The Kidney Sodium-Calcium Exchanger[a]

JONATHAN LYTTON,[b,c] SHWU-LUAN LEE,[b]
WEN-SEN LEE,[b] J. VAN BAAL,[d] RENÉ J. M. BINDELS,[d]
RACHEL KILAV,[e] TALLY NAVEH-MANY,[e]
AND JUSTIN SILVER[e]

[c]*Renal Division*
Department of Medicine
Brigham and Women's Hospital
and
Harvard Medical School
Boston, Massachusetts 02115

[d]*Department of Cell Physiology*
University of Nijmegen
The Netherlands

[e]*Minerva Center for Calcium and Bone Metabolism*
Nephrology Services
Hadassah University Hospital
Jerusalem il-91120, Israel

INTRODUCTION

The maintenance of systemic calcium balance is of essential importance to terrestrial vertebrates due to the critical involvement of this ion in a variety of both intracellular signaling and extracellular roles, as well as in the maintenance of the skeletal system. Three organ systems—intestine, bone and kidney—control body calcium under the regulation of hormones such as parathyroid hormone (PTH), 1,25-dihydroxy-vitamin D_3 ($1,25[OH]_2D_3$), and calcitonin, all coordinated by the parathyroid gland. The function of the kidney is essential to this complex network since, of all the organs, it handles by far the most calcium and is the site of conversion of vitamin D to the active metabolite, $1,25[OH]_2D_3$.[1-3]

In the kidney more than 97% of the free calcium filtered at the glomerulus is reabsorbed as the filtrate passes down the nephron. A large fraction of this calcium reabsorption occurs passively and via paracellular pathways in the proximal tubule and the loop of Henle. The final control of calcium reabsorption and its hormonal regulation, however, takes place in the distal nephron. At this site, calcium transport proceeds against an electrochemical gradient in a transcellular manner. Two transport systems on the basolateral membrane of distal nephron cells provide

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[b] An Established Investigator of the American Hearth Association. Address for correspondence: Department of Medical Biochemistry, University of Calgary Health Sciences Centre, 3330 Hospital Drive, NW, Calgary, Alberta, Canada, T2N 4N1.

the driving force for transcellular flux: the plasma membrane calcium-pumping ATPase, and the sodium-calcium exchanger. Several lines of evidence argue persuasively for a substantial involvement of the exchanger in driving calcium reabsorption.[4,5] Nevertheless, due to the lack of specific inhibitors and the potential for secondary artifacts due to the experimental manipulations, the precise relative roles of these two enzymes remain the subject of debate. Indeed, the location of expression of Na-Ca exchanger in the kidney is also controversial. Although several groups find an enrichment in membranes of distal tubules, others have presented arguments for exchanger presence in proximal tubule and collecting duct.[1,6–8]

Several factors are thought to modulate calcium reabsorption in the distal nephron, including $1,25[OH]_2D_3$, PTH, and both thiazide and amiloride diuretics.[1,8] $1,25[OH]_2D_3$ is believed to exert its effect through regulation of the expression of a 28 kDa cytoplasmic calcium binding protein, calbindin-D28k. However, regulation of transporter protein expression by $1,25[OH]_2D_3$, as has been observed in the intestine,[9] has not been carefully examined in kidney. PTH has been suggested to stimulate both the entry of calcium into distal nephron cells,[10] as well as its exit via the Na-Ca exchanger,[11] although the mechanism for these events has not been clearly elucidated. The diuretics, through their inhibition of sodium uptake at the apical membrane, stimulate calcium reabsorption indirectly. Whether this is due to potential changes across the apical membrane that increase calcium entry, or to an increase in the sodium gradient which drives basolateral exit through the Na-Ca exchanger, is unclear.[12–15]

Nevertheless, it is evident that the Na-Ca exchanger plays an important role in the active reabsorption of calcium in the distal nephron. Inasmuch as this action serves to help regulate systemic, *extracellular* calcium rather than *intracellular* calcium, the factors that regulate kidney Na-Ca exchanger activity are likely to be different from those that regulate exchange activity in other cells, such as cardiac myocytes. The investigation of such matters requires a better understanding of the molecule that underlies renal Na-Ca exchange activity, and thus in recent years several groups, including our own, have cloned the kidney Na-Ca exchanger.[16–20] Surprisingly, the molecule turns out to be an alternatively spliced product of the NCX1 gene expressed most abundantly in cardiac tissue. In this paper we describe our work on the expression of the rat Na-Ca exchanger gene in kidney, and other tissues, which suggests that regulation of this molecule is under the control of different promoters in different tissues.

METHODS

In situ hybridization was performed using a partially base-hydrolyzed ^{35}S-labeled riboprobe corresponding to the entire rat kidney F1 clone, as previously described.[21,22] Immunocytochemical procedures were as previously described.[23] The RA-RRCaBP antiserum against calbindin was a generous gift of Sylvia Christakos, University of Medicine and Dentistry of New Jersey, and the C2C12 antibody against the Na-Ca exchanger was a generous gift of Kenneth Philipson, UCLA.

Molecular cloning, RNA isolation, Northern blot hybridization, and 5′-rapid amplification of cDNA ends (RACE) were all as previously described.[17] RNase protection experiments, using ^{32}P-labeled riboprobes corresponding to our genomic clones, or to chimeras between genomic and cDNA clones, were performed according to established procedures.[24]

Models of dietary induced hypo- and hyperparathyroidism, parathyroidectomy, and vitamin-D deficiency were all induced in adult rats as previously described.[25] Following a treatment of two weeks, the animals were sacrificed, the kidneys removed and immediately frozen. Total RNA was isolated from whole kidneys according to standard procedures as previously described.[17]

RESULTS AND DISCUSSION

Our initial studies published three years ago employed the polymerase chain reaction (PCR) to identify a fragment of what proved to be the NCX1 gene that was expressed in kidney.[19] Northern blotting data (see FIG. 4, top panel) revealed that in fact NCX1 was expressed at high abundance, not only in kidney and heart, but also in various brain regions. FIGURE 1 shows the distribution of Na-Ca

