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Elevated myocardial interstitial norepinephrine concentration contributes to the regulation of Na^+,K^+-ATPase in heart failure

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Abstract

Myocardial Na^+,K^--ATPase is reduced in congestive heart failure. To study the regulation of Na^+,K^+-ATPase in congestive heart failure, we performed Western and Northern blot analyses of ventricular myocardium of dogs with pacing-induced congestive heart failure and chronic norepinephrine infusion, using isoform-specific antibodies and cDNA probes. Congestive heart failure and norepinephrine infusion caused similar increases in myocardial interstitial norepinephrine concentration and reductions of myocardial Na^+,K^+-ATPase α_3-subunit protein, but differed in their effects on myocardial Na^+,K^+-ATPase α_3-subunit gene expression. Chronic norepinephrine infusion produced no changes in the steady-state mRNA level for the α_3-subunit of Na^+,K^+-ATPase, suggesting that the changes in Na^+,K^+-ATPase protein were induced via a post-transcriptional mechanism. In contrast, down-regulation of the Na^+,K^+-ATPase α_3-subunit in the failing heart was accompanied by a decreased α_3-subunit mRNA level, indicating the presence of a transcriptional event. The α_1-subunit protein content and mRNA level were not affected by either norepinephrine infusion or rapid ventricular pacing. We conclude that, while elevated myocardial interstitial norepinephrine levels may contribute substantially to the down-regulation of the Na^+,K^+-ATPase α_3-subunit in the failing myocardium, additional regulatory factors are responsible for the decreased myocardial α_3-subunit mRNA expression in congestive heart failure.

Keywords: Na^+,K^+-ATPase; Digitalis; Heart failure; Norepinephrine

1. Introduction

Na^+,K^+-activated adenosine triphosphatase (Na^+,K^+-ATPase), a ubiquitous plasma membrane enzyme also referred to as the sodium pump, plays an important role in many fundamental cellular processes, such as the regulation of cell volume, intracellular pH, ionic gradients and transmembrane potential (Horisberger et al., 1991). It is also the receptor site for digitalis glycosides (Akera and Brody, 1977). When digitalis binds to Na^+,K^+-ATPase, it induces a conformational change of the enzyme and inhibits the active transport of Na^+ across the plasma membrane. The resultant increase in intracellular Na^+ causes cytosolic Ca^{2+} to rise via the Na^+-Ca^{2+} exchange mechanism. The increased Ca^{2+} concentration may then activate the contractile proteins and augment muscle contraction.

Na^+,K^+-ATPase exists as a heterodimer composed of an α- and a β-subunit. The catalytic activity of the enzyme resides entirely in the α-subunit, and transfection studies have established that it is the α-subunit that determines digitalis binding affinity (Kent et al., 1987). Thus far, three distinct isoforms of the α-subunit (α_1, α_2 and α_3) have been described, each a product of a different gene (Shull et al., 1986). The three isoforms differ in their binding affinity for digitalis, have distinct tissue distributions, and are regulated differently during developmental stages (Orlowski and Lingrel, 1988; Sweadner, 1989).

Diminished myocardial Na^+,K^+-ATPase activity and ouabain binding sites have been observed in patients and experimental animals with congestive heart failure (Norgaard et al., 1988; Fan et al., 1993; Kim et al., 1994). Since congestive heart failure is associated with heightened sympathetic nervous activity (Levine et al., 1982) which is known to modulate Na^+,K^+-ATPase activity (Swann, 1983), we speculate that the reduction of Na^+,K^+-ATPase in congestive heart failure is caused, at least in part, by the heightened sympathetic nervous system activity. This hypothesis is supported by our prior findings that chronic norepinephrine infusion decreased

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myocardial Na^+,K^+-ATPase (Kim et al., 1994), and that the reductions of Na^+,K^+-ATPase activity and ouabain binding sites in the failing myocardium were prevented by nadolol, a β-adrenoceptor antagonist (Fan et al., 1993).

