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Differential expression of Na^+,K^+-ATPase α- and β-subunit mRNAs in rat tissues and cell lines

(α-subunit isoforms/development)

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ABSTRACT **We have analyzed Na^+,K^+-ATPase (EC 3.6.1.3) α- and β-subunit mRNA expression in rat tissues and cell lines derived from the rat central nervous system. Substantial differences in the tissue and developmental specificity of expression were found for the genes encoding three isoforms of the α subunit. Transcripts of the α1-subunit gene were detected in all tissues tested, whereas α2- and α3-subunit mRNA species were expressed predominantly in brain. The pattern of expression of β-subunit mRNA also was complex and tissue specific but was distinct from that of any of the α-subunit mRNAs. Cell lines derived from the rat central nervous system and the pheochromocytoma PC12 expressed the mRNAs for all three α-subunit isoforms, whereas β-subunit mRNA was detected only in PC12 cells. The distinct expression patterns of rat Na^+,K^+-ATPase mRNAs suggest that different members of the ATPase family may have specialized functions.**

The Na^+,K^+-ATPase (EC 3.6.1.3) is the enzymatic activity responsible for the active transport of Na^+ and K^+ in most animal cells. The enzyme has been shown to consist of two subunits. The α subunit is a polypeptide of M_r 100,000 that contains the catalytic site for ATP hydrolysis (1). The β subunit is a glycosylated polypeptide of M_r 55,000 whose function has not yet been established. Two biochemically distinct forms of the catalytic subunit (α and α^+) have been identified in the rat (2, 3). These differ in electrophoretic mobility in $NaDodSO_4$-containing gels (2, 4) and in sensitivity to cardiac glycosides (2, 5). The two isoforms also exhibit differential tissue distribution (2, 6). The α form is the predominant species found in kidney, whereas the α and α^+ forms are present in brain (2), adipose tissue (6), and skeletal muscle (6).

Recent work has shown that Na^+,K^+-ATPase α-subunit isoforms are encoded by multiple genes (7, 8). Three α-subunit genes have been localized to different chromosomes in the mouse (7). The α1- or α-subunit gene (*Atpa-1*) is located on chromosome 3; the α2-subunit gene (*Atpa-2*) maps to chromosome 7; the α3- or α^+-subunit gene (*Atpa-3*) is located on chromosome 1 (7). The Na^+,K^+-ATPase β-subunit gene (*Atpb*) also maps to chromosome 1 but is not tightly linked to the α3-subunit gene (7).

Several lines of evidence indicate that Na^+,K^+-ATPase α- and β-subunit synthesis is coordinately regulated and that α and β subunits are expressed at approximately equal levels. Concurrent biosynthesis and rapid assembly of the two enzyme subunits have been observed in chicken sensory neurons (9). Coordinate induction of α and β subunits also occurs in response to hormones such as thyroid hormone (10) and aldosterone (11). In HeLa C^+ cells, the mRNA for α and β subunits is overexpressed (12, 13), suggesting that increased levels of synthesis of α and β subunits are required for overexpression of the enzyme. However, the separate chromosomal locations of the α- and β-subunit genes suggest that their coordinate expression is not likely to be regulated by a common cis-acting control element.

To investigate further the regulation of Na^+,K^+-ATPase biosynthesis, we have analyzed the tissue- and developmental stage-specific expression of Na^+,K^+-ATPase α- and β-subunit mRNA in rat tissues and cell lines derived from the rat central nervous system (CNS). Transcripts of the α1-subunit gene are expressed in all tissues tested thus far. In contrast, α2-subunit mRNA is present in highest abundance in the brain. The α3-subunit gene encodes three distinct mRNA species that are also expressed at high levels in brain. The β-subunit gene exhibits a complex tissue-specific pattern of mRNA expression that is distinct from that of any of the α-subunit genes. Cell lines from the rat CNS and the rat pheochromocytoma PC12 express the mRNAs for all three α-subunit isoforms, whereas β-subunit mRNA is detected only in PC12 cells. The distinct tissue distributions of α-subunit mRNAs suggest that different isoforms of the Na^+,K^+-ATPase have specialized functions. Differences in the expression patterns of α- and β-subunit mRNAs raise questions regarding the control mechanisms responsible for coordinate regulation of Na^+,K^+-ATPase subunits.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Rat CNS cell lines of neuronal and nonneuronal origin were obtained from David Schubert (Salk Institute, La Jolla, CA). Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum. PC12 cells were provided by Ed Hawrot (Yale University Medical School). PC12 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum/5% horse serum.

