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Na,K-ATPase in Several Tissues of the Rat: Tissue-Specific Expression of Subunit mRNAs and Enzyme Activity

Gregory G. Gick†, Mary Ann Hatala‡, David Chon‡, and Faramarz Ismail-Beigi‡

†Department of Biochemistry, State University of New York Health Science Center at Brooklyn, Brooklyn, New York 11203, and ‡Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Summary. The relative contents of Na,K-ATPase subunit mRNAs in rat renal cortex, ventricular myocardium, skeletal muscle (hind limb), liver and brain (cerebrum) were measured. Expressed per unit DNA, $mRNA_{\alpha 1}$ content was ~2-fold greater in the kidney and brain as compared to either heart, skeletal muscle or liver. The hierarchy of $mRNA_{\alpha 2}$ expression was brain > skeletal muscle > heart, whereas $mRNA_{\alpha 3}$ was restricted to brain. Beta1 subunit mRNA content in both kidney and brain exceeded the abundance of liver $mRNA_{\beta 1}$ by ~7-fold. In all tissues examined, the combined abundances of the alpha subunit mRNAs exceeded the content of $mRNA_{\beta 1}$. The hierarchy of Na,K-ATPase activity expressed per unit DNA was brain > kidney > skeletal muscle = heart > liver. The sum of $mRNA_{\alpha}$ as well as $mRNA_{\beta 1}$ content, expressed per g of tissue, was highest in brain and kidney. A statistically significant correlation between $mRNA_{\beta 1}$ content and Na,K-ATPase activity was evident.

Key Words sodium-potassium pump · messenger ribonucleic acid abundance · Northern blot hybridization analysis

Introduction

Na,K-ATPase (Na,K-activated ATPase, E.C. 3.6.1.3; Na,K-pump) is a ubiquitous plasma membrane-bound enzyme complex that plays a fundamental role in cellular function. The pump extrudes Na^+ from within the cell and accumulates K^+ from outside at the expense of ATP, thereby maintaining transmembrane gradients of Na^+ and K^+ and resting membrane potentials of virtually all animal cells [9]. Na,K-ATPase activity is expressed in a tissue-dependent manner and is regulated by a variety of factors [4]. Modulation of pump activity has been implicated in cell differentiation and growth and in the pathophysiology of some forms of hypertension [11, 14, 22]. This vital "housekeeping" enzyme consists of two noncovalently linked, dissimilar subunits, alpha and beta, present in equimolar amounts. The larger alpha subunit is responsible for catalysis, and tissue-specific expression of alpha subunit isoforms has been suggested to be the molecular basis of inter- and intra-tissue differences in Na,K-ATPase sensitivity to cardiac glycosides [10, 21]. The precise role of the beta subunit in pump function is unknown; however, it has been proposed that the beta subunit mediates the correct insertion of the alpha subunit in the plasma membrane [2, 8, 16]. Recently a beta2 isoform of the enzyme has been described [7, 13].

To initiate an investigation of the determinants of Na,K-ATPase expression, we first defined the wash stringency conditions for quantitative analysis of the relative abundance of subunit mRNAs in Northern blots. Using the above assay conditions, we measured the relative content of Na,K-ATPase subunit mRNAs in five tissues of the rat and compared the mRNA content values with those of Na,K-ATPase activity in the same tissues. Such a direct comparative analysis has not been previously reported and is a necessary first step in the understanding of the molecular mechanisms underlying tissue-specific regulation of Na,K-ATPase expression.

Materials and Methods

Isolation of RNA

Total tissue RNA was isolated from 200-g male Sprague-Dawley rats following homogenization of tissue in guanidine thiocyanate and centrifugation through cesium chloride as previously described [5]. RNA concentrations were quantitated by measurement of absorbance at 260 nm.

Quantitation of the Relative Abundance of Na,K-ATPase Subunit mRNA Contents

The abundances of Na,K-pump subunit mRNAs were analyzed by Northern blotting [5]. Briefly, total tissue RNA was electrophoresed in 1% agarose gels containing 6% formaldehyde and

