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Molecular cloning and characterization of the human cardiac Na^+/Ca^{2+} exchanger cDNA

(ion transporter/gene expression/heart failure/functional expression)

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ABSTRACT **The Na^+/Ca^{2+} exchanger plays important roles in Ca^{2+} handling in many excitable cells. In particular, the Na^+/Ca^{2+} exchanger is expressed at high levels in the cardiac sarcolemma and is the dominant mechanism of Ca^{2+} extrusion from the cells. In addition, the exchanger has been suggested to play key roles in digitalis action and in postischemic reperfusion injury of cardiac myocytes. We report here the isolation and characterization of the cDNA encoding the human cardiac Na^+/Ca^{2+} exchanger. Twelve overlapping clones corresponding to 5.6 kilobases of the exchanger cDNA sequence were isolated from 5×10^5 phage plaques screened. The sequence predicted a 973-amino acid polypeptide with a putative leader peptide, 11 potential membrane-spanning regions, and one large putative cytoplasmic loop between the fifth and sixth transmembrane helices. When RNA was synthesized *in vitro* from the cloned cDNA and injected into *Xenopus* oocytes, it induced expression of Na^+/Ca^{2+} exchange activity at high levels, confirming that this clone encodes the functional Na^+/Ca^{2+} exchanger. Southern blot analysis indicated that the cardiac exchanger gene exists as a single copy in the human genome, although existence of other related genes cannot be ruled out. Northern blot and S1 mapping analyses revealed that the cardiac type exchanger mRNA is expressed most abundantly in the heart and next in the brain. The cardiac-type exchanger mRNA was also expressed in the retina and in skeletal and smooth muscles at very low levels. The levels of mRNA encoding the exchanger were significantly lower in fetal hearts than in adult hearts but were unchanged in the myocardium from patients with end-stage heart failure.**

Cytoplasmic free Ca^{2+} plays crucial second-messenger functions in the regulation of numerous fundamental physiological processes, such as excitation–contraction coupling, excitation–secretion coupling, cell growth, and cell-to-cell communication (1). The Na^+/Ca^{2+} exchanger regulates intracellular Ca^{2+} concentration in many cells, including cardiac myocytes, retinal rod photoreceptors, smooth muscle cells, epithelial cells, and neurons (2, 3). The exchanger moves Ca^{2+} either into or out of the cytosol across the plasma membrane, depending on the prevailing Na^+ electrochemical gradient (2, 3). The stoichiometry of Na^+/Ca^{2+} exchange of the sarcolemma is three Na^+ to one Ca^{2+} (4, 5); thus the Na^+/Ca^{2+} exchanger is electrogenic. Voltage-clamp experiments have shown that the exchange is also voltage-sensitive (4). It has been suggested that Na^+/Ca^{2+} exchange might contribute significantly to the current carried during the cardiac action potential (6, 7).

The Na^+/Ca^{2+} exchanger of the cardiac sarcolemma is the dominant mechanism in returning the cardiac myocyte to its resting state following excitation (2). Upon depolarization of the sarcolemma, extracellular Ca^{2+} enters the cells through the voltage-gated Ca^{2+} channels, which triggers the release of Ca^{2+} from the Ca^{2+} release channel of the sarcoplasmic reticulum (SR). During relaxation, reuptake of Ca^{2+} from the sarcoplasm is mediated by the Ca^{2+}-ATPase of the SR. In addition, Ca^{2+}, which entered the cell during excitation, is extruded from myocytes primarily by the Na^+/Ca^{2+} exchanger (8). Thus, the exchanger restores the resting low intracellular Ca^{2+} concentration. Moreover, it has been suggested that the increase in intracellular Na^+ in the subsarcolemmal space through Na^+ channels may activate the Na^+/Ca^{2+} exchanger to transport Ca^{2+} into myocytes (9).