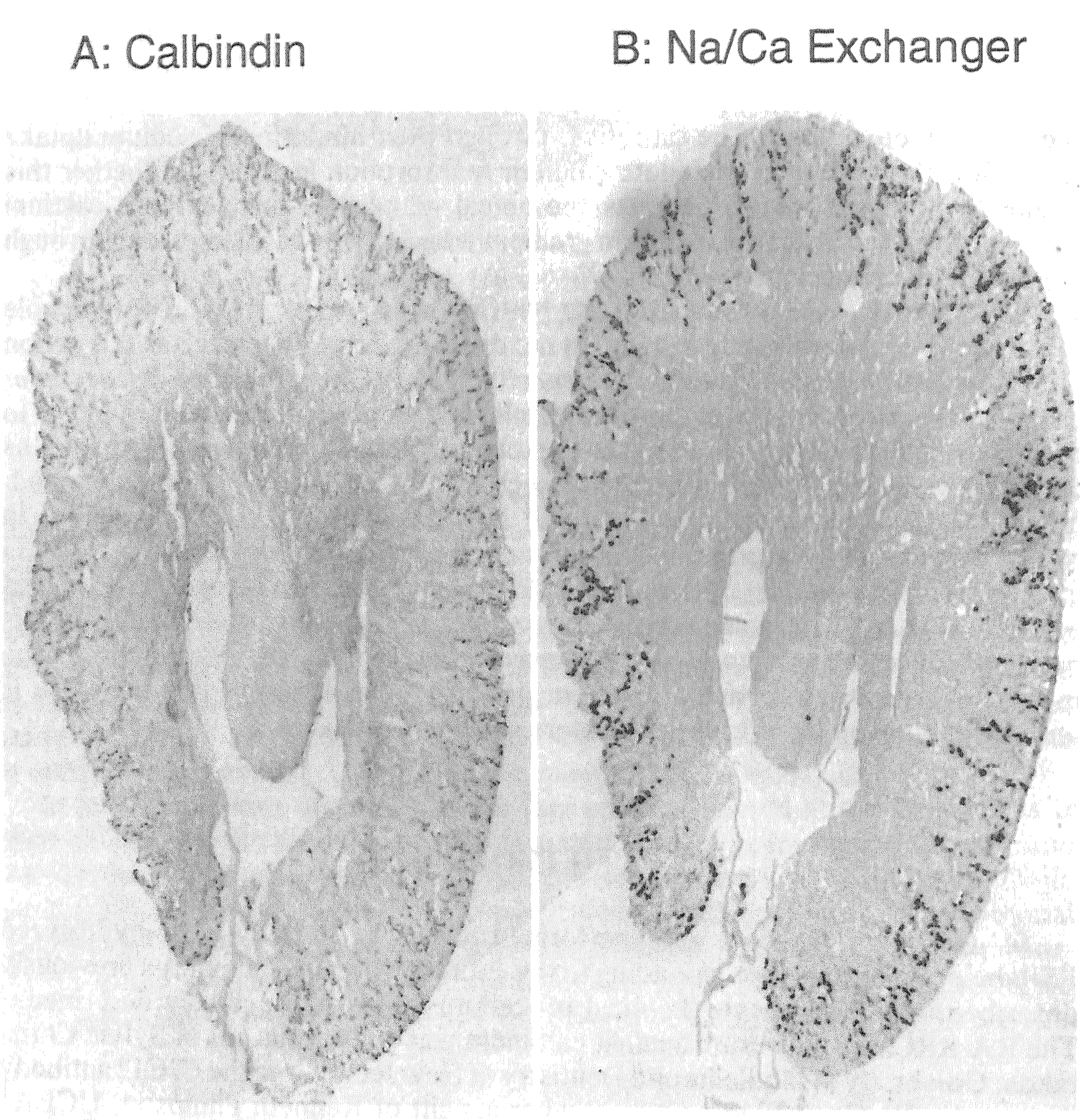


FIGURE 1. Localization of Na-Ca exchanger transcripts in rat kidney. Serial sections of fixed rat kidney were **(A)** stained for calbindin-D28k with RRCaBP antiserum by the immunoperoxidase method or **(B)** hybridized to an antisense riboprobe for Na-Ca exchanger and exposed to emulsion autoradiography. Note the significant overlap of signal for these two probes, which is particularly evident toward the *top left corner* of the figure.

exchanger transcripts in a section of rat kidney determined by *in situ* hybridization, and compared to the expression in a serial section of calbindin-D28k, a protein known to be expressed in the distal nephron, predominantly in distal convoluted tubule and connected segments. The pattern of expression of these two species is clearly similar, and demonstrates that virtually *all* of the abundant Na-Ca exchanger mRNA seen on Northern blots is restricted to only a small fraction of nephron segments—the distal tubule—while essentially no Na-Ca exchanger message is observed elsewhere in the kidney.

The distribution of Na-Ca exchanger protein in rabbit kidney is shown at higher magnification in FIGURE 2, and compared to the expression of calbindin-D28k and the plasma membrane Ca-ATPase in serial sections. The Na-Ca exchanger protein is clearly expressed on the basolateral membrane of what appear to be connecting segment cells. Similar data have also been published by others.[26,27] Interestingly, it had previously been reported that expression of the Na-Ca exchanger was restricted to the connecting segment, while expression of the plasma membrane Ca-ATPase was only in the distal convoluted tubule.[28] Our data show a remarkable overlap of expression of these two proteins in rabbit kidney, together with calbindin-D28k. Further analysis by double-label immunofluorescent staining of rat kidney sections (data not shown) indicates an almost complete overlap of expression of Na-Ca exchanger and plasma membrane Ca-ATPase. The calcium transporter positive cells form a subset of the calbindin-D28k positive cells, and overlap with expression of the thiazide-sensitive Na,Cl-cotransporter protein. These comparisons, together with the morphology of the tubule sections, suggest that Na-Ca exchanger is expressed most strongly in the late portion of the distal convoluted tubule (in true distal convoluted tubule cells), and in essentially all true connecting segment cells throughout the connecting segment, although at levels which diminish as one travels further along this segment. The extremely high level of expression of Na-Ca exchanger mRNA and protein, together with other calcium transport proteins, in precisely those nephron segments associated with active transcellular calcium reabsorption, clearly demonstrates that the exchanger plays an important role in this transport process.

Examination of our rat renal Na-Ca exchanger clone, F1, with other Na-Ca exchanger clones from various species revealed two regions of significant divergence.[17] One region corresponded to the site of alternative splicing within the central cytoplasmic loop of the molecule, which has been well characterized by the studies of Kofuji *et al.*[29] (and presented elsewhere in this volume). The other region corresponded to the 5′-untranslated portion of the transcript. To characterize further the 5′-end of Na-Ca exchanger transcripts expressed in various rat tissues, we used the technique of 5′-RACE. As illustrated in FIGURE 3, a single round of amplification revealed a single major band in RNA from heart, two major bands in kidney and three bands in brain. Analysis by subcloning, sequencing, and restriction mapping, allowed us to identify each of the major bands, indicated by the labeling to the right of the gel and the cartoon above it in FIGURE 3. Three separate species were defined by this analysis, each comprising a unique 5′-end sequence joined to a common sequence at position −34 relative to the initiating methionine codon. One such species, Kc, was isolated from kidney cortex mRNA. Another, Br (previously referred to as Br1), also was identified in brain mRNA. The third species, Ht (previously Br2), also was identified in all three tissues, as bands of different size. In heart the band at ~300 nt corresponds to Ht(s) with 112 nt of 5′-untranslated sequence. Two Ht species were identified in brain, Ht(m) and Ht(l). The latter, which was also found in kidney, contained 220 nt of 5′-untranslated sequence.