transferred to nitrocellulose paper (Schleicher & Schuell, Keene, NH). Blots were prehybridized for 16–20 hr and hybridized with full-length Na,K-pump alpha1, alpha2, alpha3 and beta1 subunit cDNA-containing plasmids [17, 24] labeled by nick-translation to near-equivalent specific activity of 5×10^8 cpm/μg DNA with thymidine 5′-[α-^{32}P] triphosphate (Amersham, Arlington Heights, IL). Following hybridization at 42°C for three days, blots were washed four times for 15 min each in 0.1 × SSC/0.1% sodium dodecyl sulfate (SDS) at indicated temperatures (1 × SSC contains 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). In experiments designed to determine the temperature at which the hybridization signal is reduced by 50% ($T_{1/2}$), blots were washed at 5°C increments between 30 to 70°C. Experiments were performed twice on RNA samples from each tissue and the results were averaged. Northern blots utilized to quantitate the relative abundance of subunit mRNAs were washed at 35°C. Various durations of film exposure were used to ensure linearity in the densitometric intensities of the resultant autoradiographs. The intensities of multiple alpha2 and beta1 subunit mRNA bands were summed [6]. To allow a quantitative comparison between hybridization data obtained with each subunit cDNA, the densitometric values were corrected for the amount of RNA analyzed and the relative proportion of thymine residues in each cDNA plasmid. Tissue protein, RNA and DNA contents were determined to enable subunit mRNA abundances and Na,K-ATPase activity to be expressed on a unit DNA basis [5] since in quiescent diploid cells DNA content is an index of the number of nuclei.

Preparation of Rat Tissue Homogenates and Na,K-ATPase Assays

Kidney cortex, heart (ventricle), skeletal muscle (hind-limb), liver and brain (cerebrum) were removed from four adult male rats, weighed and immediately frozen on dry ice. Heart and skeletal muscle tissue were minced prior to freezing. Kidney, liver and brain were homogenized in ice-cold buffer (6 ml/g wet weight) composed of 250 mM sucrose, 50 mM Tris and 1.0 mM ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) at pH 7.6 with six strokes of a motor-driven Elvehjem-Potter Teflon-glass homogenizer. Deoxycholate (0.4 mg/ml, final) was then added and homogenates were subjected to three additional strokes. Heart and skeletal muscle were initially homogenized in the above buffer (6 ml/g and 24 ml/g, respectively) in a Polytron homogenizer at maximal speed for two 20-second intervals. Deoxycholate (0.4 mg/ml, final) was next added and the tissues were subjected to 10 additional seconds of Polytron homogenization. Heart and skeletal muscle tissues were then further homogenized with six strokes of the Elvehjem-Potter homogenizer. The homogenates were diluted at 1 : 75 for kidney, 1 : 15 for heart, 1 : 2 for skeletal muscle, 1 : 8 for liver and 1 : 100 for brain in the above homogenizing buffer containing 0.4 mg/ml deoxycholate. Samples were frozen at −20°C and assayed for enzyme activity within one week. Ouabain-inhibitable ATPase activity (Na,K-ATPase) was measured according to previously described methods using triplicate 100-μl aliquots of each diluted sample in the presence and absence of 2×10^{-3} M ouabain in the assay; assay of skeletal muscle enzyme was performed in quadruplicate [3]. Greater than 50% of the ATPase activity was inhibited by 2×10^{-3} M ouabain in all of the tissue homogenates except for skeletal muscle homogenates in which the percentages averaged 26%.

Statistical Analysis

Values are expressed as means ± SE. Linear regression analysis was performed by the least-squares method. The significance of correlation coefficients was analyzed and P values < 0.05 were considered significant [1].

Results

In the present investigation of the tissue distribution of Na,K-ATPase subunit mRNAs, total RNA was isolated from adult male rat cerebrum, ventricular myocardium, kidney cortex, liver and skeletal muscle. Northern blots were hybridized with full-length Na,K-pump subunit-specific cDNAs and washed in 0.1 × SSC/0.1% SDS at 50°C (Fig. 1). These hybridization and wash conditions appear to yield specific detection of subunit mRNAs with no significant degree of cross-hybridization between the three highly homologous alpha isoform mRNAs [17]. For example, while two $mRNA_{\alpha 2}$ bands are clearly visible in 4 μg of skeletal muscle RNA, the $mRNA_{\alpha 2}$ bands were not detectable in 30 μg of the same RNA hybridized with $cDNA_{\alpha 1}$. Likewise 30 μg of kidney RNA failed to hybridize to $cDNA_{\alpha 2}$, whereas $mRNA_{\alpha 1}$ is clearly visible in 2 μg of this RNA. Finally, no $mRNA_{\alpha 3}$ band is evident in RNA samples isolated from heart, kidney, liver and skeletal muscle, whereas a $mRNA_{\alpha 3}$ band is easily detected in 4 μg of brain RNA, consistent with the absence of cross-hybridization under the conditions employed. Alpha1 and beta1 subunit mRNAs were detected in all tissues, while alpha2 and alpha3 mRNAs were expressed in a highly tissue-specific manner. The multiple beta1 mRNA bands presumably reflect various mRNA species with different 5′ and 3′ untranslated regions [24].

