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Expression of sodium pump isoforms and other sodium or calcium ion transporters in the heart of hypertensive patients

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Abstract

The sodium pump (Na^+,K^+-ATPase; EC 3.6.1.37) of animal cell membranes is the enzyme responsible for the maintenance of membrane potential, for the function of secondary active transporters, and for osmoregulation of the cell. Since inhibition of the enzyme by cardiac glycosides results in increased contractility of the heart muscle and increased blood pressure, we were interested in whether there is a correlation between hypertension and expression of the various isoforms of the sodium pump. In addition, we also examined the expression of the isoforms of the sarcoplasmic and plasma membrane Ca^{2+}-ATPase, the Na^+/Ca^{2+}- and Na^+/H^+-exchangers, and Na^+ channel and Ca^{2+} channel isoforms. Total mRNA was isolated from 50 mg tissue from the right atrium of hypertensive and normotensive patients who were undergoing cardiac surgery. After reverse transcription and subsequent amplification of ion transporter-specific cDNA fragments by polymerase chain reaction (PCR) in the presence of [α-^{32}P]dCTP, quantification of the amplified fragments was carried out by the Phosphorimager technique. The data obtained show that the αl subunit mRNA is expressed similarly in normotensive and hypertensive patients. The amount of α2 subunit mRNA, however, is increased 5-fold in hypertensive patients. In the same group, the amount of α3 isoform is also significantly increased, although not as dramatically as the α2 isoform. Besides the Na^+,K^+-ATPase isoforms, a significant increase in the expression of mRNA for the Na^+/Ca^{2+}-exchanger and the plasma membrane Ca^{2+}-ATPase isoforms was detected. It is possible that the observed changes in mRNA expression for these ion transporters reflect compensatory mechanisms to overcome a defective Na^+ and Ca^{2+} metabolism in the tissues of hypertensive patients or reflect defects directly involved in the cause of hypertension. The expression of mRNA for all other transporters investigated was unaltered.

Keywords: Hypertension; Heart; Ion transporter; Na^+,K^+-ATPase; mRNA expression; Quantitative polymerase chain reaction

1. Introduction

In industrialized countries, morbidity, invalidism and mortality are often closely associated with the phenomenon of elevated blood pressure, i.e., arterial hypertension. Hypertension is the main risk factor

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for heart insufficiency, stroke, claudicatio intermittens or kidney artery defects. Nevertheless, for only 5% of these patients is the primary cause of hypertension known, which is mostly associated with defects in kidney function. Thus, for the vast majority of hypertension cases, the primary defect and its molecular basis are unknown. These undefined cases of hypertension are referred to as primary (or essential) hypertension. Primary hypertension seems to be an inheritable disease. This conclusion is based on the fact that more than 70% of all patients with primary hypertension have a positive family history of the disease. In several cases, hypertension follows polygenic patterns of inheritance: the genes involved manifest the defect 'hypertension' via so-called intermediate phenotypes. Besides involving neurohumoral systems, these could be quantitative and also qualitative alterations in membrane-embedded ion transporting proteins. Whether independent or combined with environmental factors, these intermediate phenotypes could lead to the increase in arterial blood pressure.

In this respect, sodium chloride would be an environmental factor that could become a risk factor for hypertension when its metabolism does not follow the normal physiological pattern. An increased sodium concentration in tissues and cells has been reported to be associated with the phenomenon of hypertension [1] and on many occasions it was speculated that some defect in a sodium transporting system might cause this nonphysiological elevation. The Na^+/H^+-exchanger seems to be involved in this phenomenon [2]. A G-protein-mediated overactivation of the exchanger might cause an elevation of cytosolic sodium, leading to a secondarily induced elevation of the Ca^{2+} concentration in the cytosol and resulting in an increase in muscle contractility and an associated increase in blood pressure [2].

Despite this plausible explanation, it remains a fact that the major sodium-extruding ion transporter present in all animal cell plasma membranes is the sodium pump. Changes in its activity could also result in changes in contractility of arteries or heart muscle, something often proposed by various investigators [3]. This and the fact that various sodium pump isoforms are expressed in various tissues [4] lead to the question of whether changes in activity or expression of sodium pump isoforms might be associated with the phenomenon of hypertension. The study presented here addresses this question by investigating the expression of sodium pump isoform-specific mRNA in the heart muscle of normotensive and hypertensive patients.

2. Materials and methods

2.1. Ethics

The study was approved by the Ethics Commission of the Justus-Liebig-University of Giessen and conforms with the principles outlined in the declaration of Helsinki (BMJ 1964; ii:177). All patients

Table 1
Patient data

Patient	Sex	Age (years)	Weight (kg)	Height (cm)	Blood pressure systolic/diastolic (mmHg/mmHg)
H1	Male	66	82	175	Hypertensive 190/100
H2	Female	72	56	154	Hypertensive 190/100
H3	Male	68	80	174	Hypertensive 160/90
H4	Male	44	96	196	Hypertensive 160/80
H5	Male	55	96.5	186	Hypertensive 170/100
N1	Male	48	80	175	Normotensive 120/80
N2	Male	54	75	175	Normotensive 120/70
N3	Male	69	89	181	Normotensive 120/80
N4	Male	65	87.7	174	Normotensive 130/70
N5	Male	52	85	179	Normotensive 120/80

All patients investigated were scheduled to undergo surgery due to coronary artery disease or aortic valvular dysfunction. Tissue samples of approximately 50 mg were taken from the right atrial appendage, freed from blood in NaCl solution and immediately frozen in liquid nitrogen.

were informed and had agreed to participate in the study.

