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Tissue Distribution of mRNAs Encoding the α Isoforms and β Subunit of Rat Na^+,K^+-ATPase

Regina M. Young and Jerry B Lingrel

Department of Microbiology and Molecular Genetics,

University of Cincinnati, College of Medicine,

Cincinnati, Ohio 45267-0524

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The tissue distribution of the multiple forms of rat Na^+,K^+-ATPase was examined at the molecular level with cDNA probes specific for the α, α(+), αIII and β subunit mRNAs. Northern and slot blot analyses demonstrate that these mRNAs are produced in a tissue-specific manner. RNAs encoding the α(+) isoform are detected in kidney, brain, heart, adipose, muscle, stomach and lung, whereas αIII RNA is detected in brain, stomach and lung. Both α and β mRNAs are present in all the tissues studied, although at very different levels. Examination of heart tissue in greater detail demonstrates that the levels of mRNA encoding the α subunit are greater in the atria than in the ventricles, while the converse is true for α(+).

Na^+,K^+-ATPase transports sodium and potassium ions across the plasma membrane to generate an electrochemical gradient that is essential in maintaining the membrane potential and metabolic activity of mammalian cells. Several forms of rat Na^+,K^+-ATPase have been described which are distinct with respect to sodium affinity (1), regulation by thyroid hormone (2), and sensitivity to cardiac glycosides (3) and insulin (4). These functional differences have been correlated with the presence of two isoforms of the α subunit. Recently, three molecular forms of the rat α subunit were identified by cDNA cloning experiments (5). Two of these isoforms, α and α(+) had been detected previously in rat brain (3), adipose (4), muscle (4) and retina (6) based on differences in their apparent molecular weights and affinities for ouabain (3). The protein encoded by αIII has not yet been detected. Multiple rat β subunit cDNAs have also been isolated, but these appear to encode a single β subunit protein (7). In an effort to understand the relative expression of these various subunit forms, we determined the tissue distribution of the α, α(+), αIII and β subunit mRNAs of rat Na^+,K^+-ATPase by probing Northern and slot blots with specific DNA fragments isolated from these cDNAs.

The abbreviations used are: SDS, sodium dodecyl sulfate; kb, kilobase pair(s); T3, L-3,3' 5'-triiodothyronine; nt, nucleotides.

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Material and Methods

RNA Isolation- Total RNA was isolated from 150-200 g male CD rat (Charles River Breeding Laboratories) kidney, brain, heart (left ventricle, right ventricle, atria and whole heart), epididymal fat pad, hind limb skeletal muscle, stomach, lung and liver tissue (8). Epididymal fat pad RNA was, in addition, isolated from 200-300 g male Wistar rats (Pel-freez).

Northern and Slot Blot Analyses- Northern blots were prepared as follows; ten ug of each RNA sample was denatured with glyoxal, fractionated on 1% agarose gels, blotted onto Nytran (Schleicher and Schuell), prehybridized and hybridized according to the manufacturer's protocol, except for the inclusion of dimethylsulfoxide (7M) during denaturation. For slot blot analyses, 0.5-3ug of RNA was denatured in 4.6 M formamide, 7.5X SSC (1.1 M NaCl, 0.1 M sodium citrate, pH 7.0) at 65°C for 15 min, spotted onto Nytran using the Schleicher and Schuell apparatus, prehybridized and hybridized sequentially (9). DNA restriction fragments isolated from rat Na^+,K^+-ATPase α (5) (*Nar I-Stu I*, nt[1] 89-421), α(+) (5) (*Sca I-Nhe I*, nt 121-502 and *Xho II*, nt 4285-5026), αIII (5) (*Pst I-Sma I*, nt 51-331 and *Nco I-Apa I*, nt 3150-3487) and β subunit cDNAs (7) (*Nco I-Stu I*, nt 459-759 and *Nco I-Ssp I*, nt 459-1554) were oligolabeled (10) and used as probes for Northern or slot blots. Filters were washed in 2X SSC, 0.1% SDS at 25°C for 30 min and then at 0.1X SSC, 0.1% SDS at 50°C for 1h. After autoradiography, slot blot signals were analyzed using a Hoefer Scientific Instruments scanning densitometer. The levels of either α, α(+), αIII or β mRNA in various tissues were normalized against a titration of brain mRNA.