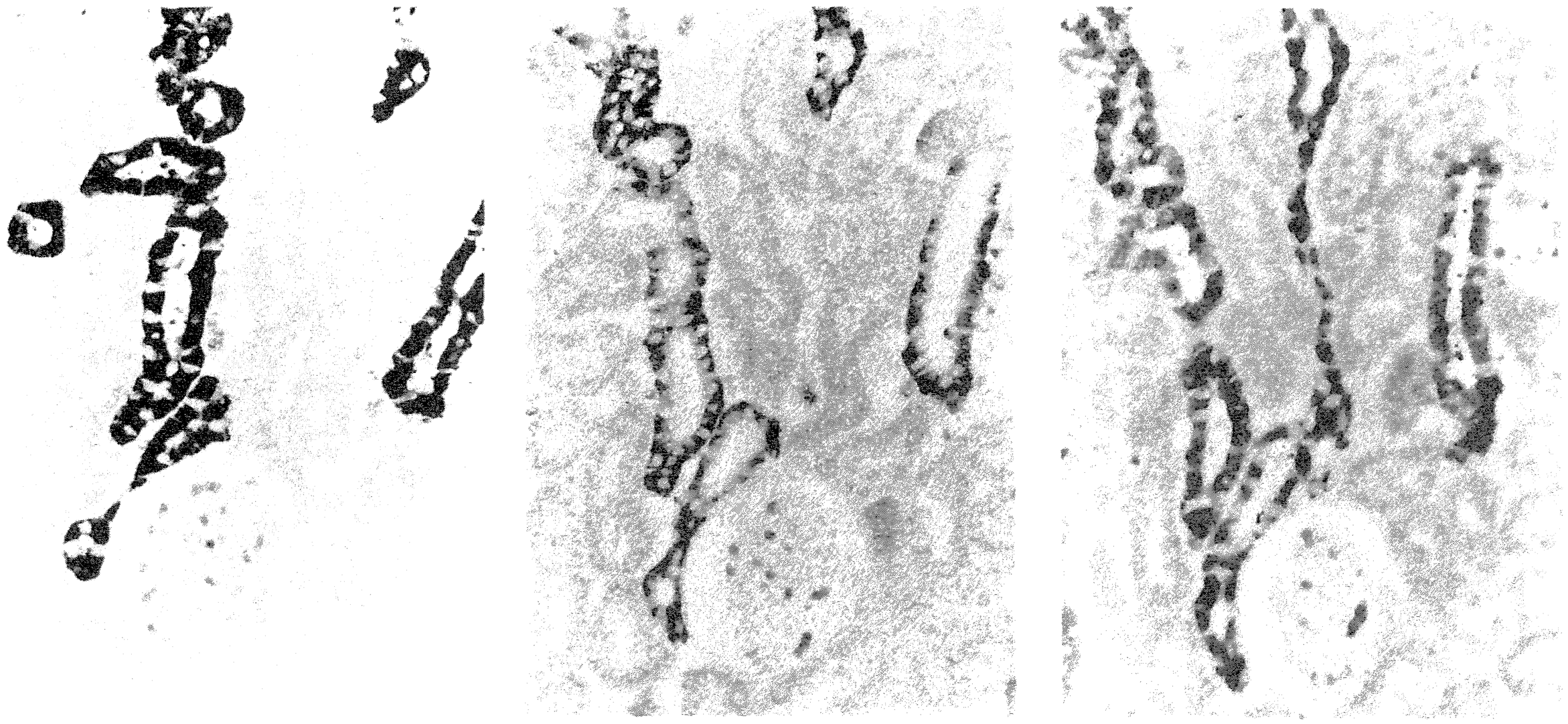


FIGURE 2. Localization of calcium transport proteins in rabbit kidney. Serial sections of fixed rabbit kidney were reacted with **(A)** antibody 0089 against calbindin, **(B)** antibody C2C12 against the Na-Ca exchanger, or **(C)** antibody 5F10 against the plasma membrane Ca-ATPase. Antibody reactivity was visualized with peroxidase staining. Note that the tubule which reacts with the plasma membrane Ca-ATPase antibody, and runs vertically toward the center of (C), cuts obliquely through these sections and only the portion toward the *top* of the figure is present in (A) and (B). Note also the intense basolateral staining with the Na-Ca exchanger antibody.

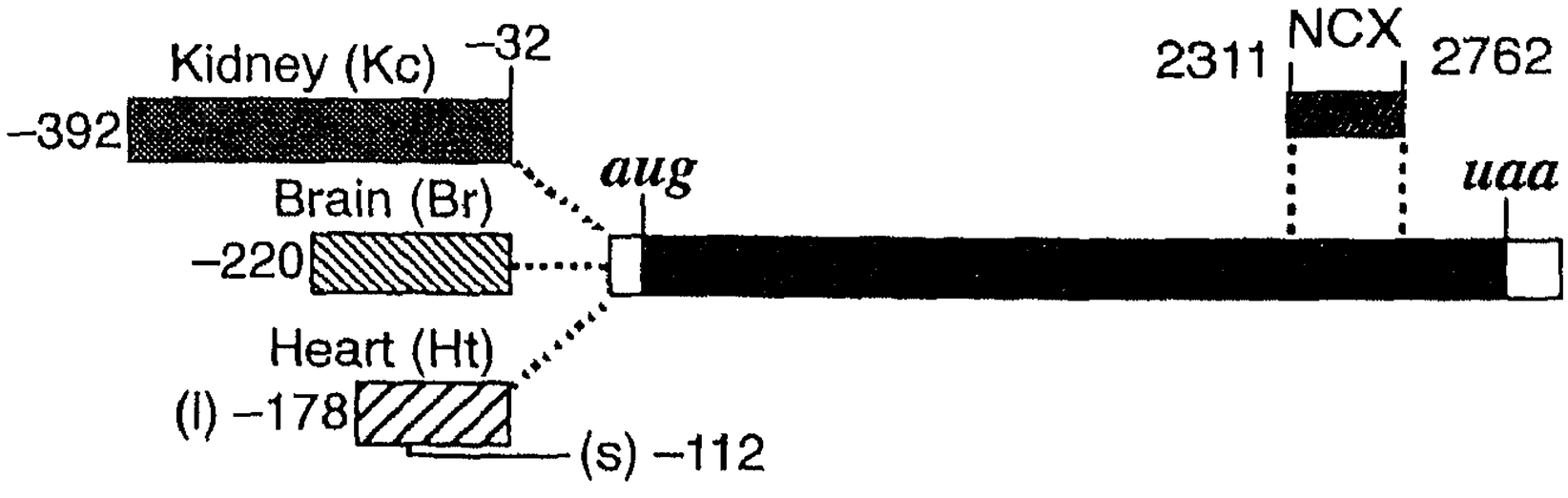


FIGURE 3. 5′-RACE amplification of Na-Ca exchanger transcripts in rat kidney, brain, and heart. The *cartoon* illustrates the coding region of the rat Na-Ca exchanger transcript in *black*, the extent of the three unique 5′-untranslated sequences, and the location of the common coding region probe, NCX-1. The *lower panel* shows an ethidium bromide stained polyacrylamide gel of the products from a typical RACE experiment. The antisense 3′ primer used for these experiments anneals beginning at nt 168, while the 5′ anchoring primer adds 35 nt to the length of the product. Thus the size of bands corresponding to the indicated Kc, Br, Ht(l) and Ht(s) products are 595, 423, 381 and 315 nt in length, respectively.

The expression of Na-Ca exchanger transcripts possessing each of these unique 5′-end sequences was examined by Northern blot analysis, as shown in FIGURE 4. The Br probe reveals a pattern of Na-Ca exchanger mRNA expression quite similar to the pattern seen with the common coding region probe, NCX-1, except for the conspicuous absence of a band in heart, and a dramatically reduced signal in kidney cortex. As expected, the Ht probe revealed a strong band in RNA from heart, but also a weaker signal in large intestine, and a weak signal in cerebrum RNA (at ~14 kb). The Kc probe revealed a strong band in only kidney cortex mRNA. Thus it is clear that Na-Ca exchanger transcripts with unique 5′-ends are expressed in a tissue-specific fashion, most likely due to the existence of three separate promoters which drive expression in heart, kidney, and elsewhere. Indeed, such a scenario would impart the potential for independent regulation of the Na-Ca exchanger, as anticipated by the different roles the molecule plays, particularly in the kidney compared to other tissues.

Investigation of selective Na-Ca exchanger expression and its regulation clearly requires an analysis of the gene, particularly the promoter regions. Toward that