RNA Blot Hybridization. RNA was isolated from rat tissues and cell lines by the guanidinium isothiocyanate method of Chirgwin *et al.* (14). Total cellular RNA was denatured by heating at 65°C for 10 min in 50% (vol/vol) formamide and was fractionated by electrophoresis through a 1% agarose/formaldehyde gel (15). The RNA was transferred to reusable hybridization membranes (Zetabind, AMF Cuno). Conditions for hybridization analysis of RNA samples were as described (16). Radiolabeled DNA probes were synthesized with the Klenow fragment of DNA polymerase I with random hexanucleotides (Boehringer Mannheim) and [α-^{32}P]dCTP (17). Blots were hybridized with 10^7 cpm (<50 ng) of the indicated hybridization probe. Blots were washed to a final stringency of 0.1 × SSCPE (15 mM NaCl/1.5 mM sodium citrate/1.3 mM KH_2PO_4/100 μM EDTA)/0.1% $NaDodSO_4$ at 65°C and exposed to Kodak XRP film at −80°C with an intensifying screen. For reprobing, blots were washed two

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Abbreviation: CNS, central nervous system.

times at 95°C for 15 min in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$.

cDNA Probes. The following Na^+,K^+-ATPase subunit probes were used: clone pA1 [3.2-kilobase-pair (kbp) rat cDNA containing the entirety of the α1-subunit coding region] (7), clone Ea5 (1.8-kbp *Eco*RI fragment of the rat cDNA encoding the amino-terminal portion of the α2 subunit) (7), clone rb13c (2.7-kpb rat cDNA encoding a portion of the α3 subunit) (7), clone rb19G (1.2-kbp cDNA containing the entirety of rat brain β-subunit coding region) (13). A physical map of each probe is diagramed in Fig. 1. Blots were also hybridized with a human γ-actin cDNA probe, pHFgammaA, provided by Larry Kedes (Stanford University School of Medicine).

RESULTS

Tissue-Specific and Developmental Expression of Na^+,K^+-ATPase α-Subunit mRNAs. To analyze expression of Na^+, K^+-ATPase α-subunit mRNAs, cDNA probes specific for each of the three α-subunit isoforms were sequentially hybridized to an RNA transfer blot containing rat tissue RNA. Virtually no cross-hybridization of the probes was observed in Southern genomic blots hybridized under the same conditions (7). The RNA transfer blotting results are shown in Fig. 2. The α1-subunit cDNA probe hybridized to a single RNA species ≈4.5 kilobases (kb) in size. In the adult, transcripts of the *Atpa-1* gene (α1 panel) were most abundant in kidney, less abundant in heart and brain, and least abundant in liver. *Atpa-1*-encoded mRNA was abundant in 2-week brain, fetal (18-day) and 2-week heart, and fetal and 2-week kidney. Fewer transcripts were present in fetal brain, fetal liver, and 2-week liver. The α2-subunit probe also hybridized to an RNA ≈4.5 kb in length. In contrast to the pattern of *Atpa-1* mRNA expression, *Atpa-2* (α2 panel) transcripts were present predominantly in 2-week brain, adult brain, and 2-week liver. Moderate levels of *Atpa-2* mRNA were detected in 2-week kidney, adult kidney, and adult liver, whereas very low levels were present in heart (all stages) and fetal kidney. No *Atpa-2* transcripts were detected in fetal brain or fetal liver.

The *Atpa-3* gene (α3 panel) encodes three distinct mRNA species, approximately 6.0, 4.5, and 4.0 kb in length. The presence of multiple *Atpa-3* (α^+) mRNAs is consistent with previous data demonstrating three potential poly(A) sites for α^+ mRNA (18). The 6.0-kb mRNA was expressed predominantly in 2-week and adult brain and at lower abundance in fetal and adult heart, fetal and 2-week kidney, and 2-week and adult liver. The 4.5-kb mRNA was expressed predominantly in 2-week brain. Lower levels of this mRNA were found in fetal and 2-week heart, 2-week and adult kidney, and 2-week and adult liver. The 4.0-kb mRNA species was observed only in adult brain, adult heart, and fetal kidney. These results demonstrate that multiple forms of the α subunit are expressed in a tissue-specific and developmentally regulated fashion. This diversity arises from expression of different genes (*Atpa-1*, *Atpa-2*, and *Atpa-3*) and probably from alternative processing of a primary transcript derived from a single gene (*Atpa-3*).