The effect of temperature of the wash solution on the specificity and intensity of the hybridization between Na,K-pump subunit mRNAs and ^{32}P-labeled cDNAs was investigated by washing replicate Northern blots in 0.1 × SSC/0.1% SDS at 5°C increments between 30 and 70°C. The result of a representative experiment utilizing brain total RNA is depicted in Fig. 2. For each subunit cDNA, wash temperature below 55°C yielded a nearly-constant intensity of autoradiographic signal with no apparent loss in specificity of hybridization. The temperature at which the hybridization signal is reduced by 50% ($T_{1/2}$) was derived from the dependence of the intensity of signal on the temperature of the wash solution. In Northern blot analysis of total RNA isolated from kidney, heart, skeletal muscle, liver and brain, the apparent $T_{1/2}$ for alpha1 and beta1 in two independent experiments averaged 56°C and 58°C, and

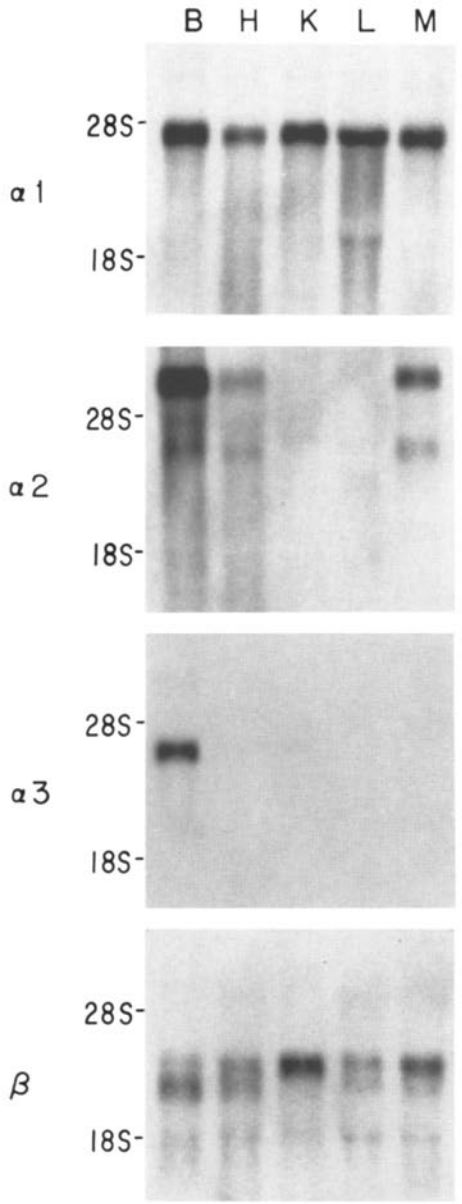

Fig. 1. Tissue distribution of Na,K-ATPase subunit mRNAs. Northern blot analysis of total RNA isolated from brain (*B*), heart (*H*), kidney (*K*), liver (*L*) and skeletal muscle (*M*). This figure depicts the results of four separate Northern blots containing different amounts of total tissue RNA. The amounts of RNA (μg) are as follows: alpha1- (*B*) 4, (*H*) 10, (*K*) 2, (*L*) 40, (*M*) 30; alpha-2- (*B*) 4, (*H*) 20, (*K*) 30, (*L*) 60, (*M*) 4; alpha3- (*B*) 4, (*H*) 20, (*K*) 30, (*L*) 60, (*M*) 4; beta1- (*B*) 2, (*H*) 20, (*K*) 4, (*L*) 60, (*M*) 30. Blots were hybridized to the respective cDNAs and washed as described in Materials and Methods.

ranged from 50 to 62°C and 53 to 63°C, respectively. The reason for the slight differences in apparent $T_{1/2}$ values for a given mRNA in RNA samples derived from different tissues is not known. The apparent $T_{1/2}$ for $cDNA_{\alpha 2}$ in heart, skeletal muscle and brain total RNA averaged 62°C, and the apparent $T_{1/2}$ for brain RNA hybridized with $cDNA_{\alpha 3}$ was 61°C. Irrespective of the wash temperature, however, $mRNA_{\alpha 2}$ was not detected in liver and kidney total RNA, and $mRNA_{\alpha 3}$ expression was limited to the brain.

Results of the above experiments indicate that washing of blots at 35°C in 0.1 × SSC/0.1% SDS yields near-maximal intensity with no significant cross-hybridization. The above conditions were used in the analysis of the relative abundance of Na,K-pump subunit mRNAs, expressed per unit RNA, in the above rat tissues (Table 1). In this analysis the relative abundance of liver $mRNA_{\beta 1}$ was lowest and was set to 1.0. Beta1 subunit mRNA was less abundant than the total of alpha isoform mRNA contents in all tissues examined. The magnitude of this disparity in subunit mRNA abundances displayed a tissue-specific pattern. For example, in rat brain the sum of alpha1, alpha2 and alpha3 mRNAs was 9-fold greater than the content of $mRNA_{\beta 1}$ while $mRNA_{\alpha 1}$ content in kidney cortex was 1.5-fold higher than that of $mRNA_{\beta 1}$. Quantitative differences were also found in the expression of alpha isoform mRNAs within a single tissue. Alpha3 mRNA expression was limited to brain and represented the predominate alpha isoform mRNA in this tissue (The small amounts of alpha3 mRNA that are present in the conduction system of adult rat heart [25] are below the level of detection by Northern blotting [Fig. 1; 23]). In both brain and skeletal muscle, $mRNA_{\alpha 2}$ content was ~2-fold greater than $mRNA_{\alpha 1}$, whereas $mRNA_{\alpha 1}$ was 1.7-fold more abundant than $mRNA_{\alpha 2}$ in ventricular myocardium.