Results

Subunit specific probes - Specific cDNA probes were developed for the α isoforms of rat Na^+,K^+-ATPase. Restriction fragments, approximately 300 nt in length (α *Nar I-Stu I*, α(+) *Sca I-Nhe I* and αIII *Pst I-Sma I*), were isolated from the 5' end of the α, α(+) and αIII cDNAs (5). DNA fragments were also prepared from the 3' untranslated region of the α(+) and αIII cDNAs (α(+) *Xho II* and αIII *Nco I-Apa I*). All of the probes exhibited limited sequence similarity to one another, to the H^+,K^+-ATPase (11) and to Ca^{2+}-ATPases (12,13). In addition, the Northern blots were washed stringently to ensure their specificity. The tissue distribution of the β subunit was analyzed using a 1.1 kb *Nco I-Ssp I* restriction fragment isolated from the protein coding region of the β cDNA (7). Slot blot analyses were performed with α isoform (α *Nar I-Stu I*, α(+) *Sca I-Nhe I* and αIII *Pst I-Sma I*) and β subunit (*Nco I-Stu I*) probes of a similar size so that the intensities of the hybridization signals could be compared.

Tissue-specific expression of mRNAs encoding the α isoforms and the β subunit- To determine the tissue distribution of the α, α(+), αIII and β subunit mRNAs of Na^+,K^+-ATPase, RNA was isolated from rat kidney, brain, heart, adipose, muscle, stomach, lung and liver and hybridized with each of the subunit specific probes. Representative Northern blots are illustrated in Figs. 1-4. The α subunit probe hybridizes to a single mRNA, 3.7 kb, in each of the tissues analyzed (Fig. 1). Two α(+) mRNA species, 3.4 kb and 5.3 kb, were detected in every tissue except liver (Fig. 2). When a 741 nt *Xho II* restriction fragment isolated from the 3' untranslated region of α(+), beyond the first potential polyadenylation site, is labeled and hybridized to rat RNA only the larger 5.3 kb band is observed (see Fig. 5). This suggests that the two α(+) RNA species result from the use of at least two of the three potential polyadenylation signals present in the longest α(+) cDNA. A single 3.7 kb hybridization signal is present in brain, stomach and

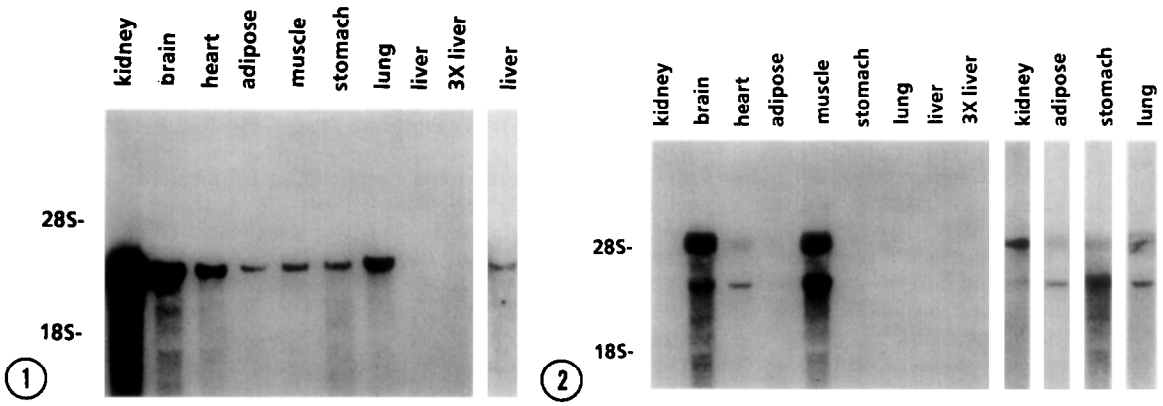

Fig. 1. Northern blot analysis of mRNA encoding the α subunit of rat Na^+,K^+-ATPase. Ten or thirty ug of total RNA was hybridized with an α specific probe isolated from the 5' end of the rat α subunit cDNA (5). A longer exposure of the signal obtained from liver tissue is illustrated to the right of the figure and the positions of the 28S and 18S ribosomal markers are indicated.

Fig. 2. Northern blot analysis of mRNAs encoding the α(+) subunit of rat Na^+,K^+-ATPase. Ten or thirty ug of total RNA was hybridized with an α(+) specific probe isolated from the 5' end of the rat α(+) cDNA (5). A longer exposure of the signals from adipose, stomach and lung tissue is illustrated to the right of the figure. The signal in the second kidney lane was obtained after 5ug of poly $(A)^+$ RNA was hybridized with an α(+) specific probe. The positions of the 28S and 18S ribosomal markers are indicated.

lung when either αIII probe is used (Fig. 3). The size of the α mRNAs are in agreement with the length of the rat α subunit cDNAs (5).