The mechanisms by which excess norepinephrine causes cardiac Na^+,K^+-ATPase down-regulation have not been adequately studied. We now report that chronic norepinephrine infusion down-regulates myocardial Na^+,K^+-ATPase α_3-subunit protein via a post-transcriptional mechanism; the steady-state mRNA level for α_3-subunit remains unaffected. In contrast, down-regulation of the Na^+,K^+-ATPase α_3-subunit in the failing myocardium involves a transcriptional mechanism as well, as evidenced by a decreased steady-state α_3-subunit mRNA level. The data suggest that, in congestive heart failure, in addition to elevated norepinephrine levels, additional regulatory factors of Na^+,K^+-ATPase are responsible for the reduction of the α_3-subunit mRNA.

2. Materials and methods

2.1. Animal preparation and study protocols

Adult mongrel dogs weighing 18.6–29 kg were used. A left thoracotomy was performed under sterile conditions for the placement of heparin-filled Tygon catheters in the left atrium, main pulmonary artery and descending thoracic aorta. In addition, a Konigsberg micromanometer (Konigsberg Instruments, Pasadena, CA, USA) was placed in the left ventricle via a stab wound at the left ventricular apex. One week later, the animals were randomized into one of the following two protocols.

2.1.1. Protocol 1

A custom-designed Medtronic multiprogrammable pacemaker (Medtronic, Minneapolis, MN, USA) was placed in a cervical pocket and connected to a bipolar pacing lead positioned in the apex of the right ventricle through an external jugular vein. The animals were randomized to either rapid pacing at a rate of 225 beats/min or control pacing at a rate of 100 beats/min. The resting heart rate is < 100 beats/min in normal dogs. The pacemaker was set in a demand mode. The heart would be electronically paced only if the animal's intrinsic heart rate fell below the assigned pacing rate. Pacing was verified weekly by ECG recordings. Congestive heart failure was assessed by the development of tachypnea and elevated left atrial pressure. The animals were allowed to adapt to the laboratory and trained to lie quietly with only minimal restraint on a table for hemodynamic measurements.

Final hemodynamic and plasma norepinephrine measurements were taken 8 weeks after implantation of the pacemaker. The animals were then killed with an intravenous injection of a lethal dose of phenobarbital (> 100 mg/kg). The heart was excised and weighed immediately. The left ventricular free wall and the interventricular septum were combined for left ventricular weight. Full-thickness muscle block samples weighing about 0.5 g each were taken from the left ventricular free wall 3 cm below the atrioventricular groove and stored in liquid nitrogen.

2.1.2. Protocol 2

An Alzet model 2ML4 osmotic minipump (Alza, Palo Alto, CA, USA) was implanted aseptically in the posterior neck region. The minipumps were used to administer either norepinephrine (0.5 μg/kg/min) or sterile normal saline subcutaneously. Because each minipump could deliver a constant volume for only 4 weeks, a second osmotic minipump was implanted 4 weeks later to ensure constant norepinephrine delivery for 8 weeks. At 8 weeks, the dogs underwent final hemodynamic studies and norepinephrine measurements, and were then killed as in Protocol 1.

The study was approved by the University of Rochester Committee on Animal Resources and conformed to the guiding principles of the American Physiological Society and the National Institutes of Health 'Guide on the Humane Care and Use of Laboratory Animals'.

2.2. Hemodynamic measurements

Final hemodynamic measurements were obtained 8 weeks after the start of cardiac pacing or norepinephrine infusion. The pacemaker (in Protocol 1) was reprogrammed 2 h before the hemodynamic measurements to a subthreshold level to allow an intrinsic heart rate. The previously implanted catheters were attached to a Spectramed P23XL (Spectramed, Oxnard, CA, USA) and an eight-channel Brush model 480 recorder (Gould, Instrument System Division, Cleveland, OH, USA) for measuring heart rate, left atrial pressure and aortic pressure. The Konigsberg transducer was connected to the Brush recorder for measuring left ventricular pressure and its first derivative (dP/dt) using an electronic differentiator. Cardiac output was measured using indocyanine green (Cardio-Green, Hynson, Westcott, and Dunning, Baltimore, MD, USA) and Lyons model D-014 dye-dilution cardiac output system (Lyons Medical Instrument, Sylmar, CA, USA). Resting hemodynamic measurements were taken in triplicate 5 min apart, and the values were averaged and used for statistic analyses.