To examine whether the expression of Na,K-ATPase subunit genes are regulated in a tissue-specific manner, it is essential to quantitatively account for any differences in the total RNA content per unit cell. Indeed, the ratio of RNA to DNA showed a significant variation in the tissues examined (Table 2). Hence, the RNA to DNA ratios were utilized to express the relative contents of subunit mRNAs on a per DNA basis, and the abundance values were normalized against $mRNA_{\beta 1}$ in liver (Table 3). Alpha1 mRNA abundance in kidney and brain was ~2-fold greater than $mRNA_{\alpha 1}$ content in either heart, skeletal muscle or liver. The relative content of $mRNA_{\alpha 2}$ in cerebrum was 6- and 1.6-fold higher than in heart and skeletal muscle, respectively. The tissue hierarchy of $mRNA_{\beta 1}$ abundance was kidney = brain > skeletal muscle > heart > liver with a maximum ~7-fold difference in relative content.

To investigate the relationship between subunit mRNA contents and Na,K-ATPase activity, homogenates were prepared from rat kidney cortex, heart, skeletal muscle, liver and brain and total Na,K-

ATPase activity, measured as ATPase activity inhibited in the presence of 2×10^{-3} M ouabain, was assayed (Table 4). When Na,K-ATPase activity is expressed either per g of tissue or per mg of protein, the rank order is kidney > brain > heart > liver > skeletal muscle, whereas the hierarchy is brain > kidney > heart = skeletal muscle > liver when Na,K-ATPase activity is expressed per mg DNA.

Linear regression analysis was performed to examine whether either the sum of alpha isoform mRNA contents or $mRNA_{\beta 1}$ abundance correlates with tissue Na,K-ATPase activity (Figs. 3 and 4). Expressed respectively per g wet weight and per mg DNA, Na,K-ATPase activity varied over a 21- and 7-fold range, relative total $mRNA_{\alpha}$ abundance varied over a 12- and 13-fold range, and relative $mRNA_{\beta 1}$ content varied over a 26- and 7-fold range. There was no statistically significant correlation between Na,K-ATPase activity and the sum of $mRNA_{\alpha}$ isoform abundances, expressed either per g of tissue or per mg DNA (Fig. 3). However, the sum of $mRNA_{\alpha}$ abundances expressed per g of tissue was highest in kidney cortex and brain, two tissues with the highest enzyme activity per g of tissue. Na,K-ATPase activity correlated with relative $mRNA_{\beta 1}$ content expressed per g of tissue ($P < 0.01$) (Fig. 4), but did not reach statistical significance when expressed per unit DNA ($0.10 > P > 0.05$). Similar to the sum of $mRNA_{\alpha}$ isoform abundances, the abundance of $mRNA_{\beta 1}$ was highest in kidney cortex and brain. It should be noted that although significant quantities of both $mRNA_{\beta 2}$ and beta2 peptide are expressed in brain [13, 18], addition of $mRNA_{\beta 2}$ abundance to the value of brain in Fig. 4 would have moved this point further to the right. Thus, it is probable that such an addition would not have significantly altered the overall findings.