Tissue-Specific and Developmental Expression of Na^+,K^+-ATPase β-Subunit mRNA. The pattern of expression of the *Atpb* (β subunit) gene is shown in Fig. 2 (β panel). The *Atpb* gene encodes four mRNA species, ranging from approximately 3.0 to 2.0 kb in length. *Atpb* transcripts were most abundant in adult brain and 2-week liver, less abundant in fetal and adult kidney, and least abundant in 2-week brain,

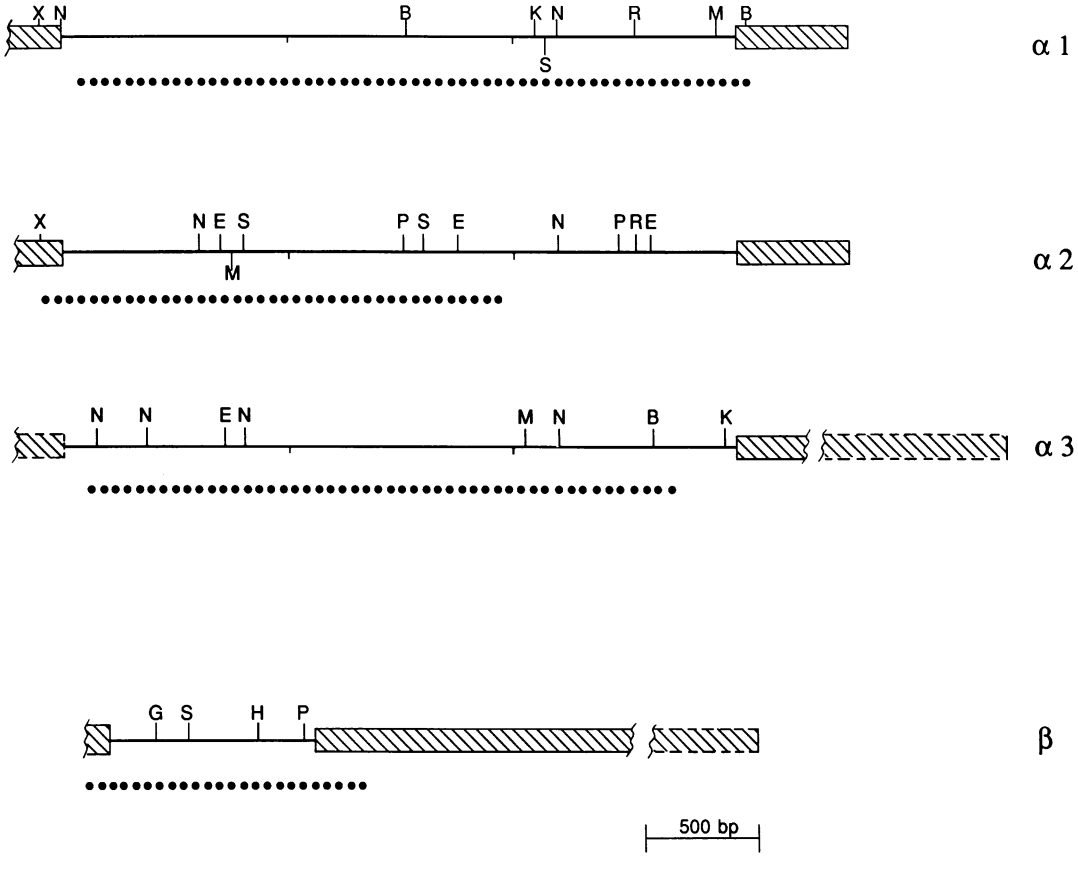

FIG. 1. Map of rat Na^+,K^+-ATPase α- and β-subunit cDNA clones. Solid lines represent the open reading frame and hatched boxes represent untranslated regions. Dotted lines represent the fragment of a clone that was used as a probe. Restriction sites: B, *Bam*HI; E, *Bst*EII; G, *Bgl* I; H, *Hin*dIII; K, *Kpn* I; M, *Mst* II; N, *Nco* I; P, *Pst* I; R, *Eco*RI; S, *Stu* I; X, *Rsr* II. bp, Base pairs.