2.2. Tissue preparation

All patients investigated were scheduled to undergo surgery due to coronary artery disease or aortic valvular dysfunction. They were between 44 and 72 years of age, and included five normotensive males, four hypertensive males, and one hypertensive female (Table 1). Tissue samples of approximately 50 mg were taken from the right atrial appendage after purse-string suture prior to venous cannulation. Immediately after removal, tissue was freed from blood in isotonic NaCl and was then immediately frozen in liquid nitrogen.

2.3. RNA extraction

Total cell RNA was isolated by the Micro RNA Isolation kit (Stratagene, Heidelberg, Germany), which is a guanidinium *iso*-thiocyanate-phenol-chloroform isolation method. The resultant RNA pellet was dried in a vacuum centrifuge and resuspended in 20 μl of RNase-free water (aqua ad injectabilia, Ampuwa, Fresenius, Bad Homburg, Germany). In order to eliminate genomic DNA, which would falsify the results of the PCR amplification, 0.5 μl (=3.75 units) of DN*ase*I (Pharmacia Biotech, Uppsala, Sweden) was added and the mixture was incubated for 10 min at 37°C. Afterwards, the sample was cooled on ice and RNA was isolated a second time using the Micro RNA Isolation kit. Then the pellet was dried in vacuum again and resuspended in 35–50 μl of the RNase-free water mentioned above. The RNA concentration in the solution was determined in a spectrophotometer by measuring the absorbance at 260 nm.

2.4. cDNA synthesis

First-strand cDNA was synthesized from 30 μg of total RNA using oligo$(dT)_{15}$ primer (4 μM), dNTP mixture (500 μM of each dNTP), 400 U Moloney murine leukemia virus reverse transcriptase (Amersham, Braunschweig, Germany) and buffer supplied by the manufacturer in a 50-μl reaction volume for 1 h at 37°C. The reaction was stopped by incubation at 95°C for 5 min, and the cDNA was stored at −20°C until use.

2.5. PCR conditions

Polymerase chain reaction (PCR) was performed in a Cyclone Temperature Cycler (Integra Biosciences, Fernwald, Germany). A total of 100 μl of the PCR mixture contained 1 μl first-strand cDNA, 200 μM of each dNTP, 20 pmol of each primer, 1.8 pmol (5.4 μCi) [α-^{32}P]dCTP (Hartmann Analytik, Braunschweig, Germany), 2 U Tfl-Polymerase (Biozym Diagnostik, Oldendorf, Germany), 1.5 mM $MgCl_2$ and buffer supplied by the manufacturer. To minimize error, a mixture of all components was made and an aliquot of this was taken for each reaction. The PCR was started with an initial denaturation (5 min at 95°C), followed by amplification with 25 cycles each consisting of the following steps: 30 s at 94°C (denaturation), 1 min at 60°C (annealing), and 2 min at 72°C (polymerization). In order to control the quality of each PCR amplification, a standard template of a defined initial amount was amplified under the same conditions. The oligonucleotide primers used are given in Table 2. They were designed for an annealing temperature of 60°C [5].

2.6. Agarose gel electrophoresis and radioactivity determinations

After amplification, 10 μl of the PCR products were size-fractionated by electrophoresis in a 1% agarose gel and then stained with ethidium bromide. The product bands were visualized under ultraviolet light, and the agarose gel was fixed in 7% trichloroacetic acid (TCA) and dried. The gel was exposed to Kodak X-Omat AR X-ray film using an intensifying screen for 2 to 48 h at −80°C to check the exposure time. A quantitative evaluation was performed with the use of a Phosphorimager BAS 1000 (Raytest Isotopenmeßgeräte, Straubenhardt, Germany). Using the radioactivity measured and the known amount of [α-^{32}P]dCTP, the quantity of the amplified double strands was calculated.

2.7. Calculation of the original mRNA/cDNA content in the cardiac tissue used for the analysis

In an optimal situation, the cDNA copies produced in each PCR cycle are twice as many as the original number of copies prior to the amplification. Thus, ideally, the number of cDNA copies follows an exponential growth rate 2^n and can be described by the formula

$$N_n = N_0 \times 2^n \quad (1)$$

In this formula, n is the number of PCR cycles, N_n the concentration of cDNA after the amplification, and N_0 the cDNA concentration prior to the amplification. This exponential PCR amplification of cDNA and the linear correlation between product concentration and original cDNA content allow one to calculate the original single-stranded (ss)DNA (= mRNA) quantity prior to the PCR amplification. Nevertheless, several parameters might influence the PCR and it is therefore necessary to define for the amplification an efficiency factor E. Taking this factor into consideration, the relationship in Eq. 1 is now written as