The Na^+/Ca^{2+} exchanger is thought to play key roles in mediating the inotropic action of digitalis glycosides and in arrythmogenesis induced by digitalis toxicity (10). Inhibition of the Na^+/K^+-ATPase by digitalis results in an increase in intracellular Na^+. This leads to an increase in intracellular Ca^{2+} and in contractility, presumably via the Na^+/Ca^{2+} exchange mechanism. With more complete (or toxic) Na^+/K^+-ATPase inhibition and a further parallel rise in internal Na^+ and Ca^{2+} concentration, an arrythmogenic transient inward current develops, which seems to be mediated by the electrogenic Na^+/Ca^{2+} exchange (11). A marked increase in systolic Ca^{2+} in ischemic or postreperfusion myocytes may also result from Na^+/Ca^{2+} exchange mechanism (10, 12). However, it is difficult to determine precise roles of the Na^+/Ca^{2+} exchanger because of the lack of specific inhibitors of the exchanger (2, 3).

Nicoll *et al.* (13) cloned the Na^+/Ca^{2+} exchanger from a canine heart cDNA library by antibody screening and elucidated its primary structure. However, its tissue distribution or alterations of the levels of expression during development and in diseased hearts are unknown. To begin molecular characterization of the human Na^+/Ca^{2+} exchanger, we cloned and characterized the human cardiac Na^+/Ca^{2+} exchanger cDNA.§ We examined its tissue distribution and the expression in human hearts during development and in diseased states.

MATERIALS AND METHODS

Cloning of Human Cardiac Na^+/Ca^{2+} Exchanger cDNA. Random- and oligo(dT)-primed human cardiac cDNA library in λZap (Stratagene) was screened with the canine Na^+/Ca^{2+} exchanger cDNA clone A4 (13). Three clones obtained by screening (λHCNC1-1, λHCNC4-2, and λHCNC8-2) were subjected to nested deletion and both strands of the cDNA inserts were sequenced by the dideoxynucleotide chain-termination reaction (14). Hydropathy plot analysis (15) of

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Abbreviation: SR, sarcoplasmic reticulum.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M91368).