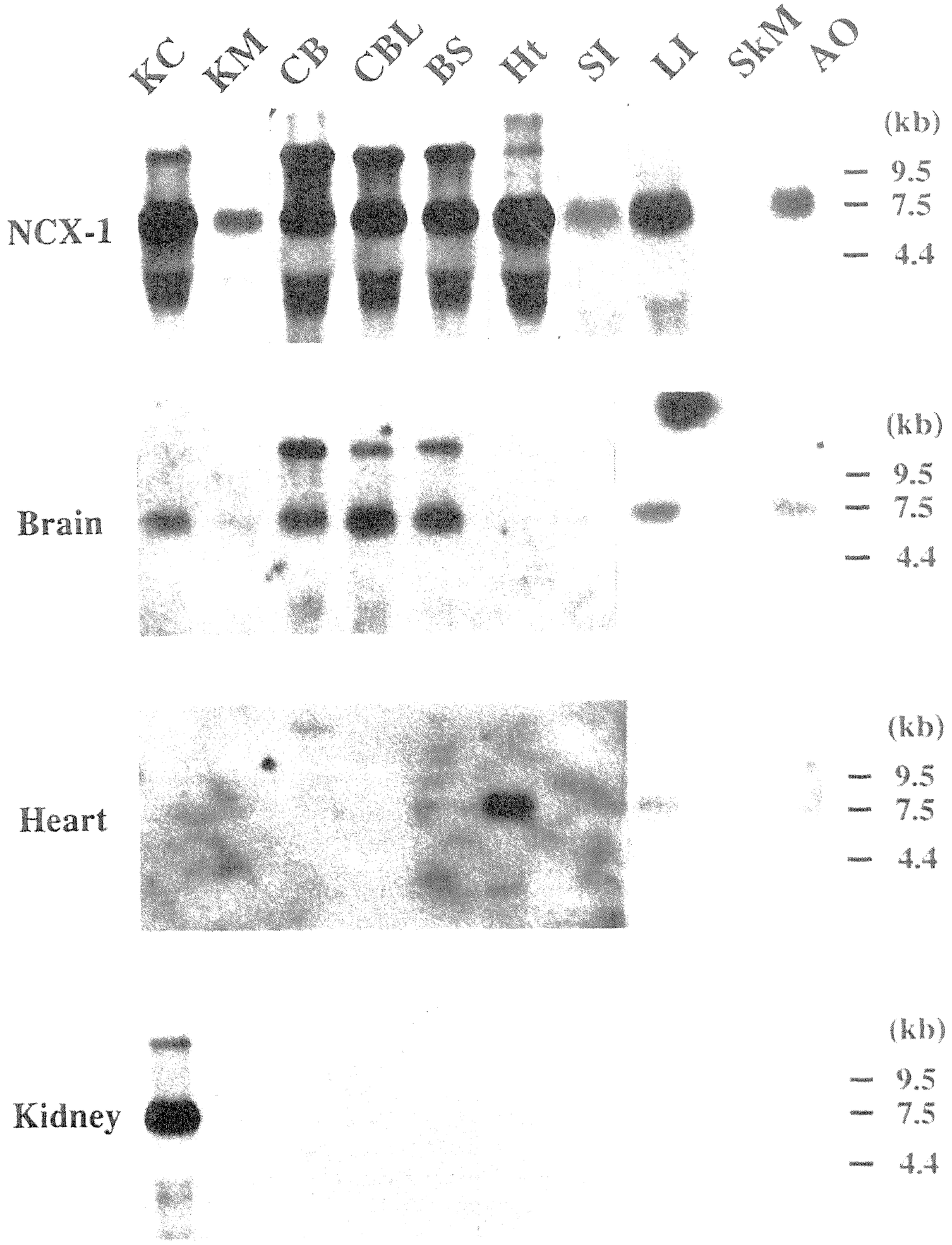


FIGURE 4. Tissue distribution of Na-Ca exchanger isoforms. Four parallel Northern blots were hybridized with probes from the common region (NCX-1) or the unique 5′-end exons corresponding to brain (Br), heart (Ht) and kidney (Kc). The brain and heart probes were short DNA probes, and hence the signal-to-noise ratio is poorer than for the longer riboprobes used for the kidney and NCX-1 probes. Ten micrograms of total RNA was loaded in each lane, isolated from the following rat tissues: KC, kidney cortex; KM, kidney medulla; CB, cerebrum; CBL, cerebellum; BS, brainstem; Ht, heart left ventricle; SI, small intestine; LI, large intestine; SkM, skeletal muscle (diaphragm); AO, aorta.

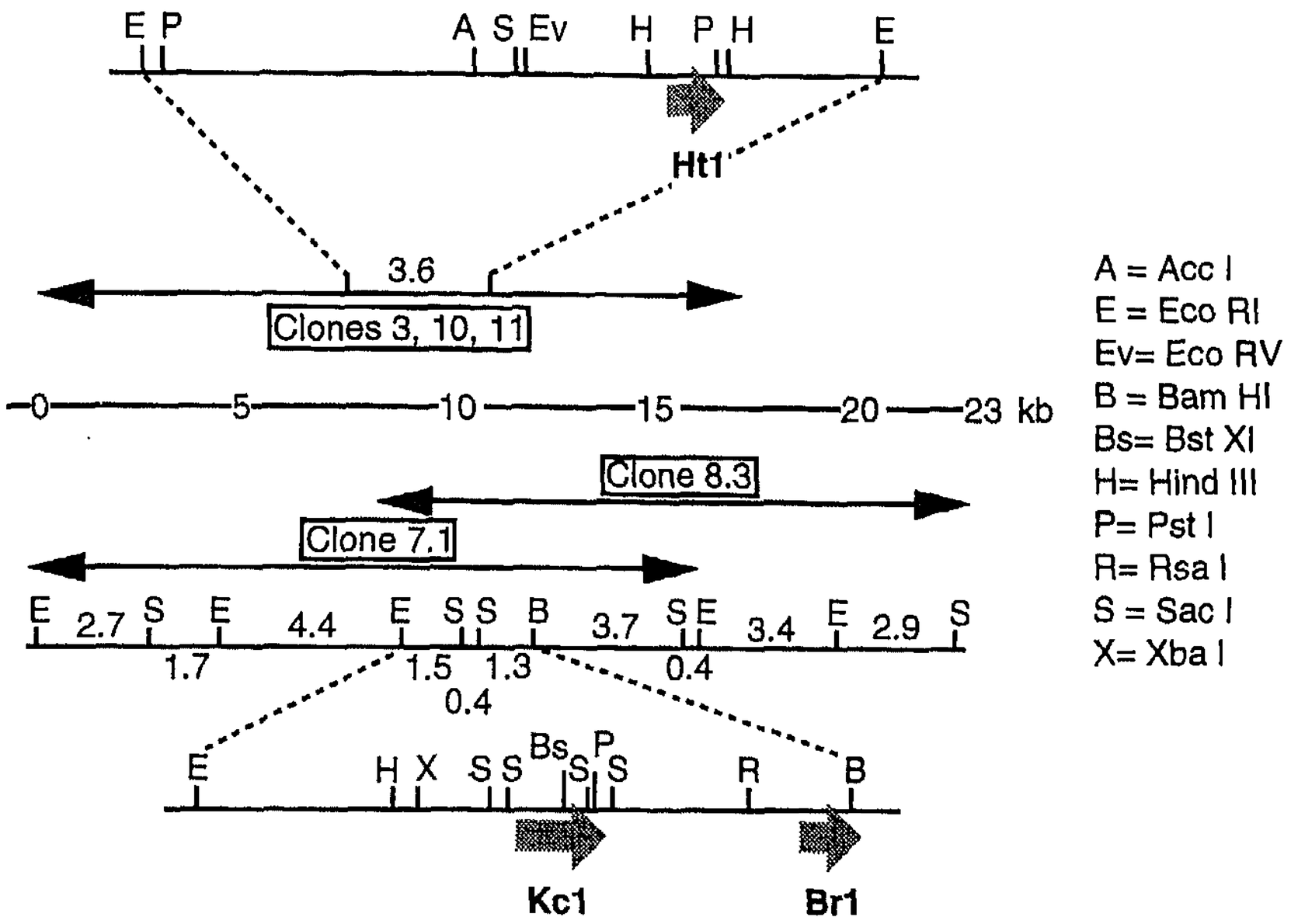


FIGURE 5. Genomic clones encoding the 5′-end exons of the rat Na-Ca exchanger gene. Partial restriction maps of two separate contigs encompassing the Ht exon (contig comprising clones 3, 10 and 11), and the Kc and Br exons (contig comprising clones 7.1 and 8.3). Exons were mapped via a combination of restriction digests, hybridization, and sequencing. The direction of transcription is indicated. Note that the two contigs do not overlap.

end, we have isolated rat genomic clones which contain each of the three unique 5′-untranslated sequences, Ht, Br, and Kc. As illustrated in the cartoon of FIGURE 5, we have located the Kc and Br exons within about 1.5 kb of each other in the center of a ~23 kb genomic contig. Clones containing the Ht exon were also isolated, but do not overlap with the Kc/Br contig, indicating that these exons are separated by at least 10 kb. Moreover, none of these clones contains sequence from the subsequent downstream exon 2, which includes the protein initiation codon. From these data we cannot order the Ht exon with respect to the Kc/Br exons. Data from Don Menick's laboratory on the Na-Ca exchanger gene of the cat (presented elsewhere in this volume) as well as anecdotal evidence based on the two bovine Na-Ca exchanger clones described by John Reeves' laboratory,[30] however, suggest that Ht lies upstream of Kc/Br. The genomic sequences match our cDNA and RACE clones exactly but all end in a common CAG sequence, suggesting that the true splice site which generates the mature Na-Ca exchanger transcripts lies at position −32 in the mRNA.