2.3. Arterial and myocardial interstitial norepinephrine

Arterial blood was collected into ice-chilled glass tubes containing reduced glutathione. The blood was centrifuged, and the plasma portion was stored at −70°C until subsequent analysis for norepinephrine using a radioenzymatic assay (Hussain and Benedict, 1985).

The myocardial interstitial norepinephrine level was

measured using a modification (Delehanty et al., 1994) of the method described originally by Cousineau et al. (1980). The method involved a bolus intracoronary injection of a mixture of [^{125}I]albumin, [^{14}C]sucrose and [^{3}H]norepinephrine and timed continuous sampling of coronary sinus blood (19 ml/min) during the ensuing 60 s. Aortic and coronary sinus blood samples were taken simultaneously for measuring norepinephrine. Myocardial interstitial norepinephrine content was measured as described before (Cousineau et al., 1980).

2.4. Protein electrophoresis and Western blotting

Left ventricular muscle blocks were trimmed, minced and homogenized in an ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 22°C). The homogenate was centrifuged at 40 000 × *g* for 15 min at 4°C. The membrane pellets were resuspended in a sample buffer containing 0.32 M sucrose, 1 mM EDTA, pH 7.35, supplemented with 0.5 mM phenylmethylsulfonyl fluoride and 1 μg/ml leupeptin. The protein content of the suspension was determined using the bicinchonic acid BCA kit (Pierce, Rockford, IL, USA), with bovine serum albumin as a standard.

Membrane fractions containing 50 μg of protein, with the addition of an equal volume of 2 × loading buffer (0.1 M Tris-HCl, pH 6.8, 20% glycerol, 4% sodium dodecyl sulfate [SDS], 10% β-mercaptoethanol and 10 μg/ml phenoblue), were heated at 95°C for 5 min and loaded onto a 4.5% polyacrylamide stacking gel and a 7.5% polyacrylamide/0.1% SDS resolving gel. The gel system was run at 10 mA for 15 h, and the proteins were transferred to polyvinylidene difluoride membranes in a transfer buffer containing 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (pH 11) and 10% methanol at 4°C for 80 min at a 500 mA constant current. The membranes were then placed in a blocking buffer (50 mM Tris-HCl, pH 7.4, 0.9% NaCl, 1 mg/l thimerosal, supplemented with 1% non-fat milk) for 1 h to minimize non-specific binding and were then probed with either anti-chicken α_1-specific antibody (α_2F, Developmental Hybridoma Bank, University of Iowa) or anti-dog α_3-specific antibody (F9G10, a generous gift from Kathleen Sweadner, Harvard University). Both antibodies are mouse immunoglobulin G in origin. The probed membranes were then washed 4 × with blocking buffer, each for 10 min, and incubated with horseradish peroxidase conjugated goat anti-mouse immunoglobulin G antibody (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). The membranes were washed with blocking buffer 4 × before reaction with 3,3′,5,5′-tetramethylbenzidine membrane peroxidase substrate (Kirkegaard & Perry Laboratories). The signal intensity of the blue precipitates was quantified by videodensitometry using a Bio-Image system (Millipore, Ann Arbor, MI, USA).

The specificity of the antibodies was tested with membrane fractions taken from the dog kidney and brain. Varying concentrations of a protein standard (membrane preparation from the dog kidney for α_1 and from dog brain for α_3) were used to make a reference standard curve by plotting videodensitometric readings against the protein concentration. The protein contents of unknown tissue samples were calculated using the reference standard curve, and expressed in μg of standard protein per mg of sample protein.