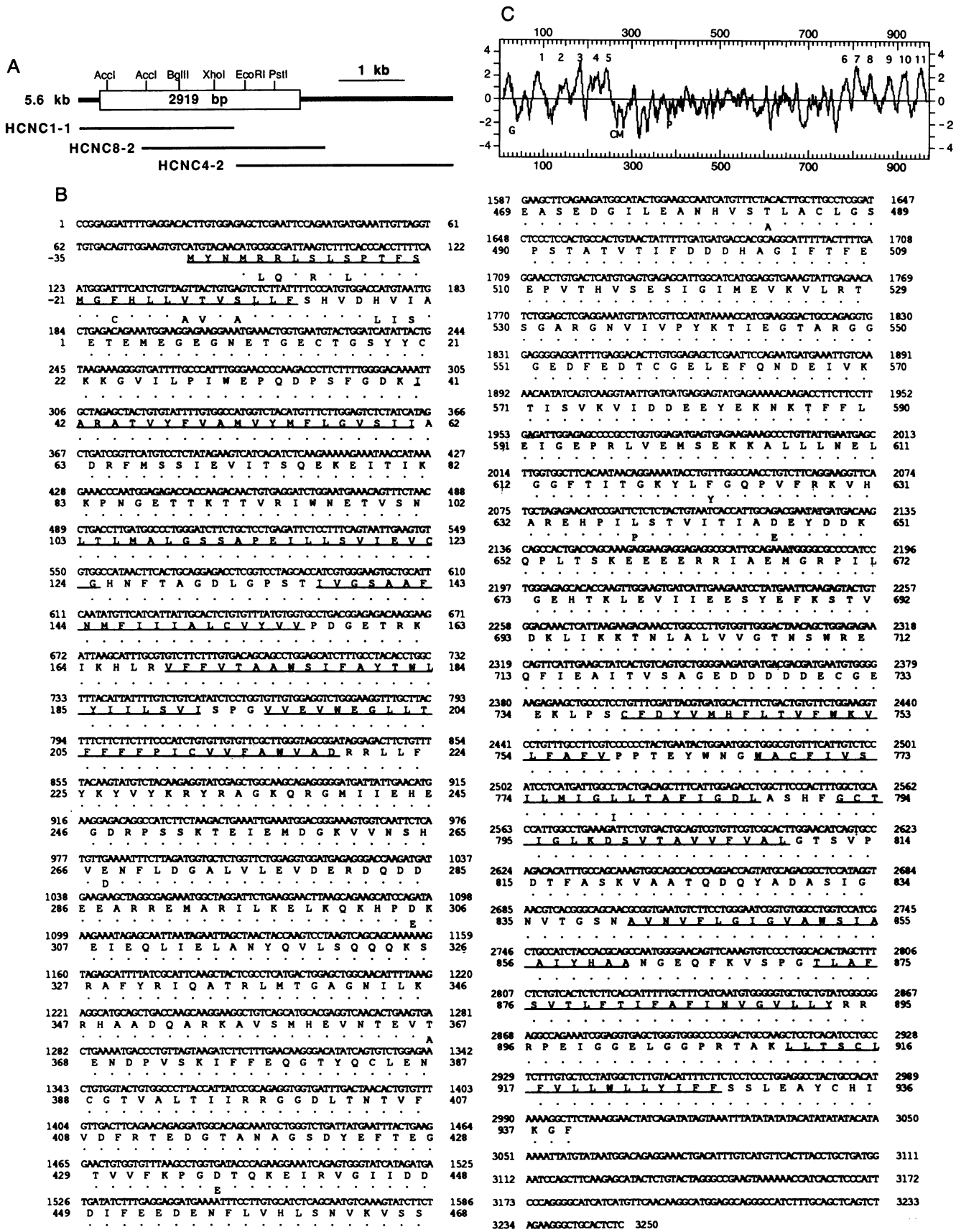

FIG. 1. (*A*) Schematic diagram of the human Na^+/Ca^{2+} exchanger cDNA. Open box, coding region; bars, untranslated regions. Only relevant restriction sites are shown. Three overlapping λ phage clones (λHCNC1-1, λHCNC4-2, and λHCNC8-2) used for DNA sequencing are shown below. (*B*) Nucleotide and amino acid sequence of the human Na^+/Ca^{2+} exchanger. The top and middle lines indicate nucleotide and amino acid sequence of the human exchanger, respectively. The bottom line shows amino acid sequence of the canine exchanger, and dots indicate identical sequences between human and dog (13). The putative leader sequence and potential membrane-spanning regions are underlined. (*C*)

the deduced amino acid sequence was performed with a window of 20 amino acids.

Expression of Na^+/Ca^{2+} Exchange Activity in *Xenopus laevis* Oocytes. The cDNA inserts of λHCNC1-1 and λHCNC8-2 were ligated to obtain the full coding sequence, and *in vitro* transcription of the cDNA was performed with T7 RNA polymerase. The *in vitro* transcribed RNA (50 ng) was injected into *Xenopus* oocytes that were then incubated for 4 days. Na^+ gradient-dependent and -independent uptake of $^{45}Ca^{2+}$ was measured as described (13).

Southern Blot Analysis. Human genomic DNA was digested with several enzymes to completion and used for Southern blot analysis (16). The 350-base-pair (bp) *Eco*RI–*Pst* I fragment and 659-bp *Pst* I–*Eco*RI fragment, which correspond to the 3′ portions of λHCNC8-2, were labeled by random priming and used as probes. After hybridization at 42°C for 15 hr with labeled probe in 50% formamide/5× standard saline citrate (SSC)/5× Denhardt's solution/50 mM Tris·HCl, pH 7.4/0.1% SDS containing salmon sperm DNA at 100 μg/ml, blots were washed twice at 50°C in 0.1× SSC/0.1% SDS.

Northern Blot Analysis, Polymerase Chain Reaction (PCR), and S1 Nuclease Mapping. Total RNAs were prepared from canine tissues and human hearts by LiCl/urea method (17). RNA samples (20 μg) were electrophoresed, transferred to a reinforced nitrocellulose filter, and subjected to hybridization as described (18). The 3′-end 1-kilobase (kb) *Eco*RI–*Eco*RI fragment of λHCNC8-2 was labeled by random priming and used as a probe. PCR amplification was carried out using synthetic oligonucleotide primers (corresponding to nucleotides 2077–2093 and 2262–2277). Approximately 10^6 recombinant phages from the human cardiac and retinal cDNA library (a gift from Jeremy Nathans, Johns Hopkins University School of Medicine) was used for the amplification. The amplification reaction—denaturation (94°C, 30 sec), annealing (48°C, 40 sec), and elongation (72°C, 40 sec)—was repeated for 30 cycles. The S1 nuclease mapping experiments were performed as described (16). Both strands were labeled at the 3′ ends by Klenow enzyme and hybridized with RNA at 5°C above the melting temperature of DNA·DNA hybrids.