To determine if our RACE and cDNA clones corresponded to full-length transcripts, we used the genomic clones as templates to perform RNase protection experiments, as illustrated in FIGURE 6. In these experiments, antisense ^{32}P-labeled riboprobes based on genomic sequences are annealed to RNA from various tissues. The annealed complexes are then digested with an RNase that will degrade any single-stranded RNA. Thus, only those genomic sequences that are present in the

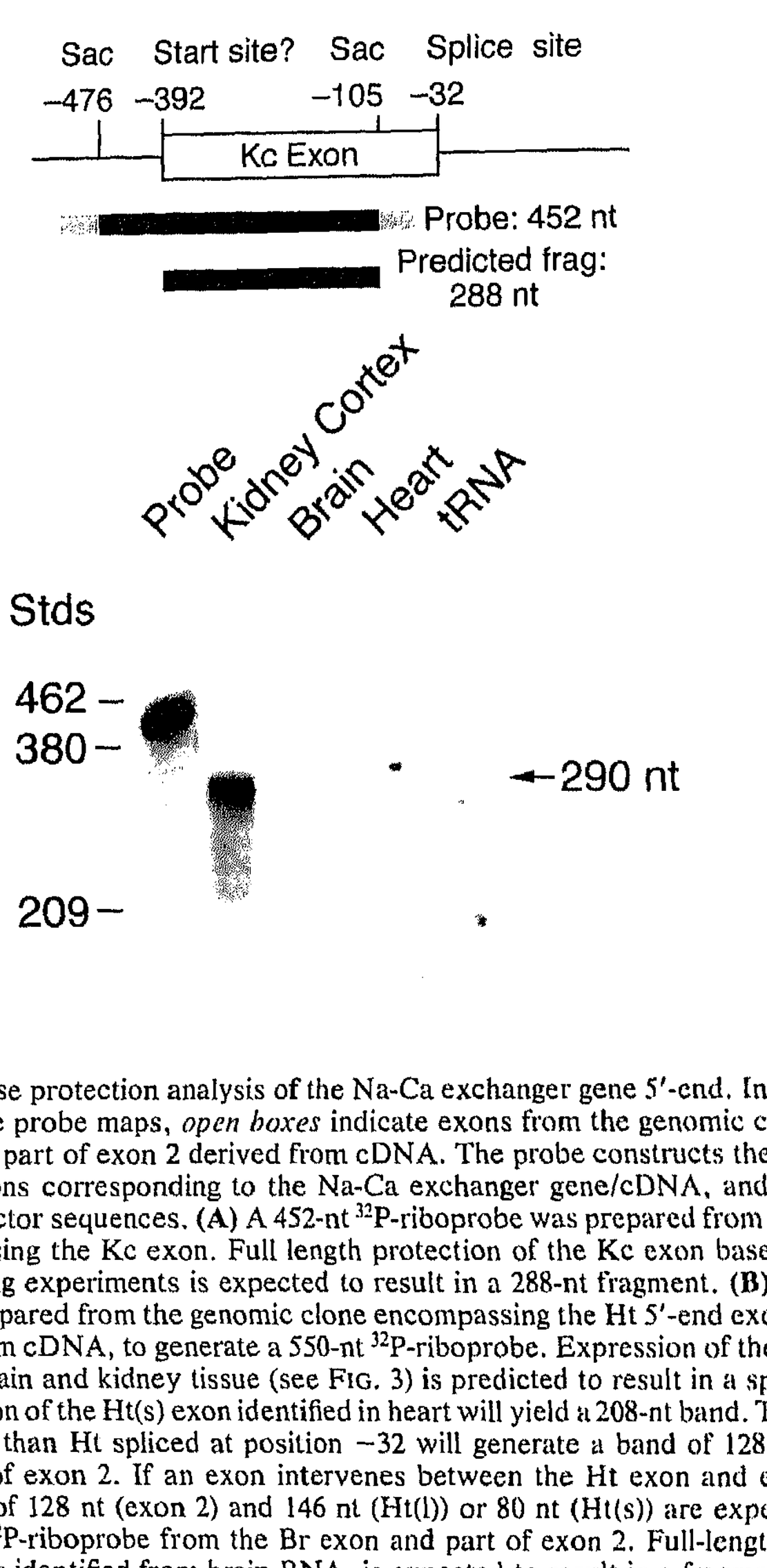


FIGURE 6. RNase protection analysis of the Na-Ca exchanger gene 5′-end. In the *cartoon* illustrations of the probe maps, *open boxes* indicate exons from the genomic clones, while the *shaded box* is part of exon 2 derived from cDNA. The probe constructs themselves are *black* in the regions corresponding to the Na-Ca exchanger gene/cDNA, and *grey* at the ends to denote vector sequences. **(A)** A 452-nt ^{32}P-riboprobe was prepared from the genomic region encompassing the Kc exon. Full length protection of the Kc exon based on cDNA and RACE cloning experiments is expected to result in a 288-nt fragment. **(B)** A chimeric construct was prepared from the genomic clone encompassing the Ht 5′-end exon, and from part of exon 2 from cDNA, to generate a 550-nt ^{32}P-riboprobe. Expression of the Ht(l) exon, as identified in brain and kidney tissue (see FIG. 3) is predicted to result in a species of 274 nt, while protection of the Ht(s) exon identified in heart will yield a 208-nt band. The presence of an exon other than Ht spliced at position −32 will generate a band of 128 nt from the common region of exon 2. If an exon intervenes between the Ht exon and exon 2, then protected bands of 128 nt (exon 2) and 146 nt (Ht(l)) or 80 nt (Ht(s)) are expected. **(C)** A chimeric 600-nt ^{32}P-riboprobe from the Br exon and part of exon 2. Full-length protection of the Br exon, as identified from brain RNA, is expected to result in a fragment of 301 nt. If another exon is spliced in place of the Br exon at position −32, a band of 113 nt is expected. Riboprobes were each annealed to 20 μg of total RNA from the indicated rat tissues, or 20 μg of tRNA (as negative control). Following RNase digestion, the protected products were separated on a 5% acrylamide-50% urea gel, together with an aliquot of the undigested probe, and exposed to X-ray film. The position of size standards (Stds), as well as the approximate size of the protected species (*arrows*), are indicated. Due to the rather high nonspecific background, two representative experiments are illustrated for the Br probe in (C).

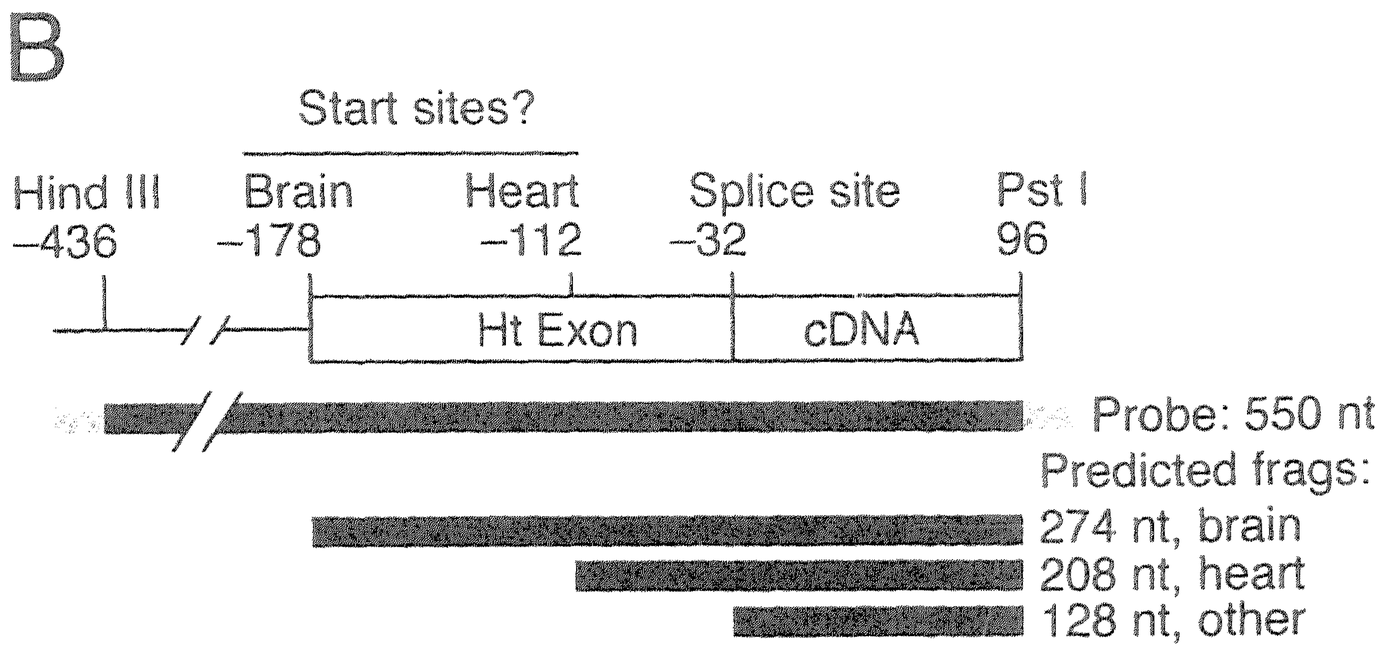


FIGURE 6. (*Continued*)

transcripts will be protected from degradation. The length of these products then defines the start of transcription (if, indeed, the probes derive from the 5′-most exon in the gene).