$$N_n = N_0(1 + E)^n \quad (2)$$

and therefore

$$\log N_n = [\log(1 + E)] \times n + \log N_0 \quad (3)$$

Table 2
Positions and sequences of PCR primers

Ion transport system	Accession no.	Position in the GenBank sequence	Sequence of the amplification primers (5′→3′)	Size of amplified fragment (bp)
YEPα2	M14512[a]	1040–1066	CTGGCTGGAGGCTGTCATCTTCTTCAT	557
		1575–1596	GGCTCTGGGGGCTGTCTTCCCT	
NaKα1	X04297	1266–1292	CTGGCTGGAGGCTGTCATCTTCTTCAT	560
		1802–1825	GTTGGGGCTCCGATGTGTTGGGGT	
NaKα2	J05096	12673–12691	CTGGCTGGAGGCTGTCATCTTCTTCAT	557
		15043–15066	GGCTCTGGGGGCTGTCTTCTCGCT	
NaKα3	X12912	1369–1395	CTGGCTGGAGGCTGTCATCTTCTTCAT	560
	M37446	31–55	ATCGGTTGTCGTTGGGGTCCTCGGT	
NACA	M96368	2160–2183	GGAGAGCACACCAAGTTGGAAGTG	570
		2707–2729	ATTGGCTGCGTGGTAGATGGCAG	
SERCA2a	M23115	1312–1335	GTTGGAGAAGCTACAGAGACTGCT	639
		1928–1950	TGACGTCACGTCCTCATCCTGCC	
PMCA1	J04027	593–616	GGAGAAGTTTCTGTTGGGGAGGAA	716
		1286–1308	GCTTTGCCAATCTGAACAGCCAG	
PMCA4	M25874	794–817	GGTCAAGTCGCAACTACCCCAGAA	701
		1472–1494	GCTTTGCCAATCTGAACAGCCAG	
NHE1	S68616	1985–2007	GACAAGAAGCACTTCCCCATGTG	589
		2551–2573	ATCTGGTTCCAGGCTTCCTCGTAG	
SCN5A	M77235	4263–4284	GCCTTTGAACTACACCATCGTG	570
		4808–4832	GCCAAGATGTTGATTTTCTCAGGAC	
SCN6A	M91556	3465–3488	GCCTTCTGTTTAACGAATCCATGC	524
		3964–3988	GAGAGCAATGGACATTTGTAGACTC	
RYR2	X98330	13773–13795	CCCATAGAATCATCGCAGTTCAC	681
		14430–14453	CAACAACAGCTAATAAGCCAACGG	
ITPR1	L38019	6961–6981	AGCATTTCGTTCAACCTGGC	714
		7654–7675	CTCTTCTCTGGGTGCAGGAGAG	
ITPR2	D26350	7109–7128	AGCATTTCGTTCAACCTGGC	710
		7796–7818	GCTGTATTTGAAGCTGGAATTGT	

The primer pairs shown amplify specific fragments for each of the ion transporters. NaKα1, NaKα2, NaKα3, sodium pump α subunit isoforms 1–3; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase; NACA, Na^+/Ca^{2+}-exchanger; PMCA, plasma membrane Ca^{2+}-ATPase; NHE1, Na^+/H^+-exchanger; SCN5, tetrodotoxin-insensitive sodium channel; SCN6, atypical sodium channel; RYR, ryanodine receptor; ITPR, inositol 1,4,5-trisphosphate receptor.

[a]Accession numbers and positions refer to the rat sodium pump isoform α2 with Locus RATATPA2, not to the plasmid.

The value for the efficiency factor E varies between 0 and 1. At 0, no amplification is achieved, whereas at 1, one would expect the ideal doubling of the cDNA in each cycle of amplification. It is therefore necessary to determine E for each amplification experiment individually.

This was done by amplifying in parallel to the unknown samples a sequence of a known vector that was subjected to a PCR amplification with an initial concentration N_0 of 2×10^{-4} pmol/100 μl of PCR reaction mixture. This amount of vector was chosen because it would produce by either 20 or 30 PCR cycles a linear area of amplification of the expected cDNA band (Eq. 3).

Using [α-^{32}P]dCTP and taking into consideration the maximally possible incorporation of radioactive dCMP into the amplified fragment, the efficiency factor E can be calculated from the formula

$$E = \sqrt[n]{N_n : N_0} - 1 \quad (4)$$

by determining the radioactivity using the Phosphorimager BAS 1000. After the determination of E, the original amount of the sample to be investigated can be calculated from the equation

$$N_0 = N_n : (1 + E)^n \quad (5)$$

2.8. Statistical analysis

Values are expressed as means with the associated standard error mediated (S.E.M.). The t-test for independent samples and analysis of variance for repeated measurements were used for statistical analysis. In some cases the nonparametric Mann–Whitney U/Wilcoxon rank sum W test was used. Values of $P < 0.05$ were considered significant.

2.9. Preparation of microsomal protein

A total of 50–100 mg derived from the right heart atrium of hypertensive or normotensive patients was immediately frozen in liquid nitrogen and ground with a mortar, also under liquid nitrogen. The tissue was then suspended in 2 ml of a buffer composed of 25 mM imidazole, 1 mM EDTA (pH 7.5), 0.5 mM phenylmethyl sulfonyl fluoride, 5 μg/ml pepstatin, 5 μg/ml leupeptin (all three protease inhibitors from Boehringer Mannheim, Mannheim, Germany), and 8% (w/v) sucrose. After a further gentle homogenization in a Potter Elevejem homogenizer held on ice, samples were centrifuged at $4000\times g$ for 20 min at 4°C. The supernatants were then transferred to new tubes and centrifuged at $30\,000\times g$ for 1 h at 4°C. The resulting pellet was suspended in 500 μl of 25 mM imidazole, 1 mM EDTA, pH 7.5. After protein determination [6], samples were stored at −20°C.

