

## Activity of cAMP-dependent protein kinase and $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase in failing and nonfailing human hearts

Uwe Kirchhefer<sup>a,\*</sup>, Wilhelm Schmitz<sup>a</sup>, Hasso Scholz<sup>b</sup>, Joachim Neumann<sup>a</sup>

<sup>a</sup>Institut für Pharmakologie und Toxikologie, Westfälische Wilhelms-Universität, Domagkstrasse 12, D-48149 Münster, Germany

<sup>b</sup>Abteilung Allgemeine Pharmakologie, Universitäts-Krankenhaus Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany

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### Abstract

**Objectives:** A hallmark of human heart failure is prolonged myocardial relaxation. Although the intrinsic mechanism of phospholamban coupling to the  $\text{Ca}^{2+}$ -ATPase is unaltered in normal and failed human hearts, it remains possible that regulation of phospholamban phosphorylation by cAMP-dependent mechanisms or other second messenger pathways could be perturbed, which may account partially for the observed dysfunctions of the sarcoplasmic reticulum (SR) associated with this disease. **Methods:** cAMP-dependent protein kinase (PKA) and  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II (CaM kinase) were characterized initially by DEAE-Sepharose chromatography in hearts from patients with end-stage dilated cardiomyopathy. We measured the activity of PKA and CaM kinase in left ventricular tissue of failing (idiopathic dilated cardiomyopathy; ischemic heart disease) and nonfailing human hearts. **Results:** Basal PKA activity was not changed between failing and nonfailing hearts. One major peak of CaM kinase activity was detected by DEAE-Sepharose chromatography. CaM kinase activity was increased almost 3-fold in idiopathic dilated cardiomyopathy. In addition, hemodynamical data (left ventricular ejection fraction, cardiac index) from patients suffering from IDC positively correlate with CaM kinase activity. **Conclusions:** Increased CaM kinase activity in hearts from patients with dilated cardiomyopathy could play a role in the abnormal  $\text{Ca}^{2+}$  handling of the SR and heart muscle cell. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** cAMP-dependent protein kinase;  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II; Heart failure

### 1. Introduction

The regulation of  $\text{Ca}^{2+}$  homeostasis in cardiac muscle cells is important for contractility and rate of relaxation. This is due to phosphorylation of cardiac regulatory proteins like phospholamban (PLB). When phosphorylated by PKA or by CaM kinase, PLB releases the cardiac  $\text{Ca}^{2+}$  pump (SERCA2a) from inhibition, resulting in an increase in  $\text{Ca}^{2+}$  transport into cardiac SR-vesicles at low ionized  $\text{Ca}^{2+}$  concentrations [1]. However, little is known about alterations of these mechanisms in end-stage heart failure. Functional studies have revealed that the positive inotropic effect of  $\beta$ -adrenoceptor agonists is reduced in terminal heart failure. This is caused by a multitude of alterations in the  $\beta$ -adrenergic pathway. Reduction of the number of  $\beta$ -adrenoceptors [2] and an increase of inhibitory GTP-

binding proteins [3,4] is thought to be responsible. This is accompanied by reduced  $\beta$ -adrenoceptor agonist-stimulated cAMP levels in failing human myocardium [5]. Furthermore, the inotropic effect of  $\beta$ -adrenergic catecholamines is reduced in end-stage human heart failure [2]. In addition to these various abnormalities,  $\text{Ca}^{2+}$  handling is most severely impaired in heart failure [6]. Interestingly, the increased resting or end-diastolic  $\text{Ca}^{2+}$  content is associated with a prolongation of the  $\text{Ca}^{2+}$  transient [7] in failing human hearts due to both idiopathic dilated cardiomyopathy (IDC) and ischemic heart disease (IHD). The disturbed  $\text{Ca}^{2+}$  handling is associated with abnormalities of  $\text{Ca}^{2+}$  transport proteins and with quantitative changes in the expression of regulatory proteins of the SR. Studies of mRNA have shown decreased levels for SERCA2a, PLB and SR- $\text{Ca}^{2+}$  release channel (RyR) in human heart failure [8–11]. Furthermore, a number of studies have demon-

\*Corresponding author. Tel.: +49-2518-3555-10; fax: +49-2518-3555-01.

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Table 1  
Clinical and anamnesis data from nonfailing heart donors

Sex	Age (years)	Diagnosis	Others
M	43	CB	Fatty liver, obesity <sup>a</sup>
M	53	Cerebral venous Thrombosis, Cerebral edema	Suspicion of cerebral Cancer
M	56	Polytrauma	Arteriosclerosis
F	46	CA, CB	Obesity, hypertension, Mild arteriosclerosis
M	36	CA, CB	Arteriosclerosis, Nicotine abuse
M	41	CB	Wernicke encephalopathy
F	27	CB	Drug addiction <sup>a,b</sup>
F	29	Polytrauma	–

The diagnoses leading to clinical death and other diagnosis in patient's medical history are given.

Abbreviations: CB, intracerebral bleeding; SA, subarachnoidal bleeding; CA, aneurysma of cerebral artery.

<sup>a</sup> No indications to any disease of the heart.

<sup>b</sup> Drugs used were alcohol, diamorphine and codeine.

strated altered SERCA2a and PLB protein levels [12–14]. In contrast, other groups could not detect any changes in the expression of genes encoding SERCA2a, PLB and RyR [9,11,15]. There are also contradictory data about the SR-Ca<sup>2+</sup> uptake which is reported to be depressed [10,16] or unaltered [17,18] in human heart failure. Therefore, it is conceivable that additional functional parameters like an altered activity of regulatory protein kinases are responsible for the disturbed Ca<sup>2+</sup> homeostasis in end-stage

human heart failure. In the work described here, we report an initial characterization of PKA and CaM kinase. The present study was undertaken to examine the activity of PKA and CaM kinase in failing and nonfailing left ventricular myocardium. Furthermore, we have addressed the question if possible alterations in enzyme activity are specific for different causes and severities of heart failure.