Panel A of FIGURE 6 demonstrates that RNA from kidney, but not from brain or heart, protects a ~290-nt fragment of the genomic clone which corresponds almost exactly to our predictions for the start site of the kidney Na-Ca exchanger transcript. To examine the heart transcript, we prepared a chimeric genomic/cDNA construct which fuses the Ht exon to the downstream exon 2. This probe results in a longer protected fragment than would be obtained using the Ht exon alone and, thus, a greater signal-to-noise ratio. Panel B shows that RNA from heart protects a band of ~205 nt, which matches very closely the predicted size based on the cDNA and RACE cloning data. No other major bands were observed in heart. Brain RNA, on the other hand, protected an Ht probe band of ~270 nt, which matched the expectations for the length of the cDNA observed. In addition, however, a strong doublet was observed at ~130/140 nt. Since the major Na-Ca exchanger transcript in brain possesses a Br exon end, the Ht probe is expected to produce only a partially protected band of 128 nt. Additionally, if another exon

C

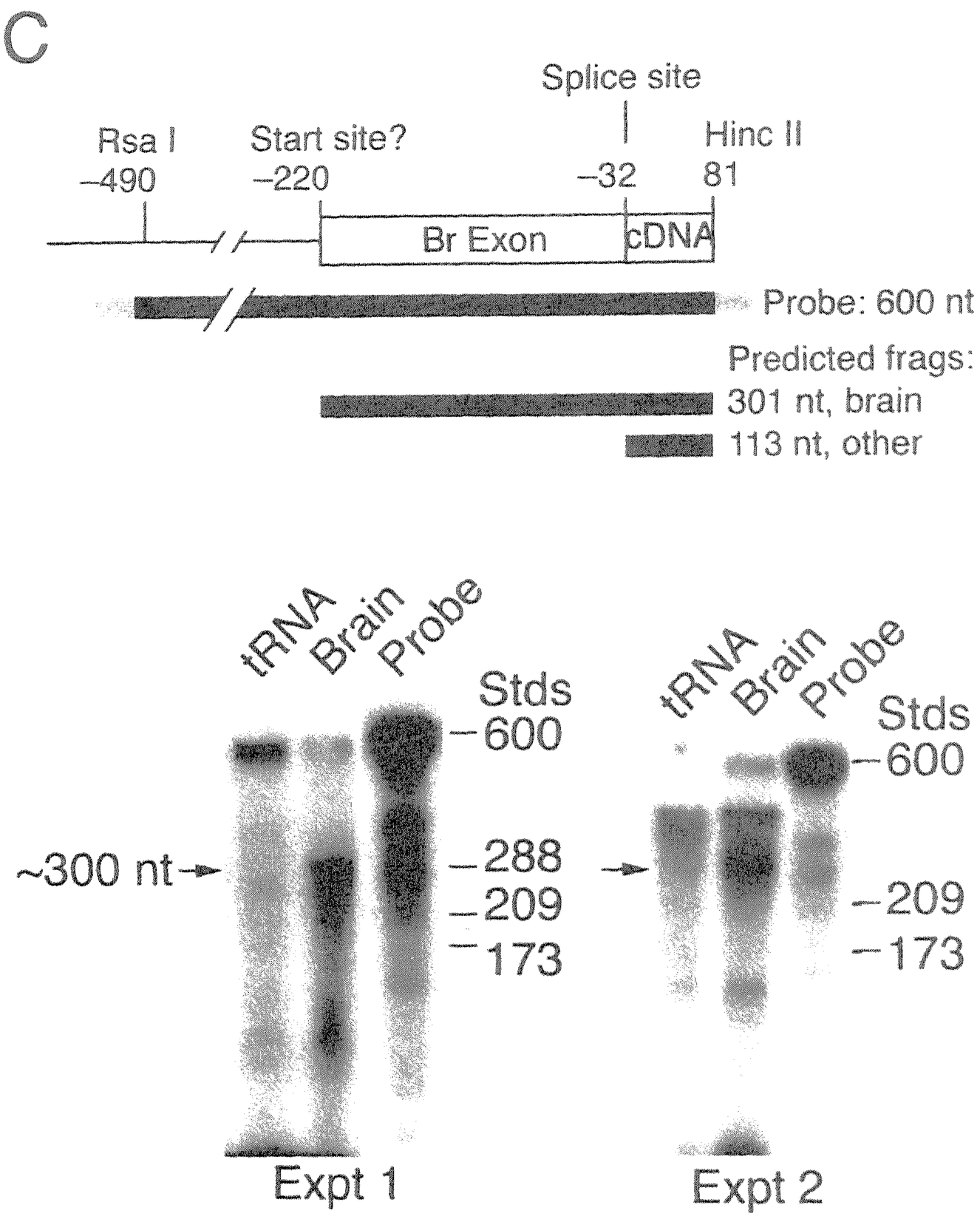
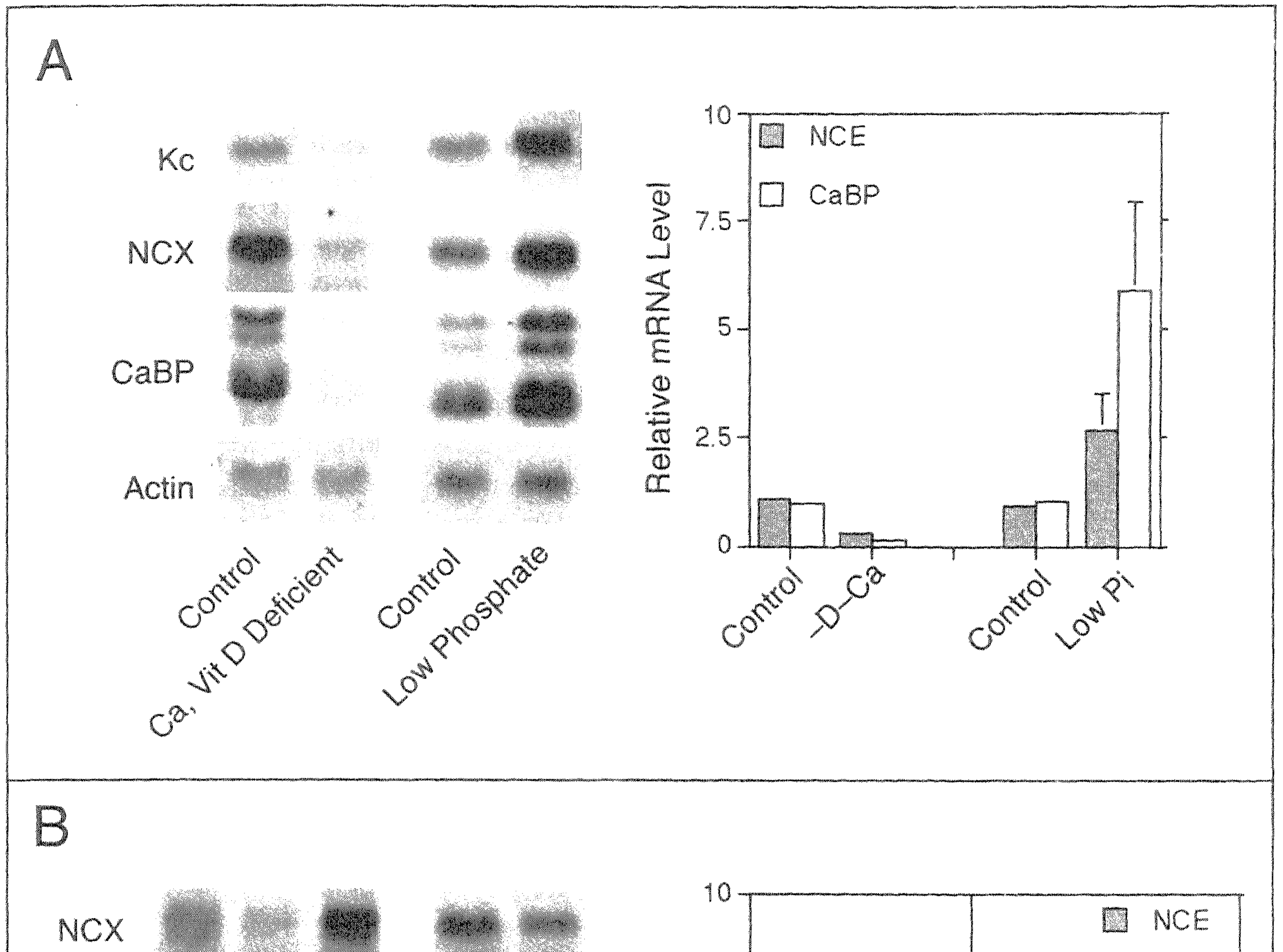