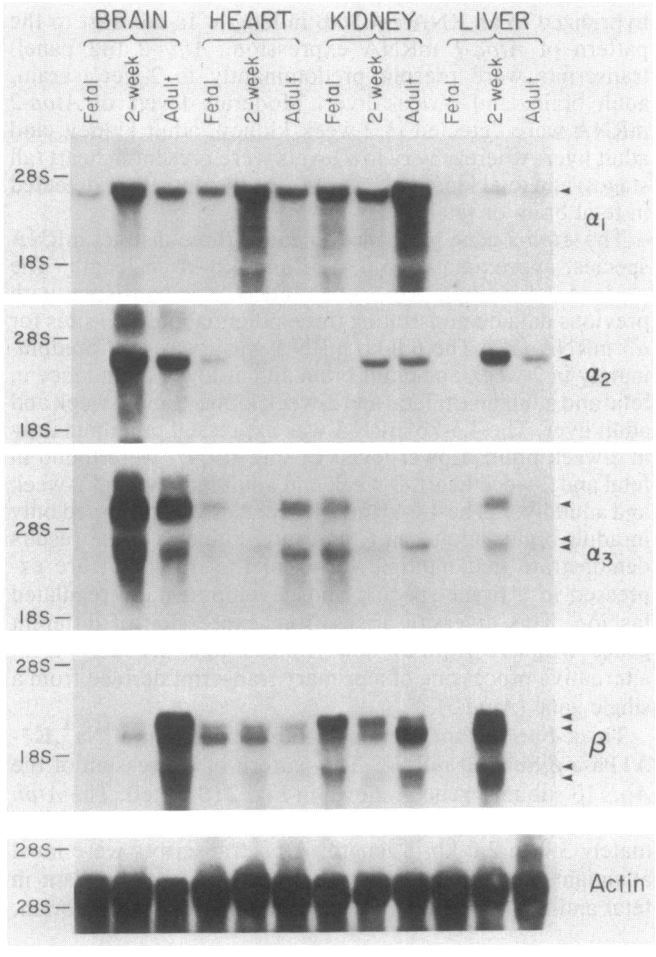

FIG. 2. Expression of Na^+,K^+-ATPase α- and β-subunit genes in rat tissues. RNA was prepared from fetal (18-day), 2-week-old, and adult rat tissues. Total cellular RNA (20 μg) was fractionated by electrophoresis through a 1% agarose/formaldehyde gel, transferred to a Zetabind filter, and sequentially hybridized with the cDNA probes indicated at the right. The positions of the 28S and 18S markers are indicated on the left. The arrowheads at the right denote the positions of the α- and β-subunit mRNA sequences.