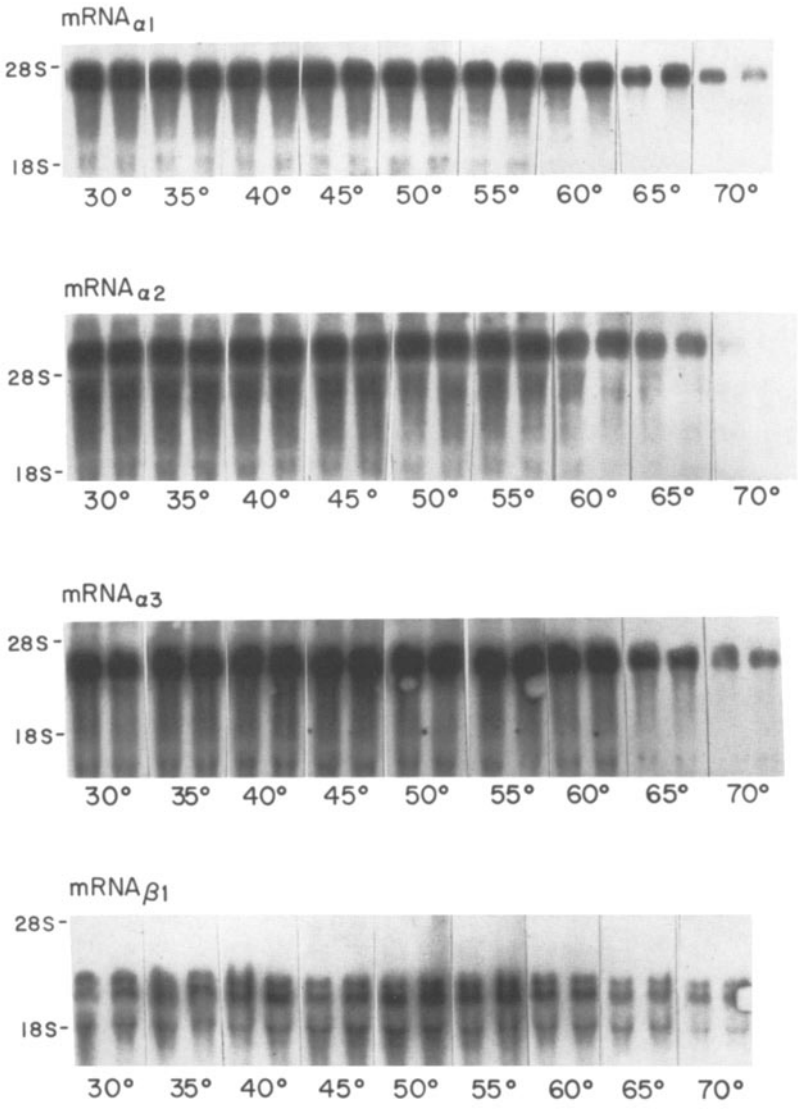

Fig. 2. Effect of temperature on Northern blot analysis of brain Na,K-ATPase subunit mRNAs. Replicate Northern blots containing total brain RNA were prepared and the resulting nitrocellulose filters were cut into strips of two lanes each before hybridization in one bag with each subunit cDNA labeled by nick-translation. The nitrocellulose strips hybridized with $cDNA_{\alpha 1}$, $cDNA_{\alpha 2}$, $cDNA_{\alpha 3}$ and $cDNA_{\beta 1}$ were then washed in 0.1 × SSC/0.1% SDS at 5°C increments between 30 and 70°C as detailed in Materials and Methods.

Discussion

The sum of Na,K-ATPase alpha subunit mRNA(s) was more abundant than $mRNA_{\beta 1}$ in all tissues examined in this study (Tables 1 and 3). The molecular basis for this disparity is unclear, and presumably reflects differences in transcriptional and/or post-transcriptional events in alpha *vs.* beta1 subunit gene expression. Moreover, the discrepancy in abundance of alpha isoform and beta1 subunit mRNAs implies that either translational and/or post-translational differences exist in the biogenesis of Na,K-pump subunits or that the total pool of alpha and beta subunits does not exist as a 1 : 1 ratio. Alternatively, if the recently described beta2 subunit is a functional component of the pump [7, 13], it is possible that the expression of beta2 peptide in the brain may account for some of the discrepancy in alpha *vs.* beta subunit mRNA expression in this tissue [18].

The content of Na,K-pump subunit mRNAs is regulated in a tissue-specific manner whether the results are expressed per unit RNA or DNA (Tables 1 and 3). The tissue abundance of subunit mRNAs expressed per unit RNA is qualitatively similar in some respects to an earlier report by Young and Lingrel [23]. For example, our analysis of $mRNA_{\beta 1}$ content is virtually identical to the reported hierarchy of $mRNA_{\beta 1}$ abundance [23]. Both qualitative and quantitative differences, however, have been identified in the present investigation. For example, Young and Lingrel [23] concluded that the level of $mRNA_{\alpha 1}$ was approximately 150-fold higher in rat kidney compared to liver. In contrast, we observed that $mRNA_{\alpha 1}$ is readily detected by Northern blot analysis of total liver RNA and the content of $mRNA_{\alpha 1}$ is only ~7-fold higher in rat kidney than in liver (Fig. 1, Table 1). Further discrepancies between these two studies are evident when the inter-tissue distribution of $mRNA_{\alpha 2}$ is investigated. In the former study, $mRNA_{\alpha 2}$ content was found to be 1.5-fold higher in skeletal muscle compared to brain, whereas we report that the content of brain $mRNA_{\alpha 2}$ is ~6-fold greater than skeletal muscle (Table 1). The reason for the discrepancies between the former and present study is not known but may reflect the use of different probe lengths and/or wash conditions.