2.10. Binding of [^{3}H]ouabain

A total of 50 μg of microsomal protein was incubated to equilibrium (60 min) at 30°C in a mixture containing 10 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM Tris/H_3PO_4, and 50 nM [^{3}H]ouabain. The total volume of each sample was 100 μl. Thereafter, the protein was pelleted by centrifugation at $13\,000\times g$ for 2 min, washed twice with H_2O at 4°C, and dissolved in 200 μl of 1 M NaOH by incubating at 80°C for 10 min. After the addition of 200 μl of 1 M HCl, the samples were mixed with 3.5 ml of scintillation cocktail (Roth, Karlsruhe, Germany) and counted for radioactivity.

3. Results

3.1. Detection and quantification of sodium pump-specific mRNA molecules

A series of experiments had to be carried out in order to isolate RNA from the tissue, convert the mRNA therein into single-stranded DNA by the use of reverse transcriptase and oligo(dT) primer, and finally amplify by PCR and by the use of specific primers (Table 2) the cDNA for α1, α2 or α3 isoforms of the sodium pump. Although laborious, these methods are well established and can be carried out by following standard protocols. Therefore, these methods have not been described in detail; rather, the results are shown in a representative way in Fig. 1A. This figure shows the autoradiography of an agarose gel containing α1-, α2- and α3-specific fragments of cDNA amplified by PCR in the presence of [α-^{32}P]dCTP. It is apparent that α1 is the dominant form of the sodium pump α subunit in this normotensive patient (Fig. 1A). In the same patient, α3 is present in a smaller amount, while the α2

isoform is almost undetectable. In order to quantify the exact amount of amplified DNA fragments, we applied the Phosphorimager technique (Fig. 1B). Using this method, it is possible to measure the radioactivity incorporated in a DNA fragment that was amplified by PCR in the presence of [α-^{32}P]dCTP. This can be done by knowing (a) the specific radioactivity of the [α-^{32}P]dCTP used and (b) the precise number of [α-^{32}P]dCMP molecules that will be incorporated into an amplified double strand of DNA. The accuracy of the method was determined prior to the actual investigation by amplifying various known amounts of cDNA coding for the rat α2 subunit of the sodium pump (not shown). Additionally, in order to avoid experimental errors, mRNA of each patient was reverse transcribed and amplified by PCR in three independent experiments. The results of these experiments are summarized in Table 3. In agreement with the results shown in Fig. 1A, the α1 cDNA is the predominant form in both normo- and hypertensive patients and is equally expressed in both groups of patients. The expression of the α2 isoform, however, is 5-fold higher in the hypertensive patients than it is in normotensives. The α3 isoform-specific cDNA is slightly but significantly elevated in hypertensive patients (Table 3). The relative expression of isoforms is summarized in Fig. 2.

3.2. *Detection and quantification of mRNA molecules coding for various Na^+ and/or Ca^{2+} ion transporters*

The rather unexpected result showing the considerable increase in expression of mRNA coding for α2 and α3 isoforms of the sodium pump (Fig. 2) in the heart of hypertensive patients prompted us to investigate the expression of mRNA molecules coding for other transporters and their isoforms. The emphasis was placed on the analysis of ion transporting systems that are involved in Na^+ and Ca^{2+} conduction: the Ca^{2+}-ATPases from the sarcoplasmic reticulum (SERCA2a) or from the plasma membrane (PMCA1 and PMCA4), the Na^+/Ca^{2+}-exchanger, the Na^+/H^+-exchanger, the tetrodotoxin-insensitive Na^+ channel SCN5, the atypical Na^+ channel SCN6, the caffeine-sensitive Ca^{2+} release channel (= ryanodine receptor; RYR2), and the inositol 1,4,5-trisphosphate-sensitive Ca^{2+}-release channels (ITPR1 and ITPR2). The results of this investigation are summarized in Table 3. For PMCA1 and PMCA4 the double-stranded (ds)DNA amplified from tissue of hypertensive patients roughly accounts for twice the amount amplified from normotensives (Fig. 2). The same applies for the Na^+/Ca^{2+}-exchanger. In contrast, no differences in amplified dsDNA were

Table 3
Active and passive transport systems of sodium and calcium amplified from the heart of normotensive and hypertensive patients