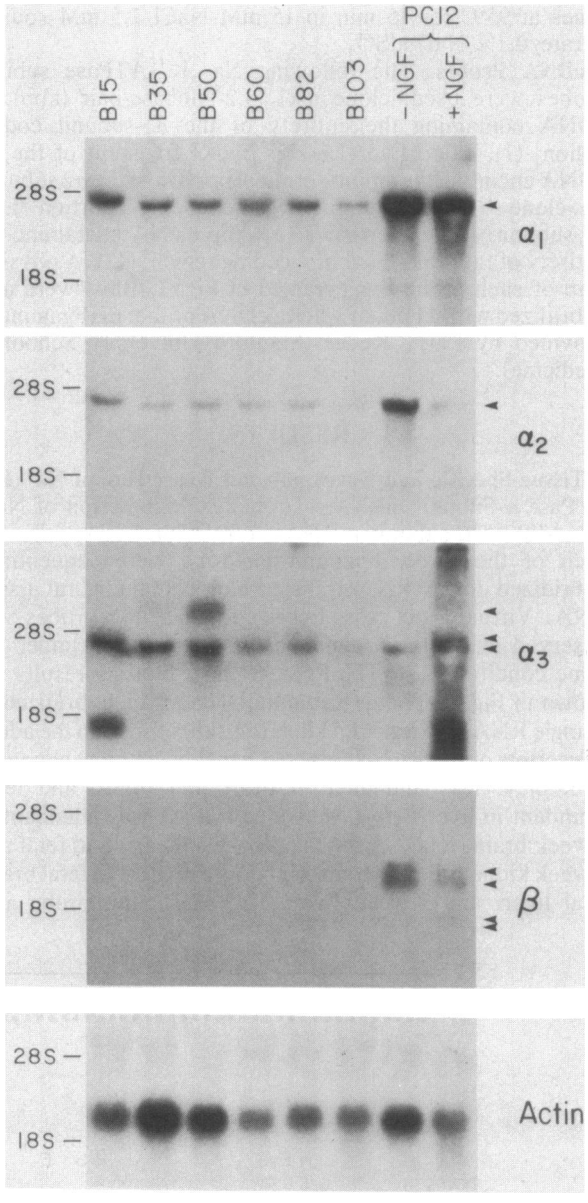

FIG. 3. Expression of Na^+,K^+-ATPase genes in rat cell lines. RNA was prepared from the rat CNS cell lines described in the text and from PC12 cells that were grown in the absence (− NGF) or presence (+ NGF) of nerve growth factor for 9 days. Total cellular RNA (20 μg) was fractionated by electrophoresis through a 1% agarose/formaldehyde gel, transferred to a Zetabind filter, and sequentially hybridized with the cDNA probes indicated at the right. The positions of the 28S and 18S markers are indicated on the left. The arrowheads at the right denote the positions of the α- and β-subunit mRNA sequences.

2-week kidney, and fetal, 2-week, and adult heart. Interestingly, there was no apparent hybridization of the probe to RNA in fetal brain, fetal liver, and adult liver. When the same blot was probed with actin cDNA (Fig. 2, actin panel), an RNA species that hybridized with approximately equal intensity was observed in all lanes. Thus the differences in intensity of hybridization obtained with each of the ATPase probes appear to be due to the relative abundance of these sequences. These results indicate that the *Atpb* gene exhibits a tissue-specific and developmental pattern of expression distinct from any member of the α-subunit gene family.

Na^+,K^+-ATPase mRNA Expression in Cell Lines Derived from the Rat CNS. Because it has been suggested that alternative forms of the Na^+,K^+-ATPase may differ in cellular or subcellular localization within the nervous system (2, 19, 20), we examined Na^+,K^+-ATPase mRNA expression in a panel of cell lines derived from the rat CNS and the rat pheochromocytoma cell line PC12. Among the cell lines tested, four (B35, B50, B60, B103) are neuronal in origin and two (B15, B82) are nonneuronal (glial) in origin (21).

A transfer blot containing RNA prepared from each of the cell lines described above was sequentially hybridized with α- and β-subunit cDNA probes. As shown in Fig. 3 (α1 and α2 panels) transcripts of the *Atpa-1* and *Atpa-2* genes were expressed in each of the cell lines tested. The *Atpa-3* gene also was expressed in each cell line (Fig. 3, α3 panel). B15, B35, B60, B82, B103, and PC12 cells expressed the 4.5-kb *Atpa-3* mRNA species, whereas B50 cells expressed the 6.0- and 4.5-kb *Atpa-3* mRNA species. Surprisingly, when the filter was hybridized with the β-subunit probe, *Atpb* mRNA sequences were detected only in PC12 cells (Fig. 3, β panel). When the blot was hybridized with actin cDNA (Fig. 3, actin panel), an RNA species that hybridized with approximately equal intensity was observed in all lanes. Thus the inability to detect β-subunit mRNA in rat cell lines appears to be due to the relatively low abundance of these sequences rather than to RNA degradation or any hybridization artifact. These results demonstrate that transcripts of all three α-subunit genes are expressed in neuronal and nonneuronal cell lines derived from the rat CNS. However, the level of expression

of β-subunit mRNA is substantially lower than the level of expression of any of the α-subunit mRNAs.

DISCUSSION

Biochemical studies have demonstrated the existence of multiple forms of the rat Na^+,K^+-ATPase that are distinct with respect to cardiac glycoside sensitivity (2) and hormonal (6, 22) and developmental (22, 23) regulation. Recent work has demonstrated that the Na^+,K^+-ATPase is encoded by a multigene family. Separate genes encoding three distinct isoforms of the α subunit and a single form of the β subunit have been identified (7). In this study we show that the mRNA products of each Na^+,K^+-ATPase subunit gene exhibit a distinct tissue-specific and developmentally regulated pattern of expression. The distinct distributions of Na^+,K^+-ATPase mRNAs may reflect important differences in the functions of individual ATPase isoforms.