Expression of Na,K-pump subunit mRNA abundances per unit tissue DNA yields novel information on the magnitude of the tissue-specific regulation of subunit mRNA contents (Table 3). In many instances the extent of differences in subunit mRNA abundances is reduced when the data are expressed per tissue DNA content rather than on a per RNA basis. For example, $mRNA_{\alpha 1}$ content in rat kidney is 11-fold greater than skeletal muscle when expressed per unit RNA (Table 1), whereas a ~2-fold difference is apparent when the data are expressed per

Table 1. Relative abundances of Na,K-ATPase subunit mRNAs expressed per unit RNA

Tissue	Na,K-ATPase subunit mRNA content			
	Alpha1	Alpha2	Alpha3	Beta1
Kidney	33.4 ± 3.7	ND[a]	ND	22.3 ± 1.5
Heart	9.6 ± 1.6	5.7 ± 1.5	ND	2.7 ± 0.6
Skeletal muscle	3.0 ± 0.6	7.2 ± 1.4	ND	2.8 ± 0.7
Liver	4.5 ± 0.1	ND	ND	1.0 ± 0.1[b]
Brain	21.0 ± 2.6	42.7 ± 2.4	72.8 ± 5.2	15.2 ± 4.6

Replicate Northern blots containing various amounts of total RNA from each tissue were hybridized with equivalent amounts of subunit cDNAs labeled to near-equivalent specific activity. Blots were washed together at 35°C in 0.1 × SSC/0.1% SDS and exposed to a single x-ray film for 16 hr. The relative amounts of subunit mRNAs were quantitated by scanning densitometry. $n = 4$ rats, means ± SE.

[a] ND indicates that the mRNA was not detectable.

[b] Relative subunit mRNA contents were normalized to a value of 1.0 for liver $mRNA_{\beta 1}$.

Table 2. Protein, RNA and DNA contents of rat tissues

Tissue	Protein (mg/g wet wt)	RNA (mg/g wet wt)	DNA (mg/g wet wt)	Prot/RNA (mg/mg)	Prot/DNA (mg/mg)	RNA/DNA (mg/mg)
Kidney	173 ± 7	3.55 ± 0.10	3.34 ± 0.25	49 ± 3	52 ± 3	1.1 ± 0.1
Heart	129 ± 8	1.46 ± 0.02	0.79 ± 0.05	88 ± 4	163 ± 6	1.9 ± 0.1
Skeletal muscle	165 ± 13	0.95 ± 0.07	0.20 ± 0.02	174 ± 8	825 ± 60	5.5 ± 1.4
Liver	201 ± 11	7.60 ± 0.16	2.20 ± 0.11	26 ± 1	91 ± 4	3.5 ± 0.1
Brain	99 ± 1	0.97 ± 0.03	0.66 ± 0.04	102 ± 2	150 ± 2	1.5 ± 0.1

Values are means ± SE for $n = 5$ for all determinations, except $n = 4$ for protein.

Table 3. Relative abundances of Na,K-Pump subunit mRNAs expressed per unit DNA

Tissue	Na,K-Pump subunit mRNA content/DNA			
	Alpha1	Alpha2	Alpha3	Beta1
Kidney	10.4 ± 1.2	ND[a]	ND	6.9 ± 0.1
Heart	5.2 ± 0.9	3.1 ± 0.8	ND	1.5 ± 0.3
Skeletal muscle	4.8 ± 1.0	11.4 ± 2.2	ND	4.5 ± 1.1
Liver	4.5 ± 0.1	ND	ND	1.0 ± 0.1[b]
Brain	9.0 ± 1.1	18.4 ± 1.0	31.3 ± 2.2	6.5 ± 2.0

The relative abundances of subunit mRNAs shown in Table 1 have been divided by the tissue RNA to DNA ratio to normalize subunit mRNA abundance per unit DNA.

[a] ND indicates that the mRNA was not detectable.

[b] Na,K-pump subunit mRNA contents expressed per unit DNA have been normalized to a value of 1.0 assigned to liver $mRNA_{\beta 1}$.