Several mRNA species encode the rat Na^+,K^+-ATPase β subunit (7) and these are observed in all of the tissues examined (Fig. 4). The largest two β subunit mRNAs are

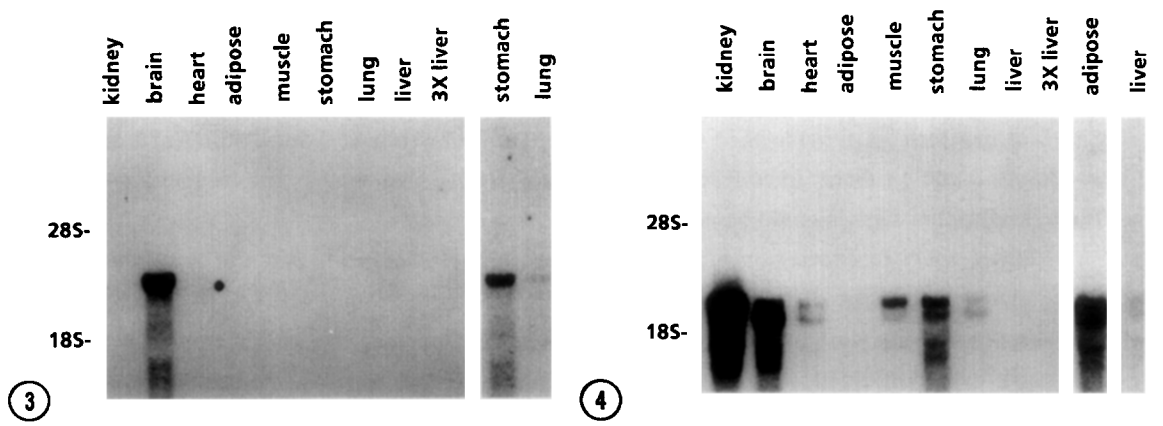

Fig. 3. Northern blot analysis of mRNA encoding the αIII subunit of rat Na^+,K^+-ATPase. Ten or thirty ug of total RNA was hybridized with an αIII specific probe isolated from the 5' end of the rat αIII cDNA (5). A longer exposure of the signals obtained from stomach and lung tissue is illustrated to the right of the figure and the positions of the 28S and 18S ribosomal markers are indicated.

Fig. 4. Northern blot analysis of mRNAs encoding the β subunit of rat Na^+,K^+-ATPase. Ten or thirty ug of total RNA was hybridized with a β subunit probe isolated from the coding region of the rat β cDNA. (7). A longer exposure of the signals from adipose and liver tissue is illustrated to the right of the figure and positions of the 28S and 18S ribosomal markers are indicated.

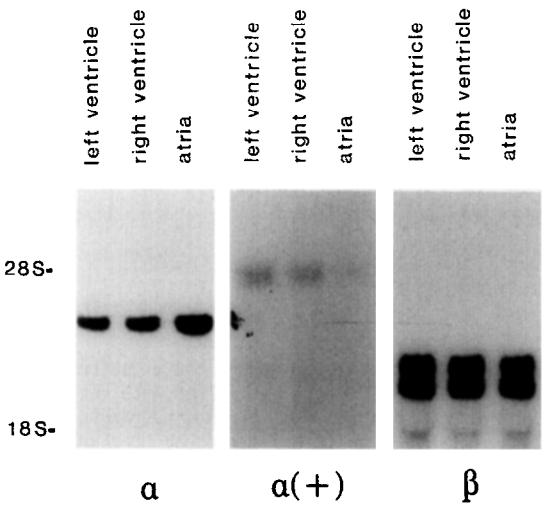

Fig. 5. Northern blot analysis of mRNAs encoding the α isoforms and β subunit of rat Na^+,K^+-ATPase in heart. Ten ug of total RNA from left ventricle, right ventricle and atria was hybridized with subunit specific probes. The positions of the 28S and 18S ribosomal markers are indicated.

transcribed from separate initiation sites which are differentially utilized in the various tissues (7). Use of the distal start site is more predominant in kidney, muscle and stomach, whereas initiation from the proximal start site is more prevalent in brain. In heart, adipose, lung and liver these initiation sites appear to be used with equal frequency. Since the levels of α and β subunit mRNAs were quite low in the liver and α(+) and αIII subunit mRNAs were not detectable, liver RNA was hybridized with a rat serum albumin probe (14) as a control. A sharp and intense signal was observed at 2.2 kb indicating that the liver RNA was intact (not shown).