Ion transporter	Isoform	Normotensive patients	Hypertensive patients	*t*-Test	PCR cycles
Na^+/K^+-ATPase	α1	0.125 (0.012)	0.152 (0.025)	n.s.[a]	25
	α2	0.010 (0.001)	0.045 (0.011)	$P = 0.008$	25
	α3	0.055 (0.005)	0.088 (0.015)	$P = 0.048$	25
Na^+/H^+-Exchanger	NHE1	1.028 (0.044)	1.197 (0.159)	n.s.	35
Sodium channel	SCN5A	0.600 (0.064)	0.701 (0.099)	n.s.	35
	SCN6A	1.023 (0.096)	1.414 (0.230)	n.s.	35
Na^+/Ca^{2+}-Exchanger	NACA	0.073 (0.007)	0.173 (0.028)	$P = 0.004$	25
Ca^{2+}-ATPase	SERCA2a	0.904 (0.095)	1.175 (0.145)	n.s.	25
	PMCA1	0.034 (0.006)	0.089 (0.020)	$P = 0.009$[b]	30
	PMCA4	0.080 (0.016)	0.164 (0.027)	$P = 0.014$	30
Calcium channel	RYR2	0.526 (0.072)	0.469 (0.090)	n.s.	25
	ITPR1	0.128 (0.026)	0.141 (0.032)	n.s.	25
	ITPR2	0.097 (0.019)	0.098 (0.019)	n.s.	25

Quantification of double-stranded DNA (dsDNA) was carried out after reverse transcription of isolated mRNAs and PCR amplification of cDNAs by the Phosphorimager technique. For more details see Section 2. Values are given in pmol dsDNA per 100 μl of PCR amplification mixture and are means of 15 measurements (five individuals in each category × three repetitions) ± S.E.M.[a]Not significant.[b]Mann–Whitney *U*/Wilcoxon rank sum *W* test.

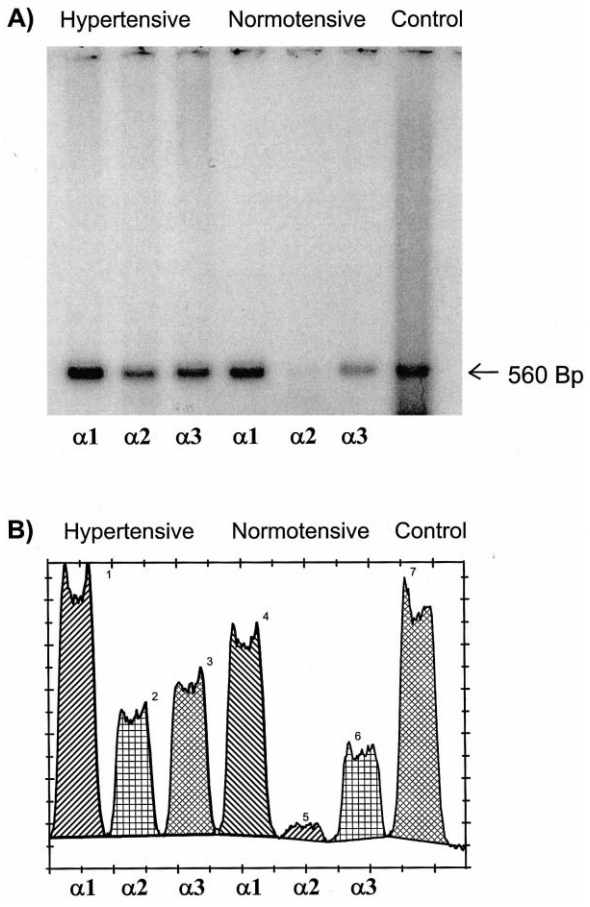


Fig. 1. Qualitative and quantitative determination of α1-, α2- and α3-specific, PCR-amplified cDNA fragments. (A) The autoradiography of an agarose gel shows the presence of α2-specific cDNA in the heart of a hypertensive patient. (B) The Phosphorimager profile further verifies this result and allows the quantification of the cDNA fragments. The control was a PCR-amplified rat α2 cDNA.

found between normotensive and hypertensive patients for any other ion channels investigated and for the Na^+/H^+-exchanger (Fig. 2).

Because PCR amplification not only depends on the amount of the starting template but also on the efficiency factor E (see Eq. 5 in the Section 2), it was important to calculate the original amount of ion transporter-specific mRNA and set this in a relationship to the total amount of RNA isolated. From Fig. 3, it is apparent that the ion transporter-specific mRNA (ssDNA) is originally present in similar proportions to that after amplification. Thus, the results in Fig. 3 confirm the results shown in Fig. 2 and Table 3.

3.3. Ouabain binding experiments

Ouabain binds with high affinity and specificity to the Na^+,K^+-ATPase, and, when used in as a radioactive ligand, is a good tool for estimating the number of pumps in membrane preparations. Ideally, one should use the substance in a Scatchard-type experiment in order to extrapolate to the maximum possible binding. Nevertheless, such experiments require