Table 4. Na,K-ATPase activity of rat tissues

Tissue	Na,K-ATPase activity (μmol P_i/h)		
	Per g tissue (wet wt.)	Per mg protein	Per mg DNA
Kidney	1923 ± 97	11.17 ± 0.56	580 ± 29
Heart	351 ± 23	2.72 ± 0.18	444 ± 30
Skeletal muscle	91 ± 15	0.55 ± 0.09	456 ± 76
Liver	253 ± 8	1.26 ± 0.04	115 ± 4
Brain	530 ± 10	5.30 ± 0.10	802 ± 15

Na,K-ATPase was measured as the difference between ATPase values in the absence and presence of 2×10^{-3} M ouabain. $n = 4$, means ± SE.

unit DNA (Table 3). Similarly, a tissue-specific 3-fold difference in rat heart and skeletal muscle $mRNA_{\beta 1}$ expression is revealed only when differences in tissue RNA to DNA ratios are considered (Table 3). Furthermore, an analysis of $mRNA_{\alpha 1}$ content expressed per RNA indicates a ~3-fold greater abundance of this mRNA in heart compared to skeletal muscle, while when expressed per unit DNA, $mRNA_{\alpha 1}$ content in these tissues is nearly equivalent.

The determinants of the abundance of Na,K-ATPase in cells and tissues is at present poorly understood. As a plasma membrane-bound enzyme, Na,K-ATPase functions to keep intracellular Na^+ and K^+ ion concentrations constant [9]. It thus may be reasoned that the abundance of the enzyme may differ among various cells on the basis of their surface area or surface to volume ratio as well as on the permeability characteristics of their plasma membrane to Na^+ and K^+ during rest and excitation. Indeed, we determined that Na,K-ATPase activity varied significantly in several tissues of the rat whether expressed per-unit-protein, per-unit-DNA or per-g-wet-weight-of-tissue (Table 4). Expression of enyzme activity per unit protein is rendered difficult because, as noted in Table 2, the protein content per unit weight is not constant among the tissues examined. Expression of Na,K-ATPase activity per unit DNA allows classification of enzyme activity per number of nuclei, but ignores the contribution of cell volume or cell surface area to the analysis. Based on the assumption that cell water content per unit wet weight is nearly constant among the tissues examined, expression of enzyme activity per g wet weight of tissue is expected to be proportional to expression of enzyme activity per unit volume, and hence could represent a valid basis for inter-tissue comparisons of enzyme activity.

In the absence of specific information on the rates of passive Na^+ influx and K^+ efflux and of the other parameters noted above that might influence Na,K-ATPase abundance among various tissues, no general inferences can be derived from comparison of Na,K-ATPase activities between the tissues examined. We can, however, address the general question whether the relative enzyme activity among these tissues correlates with the relative abundance of either (or both) of the subunit mRNAs in the same tissues. This question is of physiological and mechanistic importance for an understanding of Na,K-ATPase biogenesis, especially in view of recent suggestions that $mRNA_\beta$ and β-peptide abundance may critically regulate the biosynthesis, and thus the abundance, of the enzyme [2, 12, 15, 16, 19, 20]. Indeed, our finding that $mRNA_{\beta 1}$ abundance is less than the sum of $mRNA_\alpha$ isoform abundances in all of the tissues examined (Tables 1 and 3) is consistent with such a hypothesis.

Analysis of the data by linear analysis indicates that enzyme activity correlates with the relative abundance of $mRNA_{\beta 1}$; the weaker correlation of enzyme activity with the sum of $mRNA_\alpha$ isoforms did not reach statistical significance (Figs. 3 and 4). The correlation was strongest when relative mRNA abundances were expressed per g of tissue, a normalization that, as noted above, would be expected to generate relative mRNA abundance values that are proportional to intracellular concentrations of the subunit mRNAs. It should be emphasized that while the enzyme activity correlated more significantly with the relative $mRNA_{\beta 1}$ abundance rather than the sum of $mRNA_\alpha$ isoform abundances, the relative abundance of the sum of $mRNA_\alpha$ isoforms in tissues with high enzyme activity was markedly higher than in tissues with modest activity. Indeed,