Human Subjects. Human ventricular RNAs were isolated from 27 patients with end-stage heart failure (13 with idiopathic dilated cardiomyopathy and 14 with coronary artery disease), undergoing cardiac transplant surgery at Brigham and Women's Hospital, Boston, and from 3 organ donors who had apparently normal cardiac function, as described (19). To examine developmental changes, RNAs isolated from the ventricles of artificially aborted fetuses (gestation, 17 and 19 weeks) were also analyzed.

RESULTS

Primary Structure of the Human Cardiac Na^+/Ca^{2+} Exchanger. Twelve overlapping clones were isolated from a screen of 500,000 plaques. The restriction map of each clone indicated that they corresponded to 5.6 kb of the Na^+/Ca^{2+} exchanger. Since the restriction maps were completely identical in the overlapping portions among 12 clones, 3 clones (λHCNC1-1, λHCNC4-2, and λHCNC8-2) were used for further studies (Fig. 1*A*). The nucleotide sequence of the human cardiac Na^+/Ca^{2+} exchanger cDNA contains a 2919-bp open reading frame encoding a 973-amino acid protein (Fig. 1*B*). The first ATG is 81 bp from the 5′ end of λHCNC1-1 and there are three stop codons 45, 48, and 57 bp from the 5′ end of λHCNC1-1 in frame. The nucleotide sequence around this potential translation start site, GTCATGT, is similar to the Kozak consensus initiation site (20). The predicted human exchanger protein sequence contains 11 putative membrane-spanning regions and one large cytoplasmic loop (Fig. 1*C*). The human exchanger differs from the canine counterpart by 23 amino acid residues (Fig. 1*B*). The first hydrophobic segment has a signal sequence-like motif (21), and this segment has the most divergent sequence between the human and canine clones. Analysis of the canine exchanger by *in vitro* translation indicates that the exchanger has a cleaved leader peptide (22). The existence of a cleaved leader peptide is also supported by the work of Durkin *et al.* (23), who determined the amino terminus of the bovine cardiac Na^+/Ca^{2+} exchanger protein to be ETEMEG. This corresponds to the amino acids 36–41 in our predicted sequence. Therefore, we assign the glutamate at position 36 as the first amino acid of the mature protein.

In the putative extracellular domains, all three potential N-linked glycosylation sites are conserved between human and dog, as is one potential phosphorylation site in the large putative cytoplasmic loop. *In vitro* translation studies indicate that the exchanger is glycosylated only at the first potential N-linked glycosylation site (22). The basic region at residues 219–238, a potential calmodulin-binding domain, is also conserved. A synthetic peptide corresponding to this region can inhibit the exchanger function only at the intracellular surface, suggesting that this region is on the cytoplasmic side (24).

Expression of Na^+/Ca^{2+} Exchange Activity *in Vitro*. To confirm that the cDNA clone encodes the functional Na^+/Ca^{2+} exchanger, we synthesized RNA *in vitro* from the cloned cDNA and injected it into *Xenopus* oocytes. Four days later, we measured Na^+ gradient-dependent Ca^{2+} uptake. The magnitude of Ca^{2+} uptake in the oocytes injected with the RNA (Fig. 2, lane 3) was >40 times that in the water-injected oocytes (lane 1). There was no significant Ca^{2+} uptake without Na^+ loading (lane 5) or when Na^+ was present in the external solution (lane 4). These results indicate that this human cDNA clone codes for the functional Na^+/Ca^{2+} exchanger and that Ca^{2+} movement through the exchanger is completely dependent on the presence of a transmembrane Na^+ gradient.