FIGURE 6. (*Continued*)

(Br, Kc?) were to intervene between the Ht exon and exon 2 in brain, then both 128 nt and 146 nt protected fragments would be observed. Finally, as shown in panel C, a chimeric Br construct results in the protection of a ~300 nt fragment as predicted. The Br probe is from a very GC-rich genomic region, however, which has given us trouble with the signal-to-noise ratio. Two representative experiments are shown to help illustrate the specificity of the 300 nt fragment.

These data provide rather good evidence that transcription of the Na-Ca exchanger in kidney, brain and heart derives from different points in the gene. Further experiments are now in progress to determine promoter elements in the 5′-flanking region, and to investigate the nature of both tissue-specific and regulated expression.

Since the renal Na-Ca exchanger is implicated in the control of calcium reabsorption and systemic calcium homeostasis, we have begun experiments to investigate how alterations to body calcium balance influence the expression of the exchanger in kidney. As shown in FIGURE 7A, rats treated with a diet deficient


A
Kc
NCX
CaBP
Actin
Control
Ca, Vit D Deficient
Control
Low Phosphate
Relative mRNA Level
10
7.5
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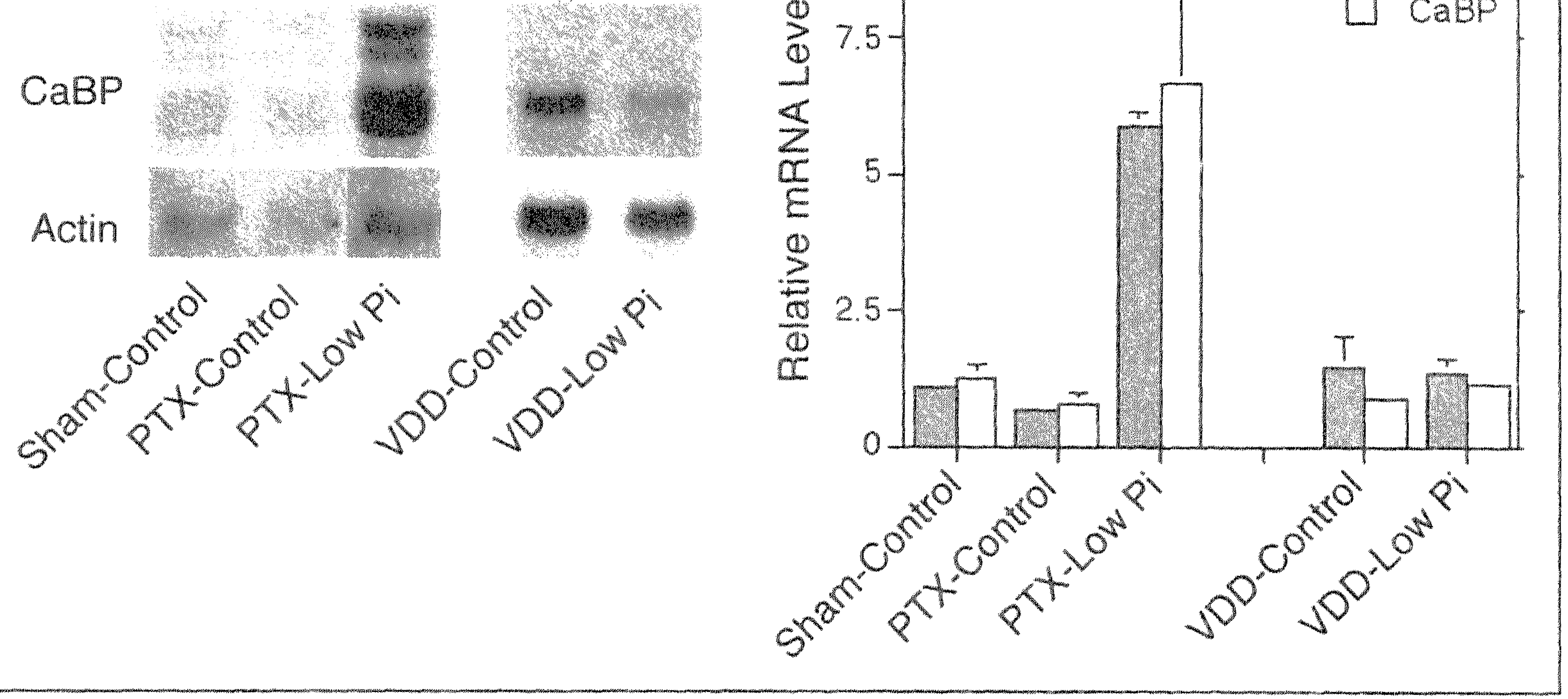

FIGURE 7. *In vivo* regulation of Na-Ca exchanger expression. Ten micrograms of total RNA isolated from kidneys of rats treated as indicated were resolved on formaldehyde-agarose gels, transferred to nylon membranes, and hybridized with probes corresponding to the common coding region of the Na-Ca exchanger (NCX), the kidney 5′-end exon of the Na-Ca exchanger (Kc), calbindin-D28k (CaBP), or β-actin (Actin). Single representative autoradiographs are shown to the *left*. The level of Na-Ca exchanger (NCE) and calbindin-D28k (CaBP) mRNA is shown quantitatively, normalized to the β-actin content with control diet levels set to 1.0 and averaged over a number of experiments, in the graphs to the *right*. *Error bars* indicate the standard error of the mean. (A) Control (n = 11) *versus* calcium and vitamin D-deficient (–D–Ca) (n = 4) and control (n = 9) *versus* low phosphate (Low Pi) (n = 4) diets. **(B)** Either sham-operated, parathyroidectomizid (PTX), or second-generation vitamin D-deficient (VDD), animals were treated with control or low phosphate (Low Pi) diets (n = 2 for each group, except the sham control, for which n = 4).

in both calcium and vitamin D show a marked reduction in the content of renal Na-Ca exchanger transcript. Conversely, rats treated with a diet deficient in phosphate had elevated levels of Na-Ca exchanger mRNA. In both cases, hybridization with either a common coding region (NCX), or the unique kidney 5′-end (Kc), probe from the Na-Ca exchanger showed similar changes, while levels of calbindin-D28k mRNA responded in parallel with those of the exchanger. Due to the known response of calbindin-D28k to $1,25[OH]_2D_3$, it therefore seemed likely that the renal Na-Ca exchanger was also controlled by levels of this hormone. Nevertheless, the above treatments result in alterations to circulating levels of PTH, as well as ionized calcium and phosphate, all of which are potential mediators of Na-Ca exchanger regulation in these whole animal experiments.