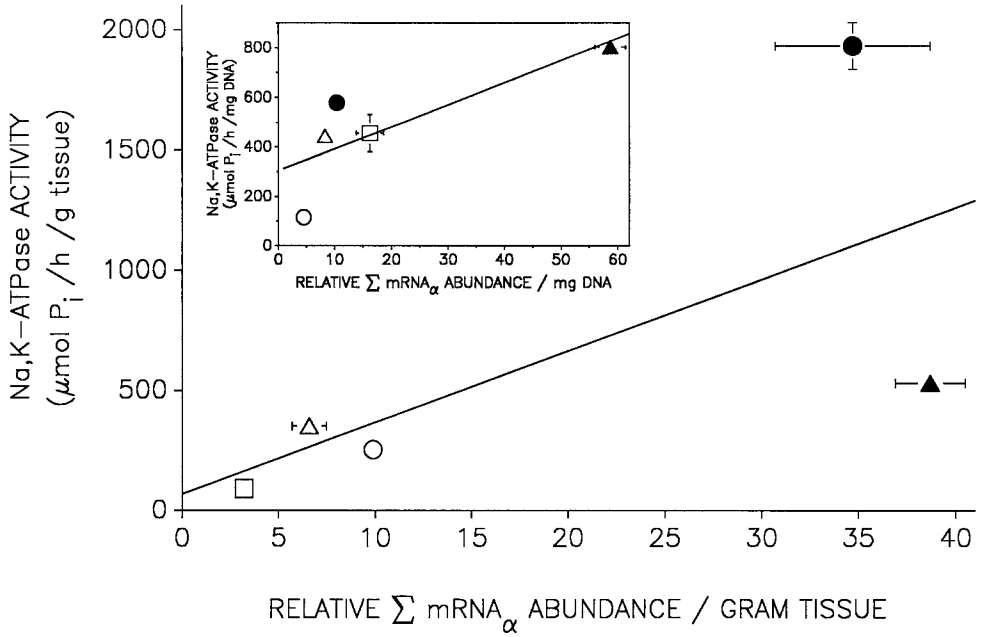

Figure 3. Correlation between the sum of Na,K-ATPase $mRNA_\alpha$ isoform abundances and Na,K-ATPase activity in five tissues of the rat. Coordinates for the sum of $mRNA_\alpha$ isoform abundances and for the enzyme activity in each of the five tissues, derived from the data given in Tables 2, 3 and 4, were plotted (means ± SE) and subjected to linear-regression analysis. Data are expressed per g wet weight of tissue ($r = 0.68$, $P > 0.10$). *Inset:* Plot of the data expressed per mg DNA ($r = 0.80$, $P > 0.10$). ●, kidney cortex; ▲, cerebrum; ○, liver; △, ventricular myocardium; □, skeletal muscle (hind-limb).

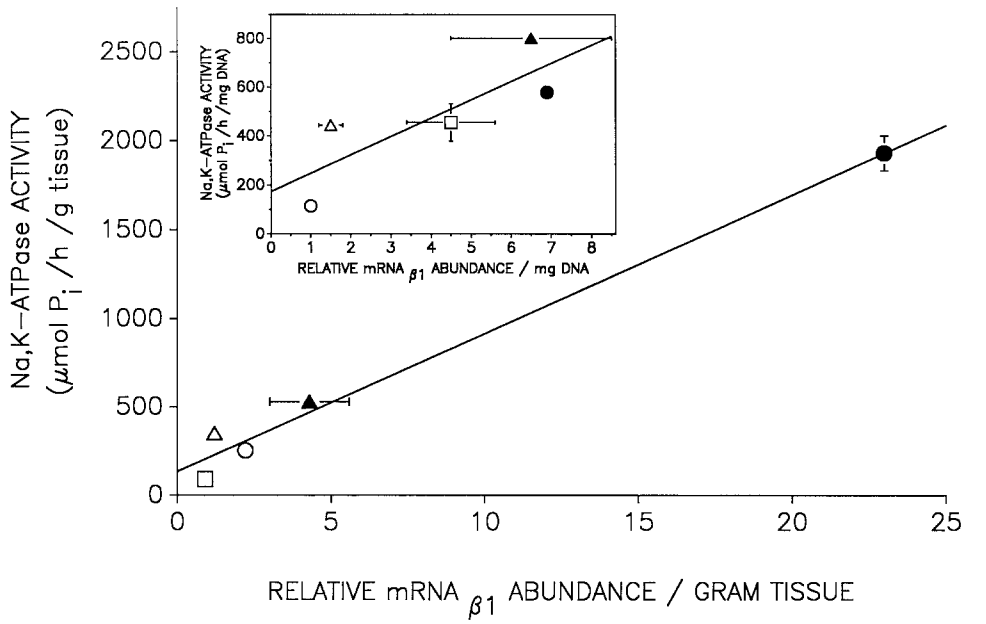

Figure 4. Correlation between Na,K-ATPase $mRNA_{\beta 1}$ abundance and Na,K-ATPase activity in five tissues of the rat. Coordinates for $mRNA_{\beta 1}$ abundance and for the enzyme activity were plotted and analyzed as described in the legend to Fig. 3. Data are expressed per g wet weight of tissue ($r = 0.99$, $P < 0.01$). *Inset:* Plot of the data expressed per mg DNA ($r = 0.83$, $0.10 > P > 0.05$). ●, kidney cortex; ▲, cerebrum; ○, liver; △, ventricular myocardium; □, skeletal muscle (hind-limb).

it can be seen that both brain and renal cortex, tissues with the highest enzyme activity per g wet weight, contain the highest relative abundance of both alpha and beta subunit mRNAs. The analysis, therefore, does not unambiguously identify either of the subunit mRNAs as playing a dominant role in determining the level of Na,K-ATPase expression. Instead, the results are consistent with the premise that Na,K-ATPase activity in various rat tissues is regulated by both alpha and beta subunit mRNA abundances.

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