2.5. RNA isolation and Northern blot analyses

Total cellular RNA was isolated using the Trisolv reagent (Biotecx Laboratories, Houston, TX, USA). Northern blot analysis was used to measure Na^+,K^+-ATPase α_1- and α_3-isoform mRNA, using a rat α_1-cDNA (kindly provided by Burton Horowitz, University of Nevada, Reno, NV, USA) and a dog α_3-cDNA, respectively. The dog α_3-cDNA was generated from myocardial RNA by reverse transcription polymerase chain reaction methodology, and cloned into a pCRII vector (Invitrogen, San Diego, CA, USA). cDNA was purified with a GeneClean kit (Bio 101, Vista, CA, USA), and radiolabeled using Klenow DNA polymerase (Boehringer Mannheim, Indianapolis, IN, USA) and [^{32}P]dCTP. The glyceraldehyde 3-phosphate dehydrogenase mRNA level, which was selected as the internal control, was measured using the human cDNA probe purchased from Clontech (Palo Alto, CA, USA).

20 μg of total RNA was denatured at 65°C for 5 min, fractionated on a 0.8% agarose gel containing 2.2 M formaldehyde, and transferred onto a positively charged nylon membrane (Boehringer Mannheim). After baking at 80°C for 3 h, the membrane was prehybridized for 3 h with a solution containing 50% formamide, 2% blocking reagent (Boehringer Mannheim), 5 × standard sodium citrate, 0.02% SDS, and 0.1% *N*-lauroylsarcosine. Hybridization was carried out by adding radioactive cDNA probes. The temperature for prehybridization and hybridization was 42°C for the α_3-probe and 40°C for the α_1-probe. After overnight hybridization, a series of washes was carried out. The final wash was in 0.1 × standard sodium citrate and 0.1% SDS at 65°C. Autoradiography was done using Kodak X-OMAT films (Kodak, Rochester, NY, USA) with intensifying screens at −70°C. The signal intensity was measured by videodensitometry using a Bio-Image system (Millipore).

The specificities of the probes were tested with cellular RNA from dog kidney and brain. The mRNA levels were normalized to the glyceraldehyde 3-phosphate dehydrogenase mRNA. The values from animals with congestive heart failure and norepinephrine infusion were then normalized by the means of their respective control groups.

2.6. Data analyses

All results are expressed as means ± S.E.M. The statistical significance of differences between groups was deter-

Table 1
Resting hemodynamic parameters in experimental animals

Parameter	Protocol 1		Protocol 2	
	Control	CHF	Saline	NE
Heart rate (bpm)	95±7	128±6 [a]	104±4	81±4 [a]
Mean aortic pressure (mm Hg)	108±3	96±2 [a]	113±3	126±4
Left atrial pressure (mm Hg)	9.7±1.0	26.7±2.4 [a]	8.0±0.6	8.3±1.0
Cardiac output (l/min)	4.5±0.4	2.6±0.2 [a]	4.8±0.3	4.1±0.5
LV dP/dt (mm Hg/min, $\times 10^{-3}$)	2.9±0.2	1.4±0.1 [a]	3.3±0.1	4.0±0.3

Data presented as means ± S.E.M., $n = 8$ in each group. CHF, congestive heart failure; NE, norepinephrine infusion; LV, left ventricular.
[a] $P < 0.05$, vs. Control or Saline, by Student's t-test for unpaired data.

mined using Student's t-test for unpaired data. Values were considered statistically significant if $P < 0.05$.