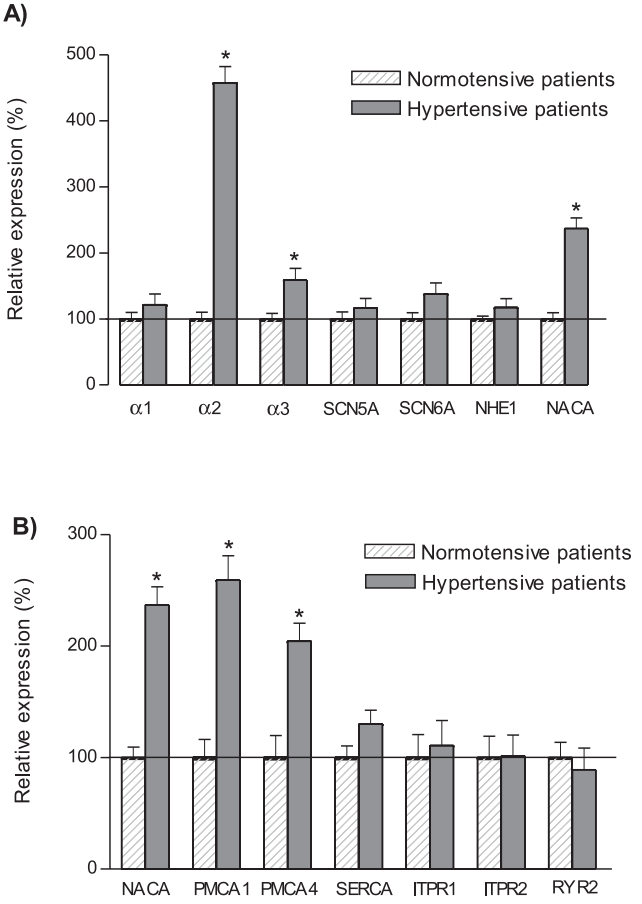
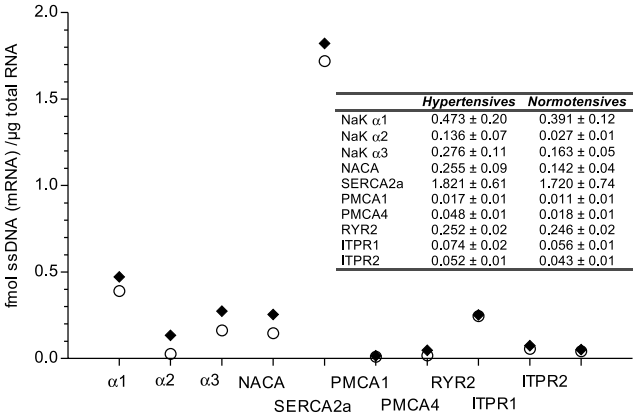


Fig. 2. Relative expression of sodium or calcium ion transporters. Values (mean ± S.E.M.) of normotensive patients are set to indicate 100% expression. (A) The sodium transporters listed are the isoforms of the α subunit of Na^+/K^+-ATPase, the sodium channel isoforms SCN5a and SCN6a, the Na^+/H^+-exchanger (NHE1) and the Na^+/Ca^{2+}-exchanger (NACA). (B) The calcium transporters investigated are the Na^+/Ca^{2+}-exchanger (NACA), isoforms 1 and 4 of the plasma membrane Ca^{2+}-ATPase (PMCA1 and PMCA4), isoform 2a of the sarcoendoplasmic Ca^{2+}-ATPase (SERCA), and the calcium channels ryanodine receptor (RYR2) and isoforms 1 and 2 of the inositol 1,4,5-trisphosphate receptor (ITPR). $*P < 0.05$.



	Hypertensives	Normotensives
NaK α1	0.473 ± 0.20	0.391 ± 0.12
NaK α2	0.136 ± 0.07	0.027 ± 0.01
NaK α3	0.276 ± 0.11	0.163 ± 0.05
NACA	0.255 ± 0.09	0.142 ± 0.04
SERCA2a	1.821 ± 0.61	1.720 ± 0.74
PMCA1	0.017 ± 0.01	0.011 ± 0.01
PMCA4	0.048 ± 0.01	0.018 ± 0.01
RYR2	0.252 ± 0.02	0.246 ± 0.02
ITPR1	0.074 ± 0.02	0.056 ± 0.01
ITPR2	0.052 ± 0.01	0.043 ± 0.01

Fig. 3. Quantification of ion transporter-specific mRNAs (ssDNAs) as a part of the total RNA isolated. The figure shows the expression of the various mRNAs in hypertensives (♦) and normotensives (○) given in fmol ssDNA per µg of total RNA. The inset includes the averaged data ± S.E. calculated from Eq. 5 (see Section 2) after taking into account the PCR efficiency factor *E*. SERCA2a is expressed in a great amount in the right atrium. Taken together, the three isoforms of the α subunit mRNAs of the Na^+,K^+-ATPase account for half of this amount.

relatively large amounts of protein and could therefore not be carried out in the current study. Instead, ouabain equilibrium binding studies were carried out as described in Section 2. Whenever sufficient protein was present, measurements were carried out in duplicate or triplicate. Although these measurements are not as precise as Scatchard binding studies, they still allow one to obtain a good approximation of the expression of sodium pumps.

Using microsomes derived from the tissue of either 11 normotensive or 14 hypertensive patients, ouabain binding was found to be 1.37 ± 0.2 pmol/mg protein for the normotensive group and 1.99 ± 0.19 pmol/mg of protein for the hypertensive group. These values for the two groups are significantly different from each other ($P = 0.046$).