Southern Blot Analysis. The genomic Southern blot was probed with a 695-bp *Pst* I–*Eco*RI fragment of λHCNC8-2, which contains the carboxyl-terminal coding and 3′ noncoding region, under high-stringency conditions. A single hybridizing band was observed in each lane (Fig. 3*B*), suggesting that the exchanger gene exists as a single copy in the human genome. When the next 5′ region, a 350-bp *Eco*RI–*Pst* I fragment of λHCNC8-2, was used as a probe, two or three hybridizing bands were observed (Fig. 3*A*). This result may suggest the existence of introns in this region of the cDNA, or the presence of other related genes in the genome. Further study on the genome structure is necessary to resolve this issue.

Expression of the Na^+/Ca^{2+} Exchanger mRNA. To examine tissue-specific expression of the Na^+/Ca^{2+} exchanger, Northern blot analysis was performed using the 1-kb *Eco*RI fragment of λHCNC8-2. A single mRNA of ≈7 kb was detected in several tissues (Fig. 4*A*). The exchanger mRNA was most abundantly expressed in the heart and to a lesser degree in the brain. Very weak hybridization signals of the same size were observed in skeletal muscle and in smooth muscle (aorta and uterus). Hybridization with poly(A)$^+$ RNA

Hydropathy plot of the human Na^+/Ca^{2+} exchanger. Hydrophobicity is indicated by positive numbers and hydrophilicity by negative numbers. Potential membrane-spanning regions are numbered 1–11. The first hydrophobic region was not numbered because it is probably cleaved after synthesis (see text). G, potential N-glycosylation site; CM, potential calmodulin binding site; P, potential phosphorylation site.

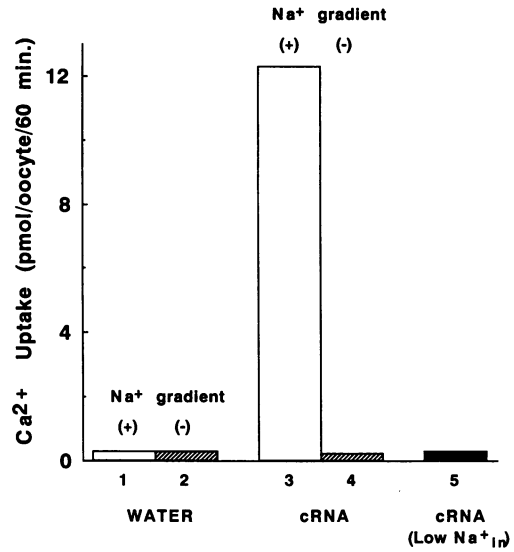

FIG. 2. Expression of Na^+/Ca^{2+} exchange activity in *X. laevis* oocytes. Na^+ gradient-dependent uptake of $^{45}Ca^{2+}$ (open bars) was measured by placing the oocytes in K^+ medium (bars 1 and 3). The background, Na^+ gradient-independent $^{45}Ca^{2+}$ uptake (hatched bars), was determined by placing the oocytes into high-Na^+ medium (bars 2 and 4). There was no significant Ca^{2+} uptake without a prior increase in internal Na^+ (Na^+_{in}) (bar 5). Bars represent means for n = 10. SEMs are <10% of the mean Ca^{2+} uptake.

confirmed the presence of the 7-kb exchanger mRNA in skeletal and smooth muscles (data not shown). No detectable hybridization signal was obtained in the liver RNA. The abundance of the exchanger mRNA roughly corresponds to the known levels of Na^+/Ca^{2+} exchanger activity in these tissues (2, 3). The retinal rod is known to express very high levels of a Na^+/Ca^{2+} exchanger that seems to differ in molecular size (215 kDa) from the cardiac exchanger (120 kDa). RNA isolated from eyes gave only weak hybridization signals. To examine whether the cardiac-type exchanger is also expressed in the retina, we performed PCR amplification with a human retinal cDNA library (25). Thirty-cycle PCR amplification gave bands of the expected size (201 bp) in the retina lane as well as in the cardiac lane (Fig. 4*B*).