Examination of the α isoform and β subunit mRNAs in the heart- A more detailed analysis of the tissue distribution of the α, α(+), αIII and β subunit mRNAs in rat heart was performed (Fig. 5). RNA isolated from left ventricle, right ventricle and atria was hybridized with probes specific for the various subunit forms. RNAs encoding the β subunit are present at equal levels in the left ventricle, right ventricle and atria of the heart. In contrast, α subunit mRNA is present at slightly greater levels in the atria and α(+) mRNAs are slightly more abundant in the ventricles. Again, no αIII mRNA was detected in the heart. To verify these data, Northern blots were sequentially hybridized with each of the probes and the same results were obtained.

Quantitation of the levels of α and β subunit mRNAs- Slot blot analyses were performed to examine the relative tissue distributions of the α, α(+), αIII and β mRNAs in a more quantitative manner. The results of these experiments are shown in Fig. 6. Several dilutions of RNA isolated from kidney, brain, heart, muscle, stomach, lung and liver and a titration of brain RNA were hybridized with each probe. Hybridization signals obtained from the various tissues were compared to a standard curve generated by plotting the hybridization intensities of the brain RNA samples *versus* the amount of

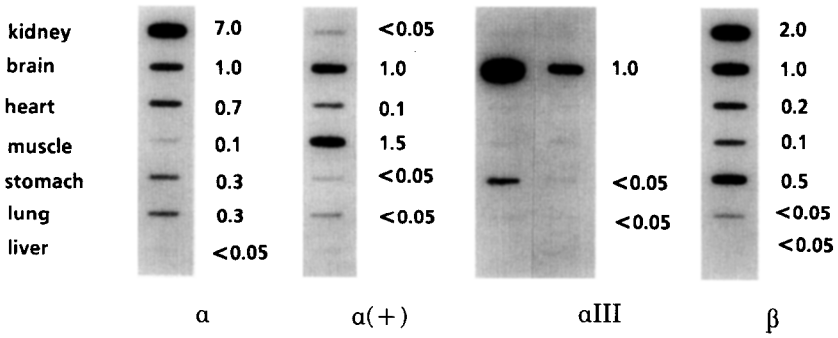

Fig. 6. Slot blot analysis of mRNAs encoding the α isoforms and β subunit of rat Na^+,K^+-ATPase. Two ug of total RNA was hybridized with rat α isoform and β subunit specific probes of a similar size and specific activity. A value of 1 was assigned to the levels of α, α(+), αIII and β mRNAs in the brain and the level of each RNA in additional tissues was normalized with respect to the brain value. These results are listed on the right of the corresponding slot blot. Filters were exposed for the same time period, however a longer exposure of the αIII slot blot is also illustrated.

RNA spotted. A value of 1 was arbitrarily assigned to the level of α, α(+), αIII and β RNA in the brain and the level of each RNA in other tissues was normalized to the brain value. As illustrated in Fig. 6, mRNA encoding the α and β subunits are most abundant in kidney and least abundant in liver. The difference in the levels of α RNA in these two tissues is greater than 150 fold. The α(+) mRNAs are most abundant in muscle and brain and in lower amounts in kidney, heart, adipose, stomach and lung. αIII mRNA is at least 20 fold more abundant in brain than stomach and lung. A probe isolated from the 3' untranslated region of the αIII cDNA was also hybridized to RNA slot blots to verify that the signal observed in the stomach did not represent cross hybridization to the H^+,K^+-ATPase.

When the relative abundance of the subunit RNAs in brain are compared, α, α(+) and αIII appear to be present at similar levels, whereas β RNA levels are approximately 5 fold higher (median of 3 experiments α: α(+): αIII: β; 1: 1.2: 0.8: 5.2). Although the probes were about the same size and specific activity, and corrections were made for any minor differences, it should be emphasized that the values given are only approximate due to experimental variation with this technique.

Discussion

These studies demonstrate that the α and β subunits of Na^+,K^+-ATPase are encoded by mRNAs which exhibit distinct tissue distributions. Although the α mRNAs are present in all the tissues examined, the steady state levels of this mRNA vary considerably amongst tissues. RNAs encoding the α(+) subunit are present in brain, heart, adipose and muscle, which is consistent with previous protein and functional studies (3,4,6,16). Low levels of α(+) mRNAs are also detected in kidney, stomach and lung. The expression of α(+) mRNAs, may be confined to a limited number of cell types in these tissues. There is some evidence in support of this hypothesis. For example,

Na^+,K^+-ATPase activity has been reported to be more sensitive to ouabain in the collecting tubules of the kidney than in more proximal segments of the nephron (15).