3. Results

3.1. Hemodynamic characteristics

Table 1 shows the final hemodynamic data. Dogs with rapid ventricular pacing (Protocol 1) displayed typical hemodynamic findings of congestive heart failure, with resting tachycardia, elevated left atrial pressure, reduced left ventricular dP/dt, and decreased cardiac output. Venous congestion was evidenced in the animals by the increases in liver weight (792 ± 49 vs. 613 ± 13 g, $t = 4.023$, $P < 0.001$) and lung weight (331 ± 35 vs. 240 ± 13 g, $t = 2.43$, $P = 0.029$), compared to the animals with control pacing. Also compared to the control animals, the congestive heart failure animals had a greater mean left ventricular weight (116 ± 5 vs. 101 ± 7 g), but the difference between the two groups did not reach statistical significance ($t = 1.84$, $P = 0.086$).

Chronic norepinephrine infusion (Protocol 2) caused a 20% reduction in heart rate, but had no significant effects on mean aortic pressure, left atrial pressure, cardiac output or left ventricular dP/dt.

Table 2
Plasma and myocardial interstitial norepinephrine levels (ng/ml)

	Protocol 1		Protocol 2	
	Control	CHF	Saline	NE
Plasma	0.31±0.07	0.98±0.21 [a]	0.31±0.03	4.14±0.38 [a,b]
Interstitial	0.20±0.07	1.12±0.15 [a]	0.26±0.06	1.78±0.43 [a]

Data presented as means ± S.E.M., $n = 8$ in each group. CHF, congestive heart failure; NE, norepinephrine infusion.
[a] $P < 0.05$ vs. Control or Saline; [b] $P < 0.05$ vs. CHF, by Student's t-test for unpaired data.

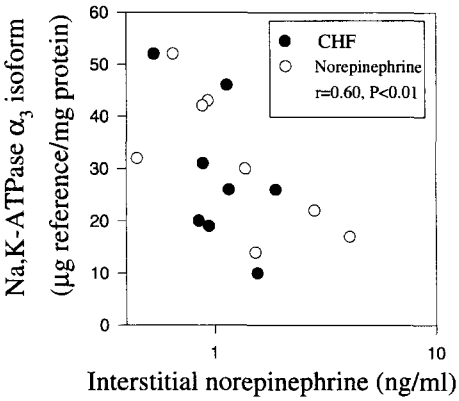

Fig. 1. Scatterplot showing the negative correlation between myocardial interstitial norepinephrine concentration (in logarithmic scale) and myocardial Na^+,K^+-ATPase α_3-isoform protein content in dogs with congestive heart failure (CHF) and norepinephrine infusion. The correlation coefficient (r) was 0.60 ($P < 0.01$). The slope of the regression line did not differ significantly between the two groups of animals.

3.2. Plasma and myocardial interstitial norepinephrine levels

Both rapid ventricular pacing and chronic norepinephrine infusion produced significant elevations in plasma and myocardial interstitial norepinephrine levels (Table 2). The plasma norepinephrine level was much higher in the norepinephrine-infused dogs than those with congestive heart failure. The mean myocardial interstitial norepinephrine level also was greater in the norepinephrine-infused dogs, but because there was a large overlap of the values between the two groups (Fig. 1), the difference in myocardial interstitial norepinephrine between the two groups was not statistically significant ($t = 1.01$, $P = 0.33$).

3.3. Western blot analysis

Table 3 shows the relative abundance of the specific isoform proteins in cardiac tissue. The α_1-isoform of Na^+,K^+-ATPase did not change significantly after either induction of pacing-induced congestive heart failure or

Table 3
Left ventricular Na^+,K^+-ATPase α_1- and α_3-isoforms

Na^+,K^+-ATPase	Protocol 1		Protocol 2	
	Control	CHF	Saline	NE
Protein				
α_1-Isoform	147±22	138±18	172±12	158±12
α_3-Isoform	57±9	29±8 [a]	51±6	31±7 [a]
mRNA				
α_1-Isoform	1.00±0.14	1.13±0.25	1.00±0.15	1.26±0.25
α_3-Isoform	1.00±0.11	0.65±0.12 [a]	1.00±0.10	0.98±0.11

Data presented as means ± S.E.M., $n = 8$ in each group. CHF, congestive heart failure; NE, norepinephrine infusion.
[a] $P < 0.05$ vs. Control or Saline, by Student's t-test for unpaired data.

norepinephrine infusion while the α_3-isoform was reduced significantly by congestive heart failure and norepinephrine infusion. There was a significant negative correlation between interstitial norepinephrine concentration and the α_3-isoform of Na^+,K^+-ATPase (Fig. 1). There was no difference in the slope of the regression line between the congestive heart failure and norepinephrine infusion groups.