Expression of the gene encoding the SR Ca^{2+}-ATPase is markedly reduced during the fetal stage and in hypertrophy or end-stage heart failure (19, 26, 27). The levels of exchanger mRNA were much lower in fetal hearts than in normal adult

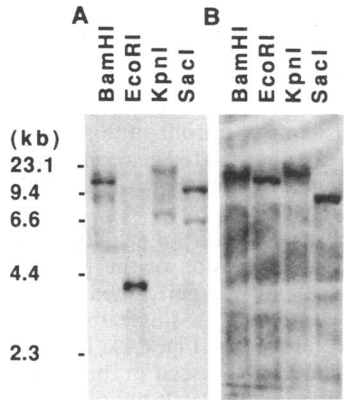

FIG. 3. Southern blot analysis. Human genomic DNA (10 μg) was digested with *Bam*HI, *Eco*RI, *Kpn* I, or *Sac* I and electrophoresed in 0.8% agarose gels. After Southern blotting, the nitrocellulose filters were hybridized with probes from the coding region (*Eco*RI–*Pst* I fragment, 350 bp) (*A*) and the 3′ coding and noncoding region of the exchanger cDNA (*Pst* I–*Eco*RI fragment, 659 bp) (*B*).

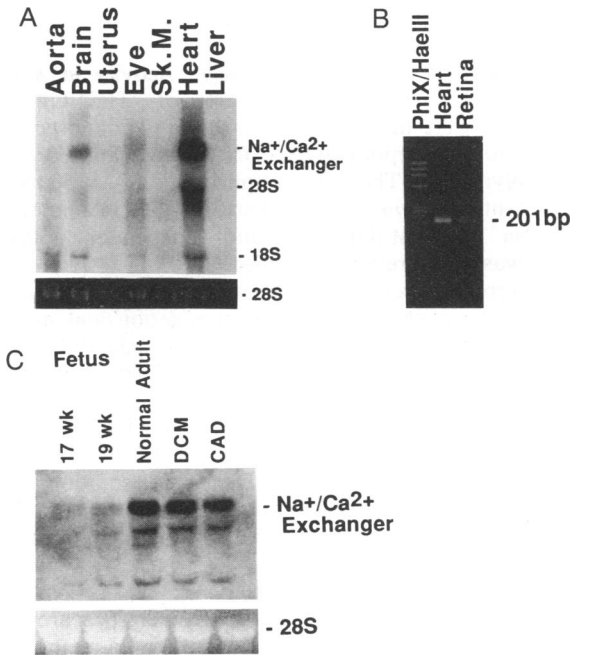

FIG. 4. Northern blot and PCR analysis of the exchanger mRNA. (*A*) Samples (20 μg) of total RNA from canine aorta, brain, uterus, whole eye, skeletal muscle (Sk.M.), heart, and liver were hybridized with a random-primed ^{32}P-labeled cDNA, 1-kb *Eco*RI fragment of λHCNC8-2. The size of the exchanger mRNA was estimated to be 7 kb. Two smaller bands seen most prominently in the heart lane probably represent a "trapping" of mRNA by rRNAs because these bands were not seen when poly(A)$^+$ RNA was used (data not shown). Ethidium bromide staining of 28S rRNA is shown in the lower panel. (*B*) PCR amplification was carried out using 10^6 recombinant phages of cardiac and retinal cDNA library. PCR products were visualized by ethidium bromide staining of the agarose gel. Left lane shows *Hae* III-digested φX174 phage DNA (size markers). (*C*) Human ventricular RNA was extracted from fetuses (17 and 19 weeks), 3 organ donors (normal adults), 13 patients with idiopathic dilated cardiomyopathy (DCM), and 14 patients with coronary artery disease (CAD). The RNA extracted from adult hearts was pooled and 20 μg of total RNA was electrophoresed. The top bands correspond to the Na^+/Ca^{2+} exchanger mRNA and the two lower bands probably correspond to 28S and 18S rRNAs. Level of the exchanger mRNA was very low in fetal heart but was not significantly changed in the failing myocardium, compared with normal adult. Similar results were obtained when RNAs from individual patients (n = 5) were analyzed separately (data not shown). Ethidium bromide staining of the gel is shown in the lower panel to indicate an equal loading of RNA in each lane.

hearts (Fig. 4*C*). In contrast, expression of the exchanger gene was not significantly down-regulated (when corrected for RNA loading) in ventricular myocardium from patients with idiopathic dilated cardiomyopathy and ischemic cardiomyopathy.