In muscle and stomach, the 3.4 kb α(+) mRNA is present at greater levels relative to the 5.3 kb mRNA than is the case in brain. This could result from the differential use of the polyadenylation signals in the 3' untranslated region of α(+) in these tissues. Alternatively, the α(+) probe may cross-hybridize with a 3.4 kb mRNA encoding a related ATPase. However, the probe used was not homologous to the H^+,K^+-ATPase or to any of the Ca^{2+}-ATPases whose amino acid sequences have been determined (11-13).

RNA encoding the third form of the α subunit, αIII, was initially identified in the brain (5). In brain, all three isoforms of the α subunit appear to be present at similar levels. If αIII is translated as efficiently as α and α(+), then it should represent a major isoform of the α subunit. To date, however, only the α and α(+) proteins have been observed. If the ouabain sensitivity and the electrophetic migration of the αIII protein overlaps that of the α or α(+) protein, this isoform would not be detected with these techniques. These studies indicate that αIII is expressed in stomach and lung, as well as brain.

Two isoforms of the α subunit of Na^+,K^+-ATPase are also present in rat heart. This is consistent with the reported presence of two inotropic receptor sites for ouabain in this tissue (16). RNAs encoding the α(+) subunit are less abundant than α mRNA, which might explain why only the α subunit protein was observed on SDS polyacrylamide gels (17). The observation that the levels of α(+) mRNA are greater in the ventricles than in the atria parallels the localization of the high affinity ouabain receptor in rat heart (16).

It is generally accepted that the β subunit is an integral component of Na^+,K^+-ATPase. This is, in part, based on the observation that the α and β subunits are coordinately synthesized both in magnitude and time course in response to T3 induction (18). This is consistent with our data that β subunit mRNAs are expressed in all the tissues examined. In kidney, brain and heart, the levels of mRNA encoding the α isoforms and the β subunit are similar, suggesting that co-regulation of α and β subunits may occur, at least in part, at the RNA level. However, this does not appear to be the case in muscle and stomach. RNA levels of the α isoforms in muscle are 3 fold greater than β subunit mRNAs. Conversely, in stomach the steady state levels of β subunit mRNAs are more than 8 fold greater than the levels of the mRNAs encoding the α isoforms. If the synthesis of the α and β subunits of Na^+,K^+-ATPase is coordinately regulated, then the mechanism is dependent upon cell type.

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References

1. Lytton, J. (1985) J. Biol. Chem. 260, 10075-10080.
2. Schmitt, C.A. and McDonough, A.A. (1986) J. Biol. Chem. 261, 10439-10444.
3. Sweadner, K.J. (1979) J. Biol. Chem. 254, 6060-6067.
4. Lytton, J., Lin, J.C. and Guidotti, G. (1985) J. Biol. Chem. 260, 1177-1184.
5. Shull, G.E., Greeb, J. and Lingrel, J.B (1986) Biochemistry 25, 8125-8132.
6. Specht, S.C. and Sweadner, K.J. (1984) Proc. Natl. Acad. Sci. USA 81, 1234-1238.
7. Young, R.M., Shull, G.E. and Lingrel, J.B (1987) J. Biol. Chem. *in press.*
8. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
9. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201-5205.
10. Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6-13.
11. Shull, G.E. and Lingrel, J.B (1986) J. Biol. Chem. 261, 16788-16791.
12. MacLennan, D.H., Brandl, C.J., Korczak, B., and Green, N.M. (1985) Nature 316, 696-700.
13. Brandl, C.J., Green, N.M., Korczak, B. and MacLennan, D.H. (1986) Cell 44, 597-607.
14. Kioussis, D., Hamilton, R., Hanson, R.W., Tilghman, S.M. and Taylor, J.M. (1979) Proc. Natl. Acad. Sci. USA 76, 4370-4374.
15. Doucet, A. and Barlet, C. (1986) J. Biol. Chem. 261, 993-995.
16. Adams, R.J., Schwartz, A., Grupp, G., Grupp I., Lee, S. and Wallick, E.T. (1982) Nature 296, 167-169.
17. Matsuda, T., Iwata, H. and Cooper, J.R. (1984) J. Biol. Chem. 259, 3858-3863.
18. Lo, C.S. and Lo, T.N. (1980) J. Biol. Chem. 255, 2131-2136.