To help distinguish among these alternatives, the experiments illustrated in FIGURE 7B were performed. Parathyroidectomy had little, if any, effect on the levels of mRNA encoding either the Na-Ca exchanger or calbindin-D28k. Moreover, parathyroidectomized animals still responded to low phosphate diet with an increase in the transcripts for these proteins. This rules out PTH as mediator of the observed changes in expression. On the other hand, second-generation vitamin D-deficient rats failed to respond to the low phosphate diet, supporting the suggestion that circulating levels of $1,25[OH]_2D_3$ may control renal Na-Ca exchanger expression.

CONCLUSIONS

Aside from its very important role in controlling intracellular calcium in cardiac myocytes and in neurons, the function of the Na-Ca exchanger is critical for renal reabsorption of calcium, and thus for the control of systemic calcium balance. An alternatively spliced product of the NCX1 gene is expressed very abundantly in kidney cortex, where it is localized to the basolateral membranes of distal nephron cells, primarily in the connecting segment. Na-Ca exchanger expression appears to be under the control of different promoters in kidney, in heart, and in other tissues, imparting the potential for independent regulation of this important transporter. In the kidney, expression of the Na-Ca exchanger responds to altered states of systemic calcium homeostasis, probably through a $1,25[OH]_2D_3$-dependent pathway.

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REFERENCES

1. FRIEDMAN, P. A. & F. A. GESEK. 1993. Calcium transport in renal epithelial cells. Am. J. Physiol. **264:** F181–F198.
2. KUROKAWA, K. 1994. The kidney and calcium homeostasis. Kidney Int. **45:** S97–S105.
3. BINDELS, R. J. M., A. HARTOG, J. TIMMERMANS & C. H. VAN OS. 1991. Active

calcium transport in primary cultures of rabbit kidney CCD: stimulation by 1,25-dihydroxyvitamin D_3 and PTH. Am. J. Physiol. **261:** F799–F807.

4. BINDELS, R. J. M., P. L. M. RAMAKERS, J. A. DEMPSTER, A. HARTOG & C. H. VAN OS. 1992. Role of Na^+/Ca^{2+} exchange in transcellular Ca^{2+} transport across primary cultures of rabbit kidney collecting system. Pflügers Arch. **40:** 566–572.
5. SHIMIZU, T., K. YOSHITOMI, M. NAKAMURA & M. IMAI. 1990. Effects of PTH, calcitonin and cAMP on calcium transport in rabbit distal nephron segments. Am. J. Physiol. **259:** F408–F414.
6. RAMACHANDRAN, C. & M. G. BRUNETTE. 1989. The renal Na/Ca exchange system is located exclusively in the distal tubule. Biochem. J. **257:** 259–264.
7. DOMINGUEZ, J. H., M. JUHASZOVA, S. B. KLEIBOEKER, C. C. HALE & H. A. FEISTER. 1992. Na,Ca-exchanger of rat proximal tubule: gene expression and subcellular localization. Am. J. Physiol. **263:** F945–F950.
8. BINDELS, R. J. M. 1993. Calcium handling by the mammalian kidney. J. Exp. Biol. **184:** 89–104.
9. CAI, Q., J. S. CHANDLER, R. H. WASSERMAN, R. KUMAR & J. T. PENNISTON. 1993. Vitamin-D and adaptation to dietary calcium and phosphate deficiencies increase intestinal plasma membrane calcium pump gene expression. Proc. Natl. Acad. Sci. USA **90:** 1345–1349.
10. FRIEDMAN, P. A. & F. A. GESEK. 1994. Hormone-responsive Ca^{2+} entry in distal convoluted tubules. J. Am. Soc. Nephrol. **7:** 1396–1404.
11. BOUHTIAUY, I., D. LAJEUNESSE & M. G. BRUNETTE. 1991. The mechanism of parathyroid hormone action on calcium reabsorption by the distal tubule. Endocrinology **128:** 251–258.
12. GESEK, F. A. & P. A. FRIEDMAN. 1992. Mechanism of calcium transport stimulated by chlorothiazide in mouse distal convoluted tubule cells. J. Clin. Invest. **90:** 429–438.
13. SHIMIZU, T., M. NAKAMURA, K. YOSHITOMI & M. IMAI. 1991. Interaction of trichlormethiazide or amiloride with PTH in stimulating Ca^{2+} absorption in rabbit CNT. Am. J. Physiol. **261:** F36–F43.
14. LAJEUNESSE, D., M. G. BRUNETTE & J. MAILLOUX. 1991. The hypocalciuric effect of thiazides: subcellular localization of the action. Pflügers Arch. **417:** 454–462.
15. COSTANZO, L. S. 1985. Localization of diuretic action in microperfused rat distal tubules: Ca and Na transport. Am. J. Physiol **248:** F527–F535.
16. KOFUJI, P., W. J. LEDERER & D. H. SCHULZE. 1993. Na/Ca exchanger isoforms expressed in kidney. Am. J. Physiol. **265:** F598–F603.
17. LEE, S-L., A. S. L. YU & J. LYTTON. 1994. Tissue-specific expression of Na/Ca exchanger isoforms. J. Biol. Chem. **269:** 14849–14852.
18. REILLY, R. F. & C. A. SHUGRUE. 1992. cDNA cloning of a renal Na^+-Ca^{2+} exchanger. Am. J. Physiol. **262:** F1105–F1109.
19. YU, A. S. L., S. C. HEBERT, S-L. LEE, B. M. BRENNER & J. LYTTON. 1992. Identification and localization of renal Na^+-Ca^{2+} exchanger by polymerase chain reaction. Am. J. Physiol. **263:** F680–F685.
20. LOO, T. W. & D. M. CLARKE. 1994. Functional expression of human renal Na^+/Ca^{2+} exchanger in insect cells. Am. J. Physiol. **267:** F70–F74.
21. WU, K-D., W-S. LEE, J. WEY, D. BUNGARD & J. LYTTON. 1995. Localization and quantification of endoplasmic reticulum Ca^{2+}-ATPase isoform transcripts. Am. J. Physiol. **269:** C775–C784.
22. LEE, W-S., M. J. BERRY, M. A. HEDIGER & P. R. LARSEN. 1993. The type I iodothyronine 5'-deiodinase messenger ribonucleic acid is localized to the S3 segment of the rat kidney proximal tubule. Endocrinology **132:** 2136–2140.
23. BINDELS, R. J. M., J. A. H. TIMMERMANS, A. HARTOG, W. COERS & C. H. VAN OS. 1991. Calbindin-D9k and parvalbumin are exclusively located along basolateral membranes in rat distal nephron. J. Am. Soc. Nephrol. **2:** 1122–1129.
24. AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. D. MOORE, J. G. SEIDMAN, J. A. SMITH & K. STRUHL, Eds. 1995. Current Protocols in Molecular Biology. John Wiley & Sons. New York, NY.
25. KILAV, R., J. SILVER, J. BIBER, H. MURER & T. NAVEH-MANY. 1995. Coordinate

regulation of the rat renal parathyroid hormone receptor mRNA and the Na/Pi-cotransporter mRNA and protein. Am. J. Physiol. **268:** F1017–F1022.

26. REILLY, R. F., C. A. SHUGRUE, D. LATTANZI & D. BIEMESDERFER. 1993. Immunolocalization of the Na^+/Ca^{2+} exchanger in rabbit kidney. Am. J. Physiol. **265:** F327–F332.
27. BOURDEAU, J. E., A. N. TAYLOR & A. M. IACOPINO. 1993. Immunocytochemical localization of sodium-calcium exchanger in canine nephron. J. Am. Soc. Nephrol. **4:** 105–110.
28. BORKE, J. L., J. T. PENNISTON & R. KUMAR. 1990. Recent advances in calcium transport by the kidney. Semin. Nephrol. **10:** 15–23.
29. KOFUJI, P., W. J. LEDERER & D. H. SCHULZE. 1994. Mutually exclusive and cassette exons underlie alternatively spliced isoforms of the Na/Ca exchanger. J. Biol. Chem. **269:** 5145–5149.
30. ACETO, J. F., M. CONDRESCU, C. KROUPIS, H. NELSON, N. NELSON, D. NICOLL, K. D. PHILIPSON & J. P. REEVES. 1992. Cloning and expression of the bovine cardiac Na,Ca-exchanger. Arch. Biochem. Biophys. **298:** 553–560.