4. Discussion

Based on clinical observations showing that salt restriction in the diet results in decreased blood pressure, and the fact that in some hypertensive patients it is possible to detect elevated Na^+ or Ca^{2+} concentrations in the cytosol of their erythrocytes or leukocytes, it was suggested earlier that electrolyte homeostasis of the cell might play an important role in the pathogenesis of the primary (essential) hypertension [7]. This physiological change could be taken as an indication for quantitative or qualitative alterations in ion transporting systems responsible for Na^+ or Ca^{2+} homeostasis. Based on this postulate, we examined the expression levels of mRNA molecules coding for the isoforms of sodium pump α subunits, sodium and calcium channels, calcium pumps, and sodium/calcium and sodium/proton exchangers. The tissue investigated was taken from the appendage of the right heart atrium of human patients that had to undergo cardiac surgery. Although the 50-mg tissue sample taken was sufficient for extraction and detection of transporter-specific mRNA molecules, it was not sufficient for detection of the corresponding proteins. Nevertheless, it was seen as an advantage at this stage to investigate the conditions in tissues where, because of the very short span of time between tissue removal and mRNA extraction, RNA degradation was able to be kept to a minimum.

Special emphasis was given to the investigation of the expression of various α subunit isoforms of the Na^+,K^+-ATPase. The sodium pump plays a key role in the physiology of animal cells. It is responsible for the maintenance of the sodium gradient and of the membrane potential and is involved in regulation of cell volume. It is the only known receptor for cardiac glycosides and is thought to be involved in the pathogenesis of hypertension [8,9]. In rats, extended treatment with ouabain [10] or a decrease in the sodium pump number results in hypertension [3]. A selective decrease in the expression of the high-affinity ouabain-binding isoform in hypertensive rats has physiological effects similar to those produced by treatment with cardiac glycosides or that observed for increased circulation rates of endogenous sodium pump inhibitors. In all these cases, the result is a reduction in sodium pump activity [11].

The expression levels of mRNA for the various sodium pump isoforms in the heart and their expression pattern in certain pathological conditions has been investigated by several groups. The objectives of these investigations were cardiac diseases like cardiomyopathy, pressure overload due to valvular defects, and hypertrophy. Fig. 4 summarizes some of these studies and sets them in relationship to our current results. In some cases, a decreased expression

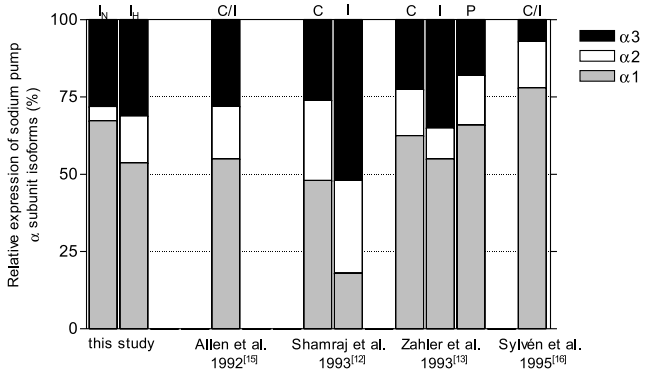


Fig. 4. Comparison of various studies. The data shown for expression of mRNA coding for α1, α2 and α3 subunits of sodium pump are normalized. The data of the current study were obtained by analyzing the expression in the appendage of the right heart atrium. All other investigators used left ventricles of human hearts. All studies, except the one of Shamraj et al. who used tissue from insufficient hearts, are in agreement that α1 is the predominant mRNA isoform of the sodium pump α subunit. I, insufficient heart with reduced pumping activity; $_N$, right atrial appendage of normotensives, $_H$, right atrial appendage of hypertensives; C, control hearts of organically healthy patients; P, pressure-overloaded hearts.

of the isoforms was found in diseased hearts [12–14], in other cases no significant changes were found in the expression of isoforms between healthy or diseased hearts [15,16]. In all of the above cases mRNA expression was mainly investigated in the left ventricles of the hearts. For detection and quantification, Northern blot analysis was used in all cases. All authors reported marked differences in the expression patterns among the various patients. Jewell et al. reported a ratio for α1 mRNA to that of total sodium pump isoforms of 29–79%, for α2 mRNA 7–43%, and for α3 mRNA 11–53% [17].

In the current study, the mRNA for the α1 subunit isoform of the sodium pump is the predominant form in the appendage of right atria. This is in good agreement with results of previous investigations [12,13,15,16]. In normotensive patients, the α2 is less abundant than the α3. The variability of expression was, however, less dramatic than in other investigations. We found a ratio for α1 of 52–72%, for α2 4–20% and for α3 26–37% (Fig. 4). In hypertensive patients, α1 is also the predominant isoform and is not expressed at a different level than in normotensives. The α2 isoform, however, is significantly elevated in hypertensives. This subunit mRNA was rarely present in normotensives (Fig. 1). The α3 isoform also shows significantly higher expression in hypertensive patients than in normotensives. Thus, the isoform expression pattern is different in the two groups of patients: the ratio α1:α2:α3 changes from 100:7:40 in normotensives to 100:30:60 in hypertensives. In the tissue investigated, hypertensive patients express more sodium pump total α subunit-specific mRNA (0.89 ± 0.38 fmol for α1+α2+α3 mRNAs per μg of total RNA) than normotensive patients (0.58 ± 0.18 fmol/μg). With membrane preparations from the same area of the heart, specific ouabain binding is elevated (1.37 ± 0.2 pmol [^{3}H]ouabain/mg protein for the normotensive group and 1.99 ± 0.19 pmol [^{3}H]ouabain/mg protein for the hypertensive group), indicating that increased mRNA indeed results in increased expression of sodium pump protein.

