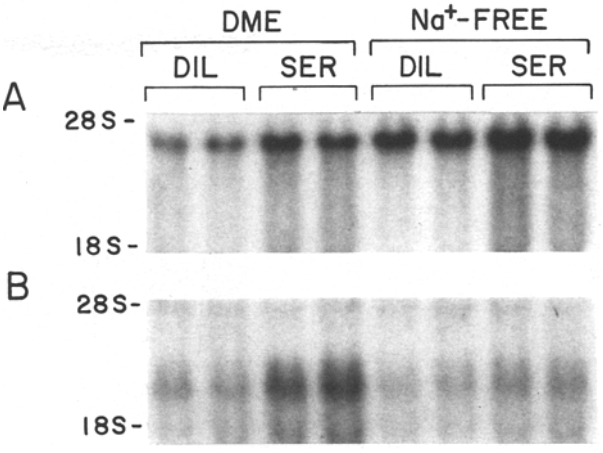

Fig. 5. Effect of serum on Na,K-ATPase $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ in Clone 9 cells incubated in Na^+-containing (DME) and Na^+-free medium. Confluent cells were preincubated for 24 hr in 8 ml of serum-free DME medium. Plates were rinsed twice with either DME or Na^+-free medium and the medium was then replaced with 8 ml of the corresponding medium. Na^+-free serum at a final concentration of 10% was added and incubation continued for 6 hr. Cytoplasmic RNA from two plates of cells for each condition was isolated and pooled; duplicate samples were prepared for each condition. RNA (10 and 40 μg) was loaded per lane in blots probed with $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$, respectively. (*A*), $mRNA_{\alpha 1}$ (*B*) $mRNA_{\beta 1}$. The positions of 28 and 18 S ribosomal RNA bands are shown.

content did not change significantly upon incubation of cells in either of the media (Fig. 7). These findings argue against the suggestion that a decrement in cell K^+ serves as a signal for $mRNA_{\alpha 1}$ induction and raise the possibility that depolarization of the plasma membrane in the presence of medium containing elevated concentrations of K^+ might account for the observed result. Depolarization of the plasma membrane has been shown to result in elevation of intracellular free calcium concentration [14, 35]. If a similar phenomenon occurs in Clone 9 cells, then the presumed rise in cell calcium could potentially lead to the observed increase in $mRNA_{\alpha 1}$ content of cells incubated in Na^+-free medium. This inference is supported by the recent observation that in rat renal tubular cells elevation of intracellular calcium concentration enhances the transcription of Na,K-ATPase α1, but not the β1, gene [27].

The results reported here support the conclusion that the serum-induced increment in Na,K-ATPase $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ content is mediated at transcriptional and post-transcriptional levels, respectively. The results further indicate that while an early action of serum is to stimulate Na^+ influx (thereby increasing cell Na^+ content and stimulating active Na,K transport), and while elevations in cell Na^+ concentration can lead to an induction of Na,K-ATPase and its subunit mRNAs, the increase in cell Na^+ concentration is not *necessary* for the induction of $mRNA_{\alpha 1}$ by serum. In

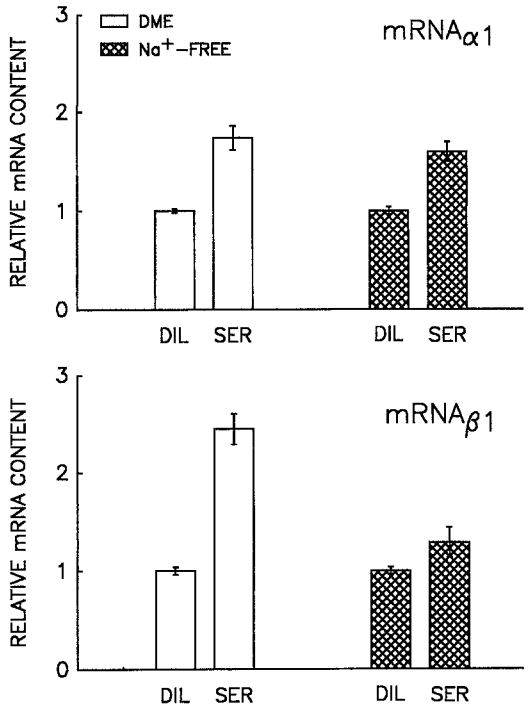

Fig. 6. Quantitation of the effect of serum on the relative abundances of $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ in Clone 9 cells incubated in DME and Na^+-free medium. Cells were treated as described in the legend to Fig. 5. The experiment was repeated four times and the relative content of each subunit mRNA in cells treated with serum was normalized against its respective content in DME medium-incubated control cells. The data are presented as means ± SEM. *DIL* and *SER* denote diluent- and serum-treated cells, respectively. (*Upper panel*) $mRNA_{\alpha 1}$ content. (*Lower panel*) $mRNA_{\beta 1}$ content. The serum-induced increment in the $mRNA_{\alpha 1}$ content of cells incubated in Na^+-free medium was not significantly different from that of cells incubated in DME medium. In contrast, the $mRNA_{\beta 1}$ content of cells incubated in Na^+-free medium was not significantly affected by the addition of serum. $mRNA_{\alpha 1}$ content increased 2.0 ± 0.2-fold in cells incubated in Na^+-free medium alone, whereas $mRNA_{\beta 1}$ content remained unchanged.

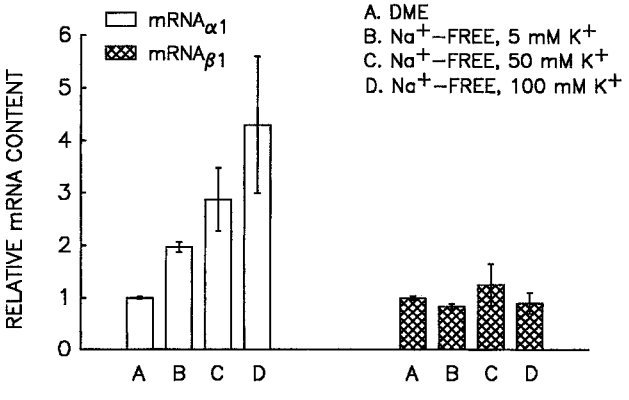

Fig. 7. Effect on $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ content of incubation of Clone 9 cells in Na^+-free medium containing varying concentrations of K^+. Cells were treated as described in the legend to Fig. 5. Na^+-free medium containing increasing concentrations of K^+ was prepared by isosmolar substitution of KCl for choline chloride. The data are presented as means ± SEM; $n = 4$.

contrast to $mRNA_{\alpha 1}$, the induction of $mRNA_{\beta 1}$ by serum requires the presence of external Na^+, suggesting a divergence of the regulatory steps mediating the response of the two subunit mRNAs to serum.

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