The genes encoding the cardiac Ca^{2+}-ATPase and the cardiac Ca^{2+} channel are known to produce multiple mRNAs by alternative splicing. To examine whether the cardiac Na^+/Ca^{2+} exchanger transcript also undergoes alternative splicing, we performed S1 nuclease mapping with RNAs isolated from heart, brain, and smooth muscle (intestine). When three DNA fragments (probe A, a 528-base *Acc* I fragment corresponding to the first five transmembrane regions; probe B, a 474-base *Bgl* II–*Xho* I fragment corresponding to the middle of the cytoplasmic loop; probe C, a 1009-base *Eco*RI fragment covering all of the carboxyl-terminal transmembrane regions) were used as probes, only fully protected bands were seen in all tissues examined (data not shown). Therefore, for the portions corresponding to the probes used (which cover all transmembrane regions and a

part of the cytoplasmic loop), there appear to be no alternatively spliced products of the exchanger mRNA, although it is possible that such transcripts may be present in the remaining portions.

DISCUSSION

We have isolated the full coding cDNA of human cardiac Na^+/Ca^{2+} exchanger. Expression studies using *Xenopus* oocytes have demonstrated that the cDNA clone encodes the functionally active Na^+/Ca^{2+} exchanger. A single open reading frame of 2919 nucleotides encodes a protein of 973 amino acids, 3 amino acids longer than the canine exchanger. Sequence homology between human and canine exchangers is extremely high except in the first hydrophobic segment, which seems to be a cleaved leader peptide. All structural characteristics of the exchanger were conserved between the human and canine clones.

Not only the putative transmembrane segments but also the large central cytoplasmic loop are very highly conserved between the human and canine exchanger. This suggests that the central loop also plays functionally important roles. On the other hand, a deletion construct that lacks most of this cytoplasmic loop is capable of conferring Na^+/Ca^{2+} exchange activity when expressed in *Xenopus* oocytes (22). Therefore, this cytoplasmic domain may play other regulatory roles that are important for the function of the exchanger *in vivo*.

Southern blot analysis using the carboxyl-terminal coding and 3′ noncoding region suggested that the cardiac Na^+/Ca^{2+} exchanger exists as a single copy in the genome. Northern blot analysis showed that the exchanger mRNA was expressed in all excitable tissues examined and was especially abundant in the heart. S1 mapping analysis suggested that the exchanger mRNAs expressed in the heart, brain, and smooth muscle are coded by the same gene. Although the Northern blot analysis gave a "hazy" hybridization signal in RNA obtained from eyes, PCR amplification using the human retinal cDNA library confirmed the existence of the same or a similar exchanger mRNA in the retina. Although Na^+/Ca^{2+} exchange activity is very high in the retina (28), the relative amount of the exchanger mRNA in the retina appeared very low. This is probably due to the existence of an additional, retina-specific, Na^+/Ca^{2+} exchanger that has little sequence homology to the cardiac-type exchanger (31). The molecular mass of the exchanger of cardiac sarcolemma (120 kDa) is different from that of the retinal exchanger (215 kDa) (13, 28), and antibodies against the cardiac Na^+/Ca^{2+} exchanger crossreact poorly with the purified rod exchanger (29). Further, the retinal rod exchanger cotransports K^+, but the exchangers from other tissues do not (28). The distinct retinal exchanger seems to coexist with the cardiac-type exchanger in the retina. However, it remains to be determined whether the cardiac- and the retinal-type exchangers are coexpressed in the same cells in the retina.

We examined the levels of cardiac exchanger mRNA during development and in diseased states of humans. The mRNA levels of the exchanger were much lower in the early fetal heart than in the adult heart. It has been reported that in experimental animals the resting tension and cytosolic Ca^{2+} are higher, and that the half-time to relaxation is longer in the fetal myocardium than in the adult muscle (30). The high diastolic cytosolic Ca^{2+} concentration and subsequent impaired relaxation of the fetal myocardium may be in part due to low levels of expression of the Na^+/Ca^{2+} exchanger and SR Ca^{2+}-ATPase (26). The levels of the exchanger mRNA were not significantly decreased in idiopathic dilated cardiomyopathy and ischemic heart disease. It remains to be determined how these Ca^{2+} regulatory protein genes are regulated during normal development and in various pathophysiological states in humans.