The Na^+,K^+-ATPase plays a critical role in the functioning of the brain. The enzyme is found in high concentrations in neurons, where it maintains the sodium and potassium gradients that are essential for nerve impulse generation (24), and in glial cells, which are involved in the buffering of extracellular potassium following nerve activity (25). A number of studies have demonstrated the presence of α- (α1) and α^+- (α3) subunit polypeptides in rat brain (2, 22). Our results indicating that mRNA encoding the ATPase α2-subunit isoform is expressed in developing and adult rat brain clearly make it interesting to determine whether the α2-subunit polypeptide is also expressed in this tissue. The development of isoform-specific antibodies should make it possible to determine the relative abundance and anatomical localization of Na^+,K^+-ATPase isoforms within the brain and other tissues.

The differences in tissue specificity of expression of the α-subunit genes may reflect differences in the cellular and/or subcellular localization of α-subunit isoforms. Specht and Sweadner (19) have shown that the α^+ form of the ATPase exhibits increased levels of axonal transport compared to the α form of the enzyme. This suggests that the α and α^+ isoforms may differ in localization within neurons. We find that mRNAs encoding multiple α-subunit isoforms are expressed in neuronal and nonneuronal cell lines derived from the rat CNS. This observation is consistent with the view that multiple forms of the ATPase may be present in the same cell type. The availability of isoform-specific antibodies should allow identification of the ATPase polypeptides present in various cell types and their patterns of subcellular distribution.

A number of lines of evidence indicate that α- and β-subunit polypeptides are coordinately synthesized (9–11), and the molar ratio of α/β subunits in the holoenzyme is 1:1 (1). However, the differences in the level of expression of α- and β-subunit mRNAs suggest that coordinate regulation of the subunits does not occur at the transcriptional level. Schmitt and McDonough (22) have demonstrated that a 10-fold increase in Na^+,K^+-ATPase activity occurs in rat brain between 18 days of gestation and 20 days after birth. We find that the level of β-subunit mRNA in brain increases approximately 5- to 10-fold between 18 days of gestation and 14 days after birth. However, the level of α-subunit isoform mRNA increases approximately 25- to 50-fold during this same period. Our results indicate that the levels of α- and β-subunit mRNA do not exhibit a direct correlation with Na^+,K^+-ATPase activity and suggest that the availability of β-subunit mRNA plays an important role in the regulation of Na^+,K^+-ATPase expression in the brain.

Differences in the tissue distribution of α- and β-subunit mRNA raise important questions regarding the overall control of Na^+,K^+-ATPase expression. In rat CNS cell lines in particular, the level of mRNA expression of the only β-subunit gene that has been identified to date (7) is substantially lower than the level of expression of any of the α-subunit genes. If relatively equal levels of expression of α- and β-subunit mRNAs are required for Na^+,K^+-ATPase activity, then it is possible that the β-subunit gene may also be a member of a multigene family. Hubert *et al.* (26) have presented evidence suggesting that the liver contains structural variants of the β subunit. If an alternate form of the β-subunit gene is identified, it will be of interest to determine whether its pattern of expression is different from the β-subunit gene characterized in this study.

The distinct tissue-specific and developmental pattern of expression of the Na^+,K^+-ATPase gene family has important implications regarding the functional significance of Na^+,K^+-ATPase isoform diversity. The multiplicity of cellular processes in which the enzyme is involved may reflect differences in the functions of the various Na^+,K^+-ATPase isotypes. Alternatively, the various isoforms may each carry out the same functions but their activities may be differentially regulated. For example, the α and α^+ forms of the Na^+,K^+-ATPase appear to be present in fat cells, where the α^+ form is selectively sensitive to stimulation by insulin (6). The α (α1) and α^+ (α3) isoforms from rat brain exhibit markedly different affinities for the cardiac glycoside drugs (2, 5), and a cDNA encoding the α1 subunit of the mouse Na^+,K^+-ATPase has been shown to confer ouabain resistance to ouabain-sensitive monkey cells (27). The isolation of full-length cDNA clones for three α-subunit isoforms from rat brain (7, 18) and the development of an expression system to test the biological activity of α-subunit genes by way of DNA transfer (27) should now make it feasible to analyze the functional properties of these molecules.

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