Thus, in the current study, there is no indication that hypertension is necessarily associated with reduced amounts of Na^+,K^+-ATPase [3,9]. Assuming that our observations reflect a physiologically relevant phenomenon, one has to ask what is the signal that triggers the elevated expression of sodium pump-specific mRNAs. One obvious possibility is that increased expression reflects a compensatory reaction to an elevated intracellular Na^+ concentration. Since in this case [Ca^{2+}] should be elevated in the cytosol due to the decrease of the Na^+ gradient, one would expect that in hypertension the expression of Ca^{2+}-specific transporters would be also affected. Increased intracellular [Ca^{2+}] in hypertensives has been shown before [18,19]. For this reason, besides the sodium pump isoforms and other Na^+ transporting systems, we decided to investigate also the expression of various Ca^{2+} transporters.

One of the various Na^+ transporting systems is the Na^+/H^+-exchanger, which is thought to be involved in the pathogenesis of hypertension [20,21]. Elevated activity of this exchanger would result in elevated intracellular [Na^+] and therefore also elevated [Ca^{2+}], a phenomenon observed in investigations of some patients with hyperactivity of this ion-transporting system [2,22–24]. An increased activity is not associated with elevated mRNA or protein levels for this ion transporter [21,25], but rather is possibly due to changes in the action of G-proteins [26]. Although the latter possibility cannot be addressed

in our investigation, in agreement with the previous reports we did not detect any hypertension-associated alterations in the expression of this transporter (Fig. 2).

The same applies for the expression of the sodium channels SCN5 and SCN6. No differences were found between hypertensive or normotensive patients (Fig. 2 and Table 3). There are three mutations, two point mutations and a KPQ-deletion in SCN5, known to be associated with a persistent influx of Na^+ that might play a role in hypertension [27,28]. In our study, we investigated but were not able to confirm the KPQ deletion (not shown).

The Na^+/Ca^{2+}-exchanger is the connecting link between Na^+ and Ca^{2+} homeostasis and is believed to play a role in the action of cardiac glycosides or in the development of hypertension [29]. In our investigation, the tissue from hypertensive patients expresses 2.4-fold higher Na^+/Ca^{2+}-exchanger-specific mRNA than the control group (Fig. 2 and Table 3). Changes in the expression of the Na^+/Ca^{2+}-exchanger have been investigated previously but the results are not consistent. While some have demonstrated increased exchanger-specific mRNA and protein levels associated with heart diseases [30], others have failed to confirm this observation [31,32]. If we assume that elevated mRNA is an indication for elevated protein expression, our results are consistent with our hypothesis that increased expression of Na^+ or Ca^{2+} transporting systems takes place as a compensatory mechanism in the face of elevated cytosolic concentrations of these ions. Along these lines, long-term treatments with ouabain were shown to cause higher expression of the exchanger [33,34].

Of all active Ca^{2+} transporting systems investigated, differences in expression between normo- and hypertensives were only found for the plasma membrane Ca^{2+}-ATPase. In hypertensives the mRNA coding for PMCA1 and PMCA4 isoforms are significantly increased (Fig. 2 and Table 3). Plasma membrane Ca^{2+}-ATPases have been found to be elevated on the mRNA level in spontaneously hypertensive rats [35]. Nevertheless, not much is known about the function and regulation of the various isoforms of the plasma membrane Ca^{2+}-ATPases. In contrast to the results for the PMCA isoforms, the expression of the sarcoendoplasmic Ca^{2+}-ATPase SERCA2a is the same in both groups of patients (Fig. 2 and Table 3). The same applies for the other sarcoendoplasmic ion transporting systems investigated here, the Ca^{2+}-channels RYR2, ITPR1 and ITPR2. These systems were preferred for investigation over others because a reduced expression of RYR2 mRNA and elevated ITPR1 mRNA in the ventricle were reported in connection with heart diseases [31,36]. In our investigation, however, no differences were found between normotensive and hypertensive patients.

Currently, we do not have a plausible explanation as to why all hypertension-related differences in mRNA expression we have seen thus far are associated with ion transporters located in the plasma membrane. The results, however, are consistent with the idea of compensatory responses to elevated cytosolic Na^+ and Ca^{2+} concentrations. The reason for such changes, which have been demonstrated to occur in connection to hypertension in various investigations [18,19,37,38], is unclear. One might think that endogenous cardiac steroids, if produced at higher levels, might inhibit the sodium pump, causing elevation of Na^+ and subsequently Ca^{2+} concentrations in the cytosol. In such a case, the heart might respond with upregulation of plasma membrane Na^+ and Ca^{2+} transporters to overcome the unphysiological imbalance of these ions. Such endogenous inhibitors of the sodium pump have been described in many investigations [39,40], and it is thought that their physiological production stimulates heart contraction and that increased biosynthesis might cause hypertension [9,29]. Nevertheless, alternative mechanisms of sodium pump regulation, as proposed or shown by various investigators [41–45], or the elevated activity of the Na^+/H^+-exchanger [26] might (also) be linked to cytosolic changes in $[Na^+]$ or $[Ca^{2+}]$. Although the investigation presented here, because of its nature, does not address these possibilities, it is in good agreement with the overall tenor of numerous investigations suggesting a link between electrolyte homeostasis and hypertension and encourages the continuation of the work under physiologically controlled conditions.

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