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1. Campbell, A. K. (1983) *Intracellular Calcium: Its Universal Role as Regulator* (Wiley, New York).
2. Allen, T. J. A., Noble, D. & Reuter, H. (1989) *Sodium-Calcium Exchange* (Oxford Univ. Press, Oxford).
3. Sheu, S.-S. & Blaustein, M. P. (1986) in *The Heart and Cardiovascular System: Scientific Foundations*, eds. Fozzard, H. A., Haber, E., Jennings, R. B., Katz, A. M. & Morgan, H. E. (Raven, New York), pp. 509–535.
4. Reeves, J. P. & Hale, C. C. (1984) *J. Biol. Chem.* **259,** 7733–7739.
5. Kimura, J., Noma, A. & Irisawa, H. (1986) *Nature (London)* **319,** 596–597.
6. Eisner, D. A. & Lederer, W. J. (1985) *Am. J. Physiol.* **248,** C189-C202.
7. Noble, D. (1984) *J. Physiol. (London)* **353,** 1–50.
8. Darnell, J., Lodish, H. & Baltimore, D. (1990) *Molecular Cell Biology* (Sci. Am. Books, New York), pp. 865–879.
9. Leblanc, N. & Hume, J. R. (1990) *Science* **248,** 372–376.
10. Smith, T. W., Braunwald, E. & Kelly, R. A. (1988) in *Heart Disease: A Textbook of Cardiovascular Medicine*, ed. Braunwald, E. (Saunders, Philadelphia), pp. 489–507.
11. Kass, R. S., Lederer, W. J., Tsien, R. W. & Weingart, R. (1978) *J. Physiol. (London)* **281,** 187–208.
12. Kleber, A. G. (1983) *Circ. Res.* **52,** 442–450.
13. Nicoll, D. A., Longoni, S. & Philipson, K. D. (1990) *Science* **250,** 562–565.
14. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
15. Kyte, J. & Doolitte, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
16. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
17. Auffray, C. & Rougeon, F. (1980) *Eur. J. Biochem.* **107,** 303–314.
18. Komuro, I., Kurabayashi, M., Takaku, F. & Yazaki, Y. (1988) *Circ. Res.* **62,** 1075–1079.
19. Takahashi, T., Allen, P. D. & Izumo, S. (1992) *Circ.Res.*, in press.
20. Kozak, M. (1989) *J. Cell Biol.* **108,** 229–241.
21. von Heijne, G. (1986) *Nucleic Acids Res.* **14,** 4683–4690.
22. Nicoll, D. A. & Philipson, K. D. (1991) *Ann. N.Y. Acad. Sci.* **639,** 181–188.
23. Durkin, J. T., Ahrens, D. C., Pan, Y.-C. E. & Reeves, J. P. (1991) *Arch. Biochem. Biophys.* **290,** 369–375.
24. Li, Z., Nicoll, D. A., Collins, A., Hilgemann, D. W., Filoteo, A. G., Penniston, J. T., Weiss, J. N., Tomich, J. M. & Philipson, K. D. (1991) *J. Biol. Chem.* **266,** 1014–1020.
25. Nathans, J. & Hogness, D. S. (1983) *Cell* **34,** 807–814.
26. Komuro, I., Kurabayashi, M., Shibazaki, Y., Takaku, F. & Yazaki, Y. (1989) *J. Clin. Invest.* **83,** 1102–1108.
27. Mercadier, J. J., Lompre, A. M., Duc, P., Boheler, K. R., Fraysse, J. B., Wisnewsky, C., Allen, P. D., Komajda, M. & Schwartz, K. (1990) *J. Clin. Invest.* **85,** 305–309.
28. Nicoll, D. A. & Appleburry, M. L. (1989) *J. Biol. Chem.* **264,** 16206–16213.
29. Vemuri, R., Haberland, M. E., Fong, D. & Philipson, K. D. (1990) *J. Membr. Biol.* **118,** 279–283.
30. Nakanishi, T. & Jarmakani, J. M. (1984) *Am. J. Physiol.* **246,** H615-H625.
31. Achilles, A., Friedel, U., Hasse, W., Reilander, H. & Cook, N. J. (1991) *Ann. N.Y. Acad. Sci.* **639,** 234–244.