3.4. Northern blot analysis

Table 3 also shows the relative abundance of α_1- and α_3-mRNA in different groups of animals. The table shows that α_1-mRNA did not change in dogs after either rapid ventricular pacing or chronic norepinephrine infusion compared to the control animals. Norepinephrine infusion also did not affect myocardial α_3-mRNA. In contrast, α_3-mRNA showed a significant reduction in animals with pacing-induced congestive heart failure compared to the control dogs.

4. Discussion

The present study demonstrated two aspects of the regulation of Na^+,K^+-ATPase in the canine myocardium. First, the myocardial Na^+,K^+-ATPase α_1-isoform was unaffected, with regard to protein content and mRNA levels, by either pacing-induced congestive heart failure or chronic norepinephrine infusion. Second, the myocardial α_3-subunit protein content was reduced both in congestive heart failure and after chronic norepinephrine infusion; however, based on the mRNA level the α_3-isoform was reduced only in congestive heart failure. Thus, there is an apparent isoform-specific down-regulation of Na^+,K^+-ATPase in failing myocardium. The canine myocardium lacks the α_2-isoform (Kim et al., 1994). The data also indicate that although the elevated myocardial interstitial norepinephrine level accounts to a large extent for the decrease in α_3-subunit protein in failing myocardium, additional factors are responsible for the decreased expression of the α_3-subunit mRNA in congestive heart failure.

Animals received 8 weeks of either cardiac pacing or norepinephrine infusion. This duration of rapid cardiac pacing was sufficient to produce stable chronic heart failure, increase myocardial interstitial norepinephrine and reduce myocardial β-adrenoceptor density by 43% (Delehanty et al., 1994). We have further shown that norepinephrine infusion for 8 weeks causes decreases in myocardial catecholaminergic histofluorescence profile, immunoreactive tyrosine hydroxylase profile, and neuronal norepinephrine uptake activity similar to those that occur in congestive heart failure (Himura et al., 1993). Furthermore, our present study showed that myocardial interstitial norepinephrine was elevated in dogs with congestive heart failure and norepinephrine infusion, and that the interstitial norepinephrine levels did not differ significantly between the two groups of animals. Thus, we believe that it was reasonable to choose the 8-week time point for the present study. Other time points have not been systematically studied.

Dogs tolerated the 8-week infusion of the subhypertensive dose of norepinephrine. At this dose, norepinephrine infusion caused no deaths. Nor did it cause myocardial hemorrhage or necrosis (unpublished observations). There were no significant changes in mean aortic pressure, left atrial pressure, left ventricular dP/dt, and cardiac output. The findings suggest that an elevated ventricular filling pressure is not a prerequisite for the down-regulation of the Na^+,K^+-ATPase α_3-isoform. Our results indicate that the reduction of α_3-subunit in failing myocardium is caused, at least in part, by the heightened sympathetic nervous system activity. This is consistent with the finding in our prior study of right-sided congestive heart failure that the reductions of Na^+,K^+-ATPase activity and ouabain binding sites were prevented by the non-selective β-adrenoceptor antagonist, nadolol (Fan et al., 1993).

The results of our study of chronic norepinephrine infusion effects on Na^+,K^+-ATPase, however, differ significantly from those following acute administration of norepinephrine. Prior studies have shown that acute infusion of norepinephrine increases cerebral ouabain binding in intact rats (Swann and Steketee, 1989). Similarly, Svoboda et al. (1986) showed that isoproterenol, norepinephrine, and epinephrine, all acutely stimulated brain Na^+,K^+-ATPase activity. Wanless et al. (1985) showed that the stimulatory effect of norepinephrine on myocardial Na^+,K^+-ATPase was blocked by the β-adrenoceptor antagonist, propranolol, suggesting that the effect was mediated through β-adrenoceptors. In contrast, administration of norepinephrine (5 mg/kg/day) to dogs for 7 days decreased myocardial Na^+,K^+-ATPase activity (Swann, 1983). The latter change is consistent with the reduction of myocardial Na^+,K^+-ATPase observed in our present study.

The precise mechanism by which chronic norepinephrine decreases Na^+,K^+-ATPase α_3-isoform remains to be elucidated. Results of our prior study with nadolol (Fan et al., 1993) suggested that β-adrenoceptors play a role in the cascade of events that lead to decreased synthesis and/or increased degradation of α_3-subunit protein. Indeed, norepinephrine has been shown to increase intracellular cyclic AMP and causes the reduction of Na^+,K^+-ATPase through a phospholipase A_2-mediated pathway (Lingham and Sen, 1982; Satoh et al., 1992; Satoh et al., 1993). Alternatively, norepinephrine may cause Na^+,K^+-ATPase reduction via increased formation of oxygen free radicals (Rump and Klaus, 1994; Haggendal et al., 1987). Oxygen free radicals have been shown to inhibit Na^+,K^+-ATPase activity (Huang et al., 1992). Since many β-blockers are also antioxidants (Mark and Weglicki, 1988), the effect of nadolol on Na^+,K^+-ATPase down-regulation in congestive heart failure (Fan et al., 1993) could have been caused by its antioxidant properties.

Unlike congestive heart failure, chronic norepinephrine at the dose administered produced no reduction of α_3-isoform mRNA in the heart. It is possible that local stress in the failing myocardium could trigger paracrine and/or autocrine systems that exert their regulatory effects on gene expression (Sadoshima et al., 1993). These local stress factors are also known to activate the transcriptional protooncogenes, such as c-fos and c-jun (Kolbeck-Ruhmkorff et al., 1993; Shida and Isoyama, 1993). The 5′-flanking region of the human and rat α_3-subunit of Na^+,K^+-ATPase contains a binding site for the transcriptional factor AP-1, which is composed of c-fos and c-jun (Pathak et al., 1990).

Little is known of the specific physiological function of each of the three Na^+,K^+-ATPase isoforms. Using in situ hybridization techniques, Zahler et al. (1992) have shown that, in adult rat hearts, the mRNAs for the α_2- and α_3-isoforms are preferentially localized to the cardiac conduction system. Additional studies are warranted to determine whether a selective loss of Na^+,K^+-ATPase α_3-isoform may render the failing heart more susceptible to the arrhythmogenic effect of cardiac glycosides.

In summary, myocardial Na^+,K^+-ATPase protein is reduced in dogs with chronic norepinephrine infusion and pacing-induced congestive heart failure. The change is limited to the α_3-isoform. Chronic norepinephrine infusion down-regulates myocardial Na,K-ATPase α_3-subunit proteins via post-transcriptional mechanisms while down-regulation of the Na^+,K^+-ATPase α_3-subunit in the failing heart involves a transcriptional mechanism as well, as evidenced by a decreased steady-state α_3-subunit mRNA level. The data suggest that, in congestive heart failure, an elevated myocardial interstitial norepinephrine level is an important but not the sole factor contributing to the down-regulation of Na^+,K^+-ATPase α_3-subunit. Additional factors are present in congestive heart failure, causing alterations of the transcriptional factors and reductions of myocardial α_3-subunit mRNA. Further studies using different experimental animal models or humans are needed to determine if our findings are applicable to other forms of congestive heart failure.

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