## 2. Methods

### 2.1. Human myocardial tissue

Procedures for obtaining human tissue complied with the Helsinki Declaration. Permission for these studies was obtained from the Ethics Committee of the University Hospital of Hamburg (Az 532/116/9.7.1991). Myocardial tissue from nonfailing hearts (NF) that could not be transplanted due to surgical reasons (size) or blood group incompatibility was obtained from organ donors. On inspection these hearts appeared to have normal ventricles. Aortic and pulmonary valves were excised and later used for valve replacement. Clinical data for these patients are reported in Table 1. Failing hearts were obtained from patients undergoing orthotopic heart transplantation due to end-stage heart failure (NYHA IV) resulting from IDC or IHD. Clinical data are reported in Table 2. Medical treatment for the patients with heart failure consisted of nitrates, cardiac glycosides, diuretics and angiotensin-con-

Table 2  
Hemodynamic data from patients with end-stage heart failure (NYHA IV)

Sex	Age	PCW (mm Hg)	EF (%)	CI (l/min×m <sup>2</sup> )
<i>Idiopathic dilated cardiomyopathy</i>				
M	46	19	16	2.1
M	54	21	13	1.6
W	38	11	15	1.7
M	40		22	2.3
M	47	15	25	2.2
W	35	23	25	1.9
M	59	35		1.7
M	59		24	
M	42	30		2.4
M	55	26		1.2
Mean±S.E.M.	47.5±2.7	22.5±2.7	20.0±1.9	1.9±0.1
<i>Ischemic heart disease</i>				
M	50	25	23	2.3
M	54	27	23	2.7
M	53	35	17	1.7
M	49	20	18	1.7
M	52	28	17	2.8
M	46	44		1.7
M	64		23	
M	57		25	
M	62			1.6
M	55	24		1.9
Mean±S.E.M.	54.2±1.7	29.0±3.0	20.8±1.2	2.0±0.2

Abbreviations: PCW, pulmonary capillary wedge pressure; EF, radionuclide-determined left ventricular ejection fraction; CI, cardiac index.

verting enzyme inhibitors. Patients did not receive  $\beta$ -adrenoceptor blocking therapy before transplantation. Cardiac surgery was performed with neuroleptic narcotic combination (haloperidol, fentanyl, nitrous oxide). Left ventricular tissue was quickly transferred in gassed bathing solution [5] at 4°C from the operating room to the laboratory. Care was taken not to use scarred, fibrotic, adipose tissue, endocardium, epicardium or great vessels. Tissue was frozen in liquid nitrogen and stored at –80°C for biochemical assays. The tissue used for this study was frozen within 1.5 h of explantation.

## 2.2. DEAE-Sepharose chromatography

Characterization of PKA isozymes was performed similar to Corbin et al. [19]. All steps were carried out at 4°C. Briefly, 2 g of frozen left ventricular myocardium were homogenized for 3×30 s in 10 ml of a buffer containing 1 mM EDTA and 5 mM Tris–HCl. The homogenate was centrifuged for 20 min at 14 000 *g*. The clarified supernatant was applied to a DEAE-Sepharose column (2.5×20 cm, Bio-Rad, Richmond, CA, USA) equilibrated in the same buffer. After washing the column with 60 ml of buffer, a linear gradient of NaCl (0 to 0.5 M) was started. Fractions of 2 ml volume were collected at a flow-rate of 60 ml/h and the PKA activity determined. A 10- $\mu$ l aliquot of every second fraction was added to 50  $\mu$ l of a medium (with and without 2  $\mu$ M cAMP) containing 20 mM NaF, 10 mM MgCl<sub>2</sub>, 40 mM Tris–HCl (pH 7.4), 160  $\mu$ M 3-isobutyl-1-methylxanthine, 0.1 mM ATP and 5  $\mu$ Ci [ $\gamma$ <sup>32</sup>P]ATP using histone IIA (8.3 mg/ml) as substrate. The assay was carried out as described in Section 2.3. In addition, protein elution was monitored by the absorbance at 280 nm in 10-fold diluted fractions (data not shown).

Characterization of CaM kinase was done by a modification of the method of Gupta and Kranias [20]. The left ventricular myocardium (2 g) was homogenized for 3×30 s with 10 ml of a buffer (in mM: Tris–HCl 50, MgCl<sub>2</sub> 5, EGTA 1, EDTA 1,  $\beta$ -mercaptoethanol 10, imidazole hydrochloride 1, pH 7.4) in the presence of protease inhibitors (0.4 mM PMSF and 15  $\mu$ M benzamidine). The tissue was then centrifuged for 20 min at 14 000 *g*. The resultant supernatant was bound to 10 ml of DEAE-Sepharose, which was preequilibrated with the buffer above (without protease inhibitors). The column (2.5×20 cm) was washed with 50 ml of the buffer. The bound protein was eluted from the column with a linear gradient of NaCl (0 to 0.5 M). Fractions (2 ml) were collected at a flow-rate of 60 ml/h. CaM kinase activity was determined in a 10- $\mu$ l aliquot of every second fraction as described in Section 2.3.

## 2.3. Protein kinase assays

All homogenates from nonfailing and failing hearts were prepared and assayed in parallel for determination of both

PKA and CaM kinase activity. PKA activity was determined by modification of a previously described method [21]. A 60-mg amount of frozen tissue powder was thawed and homogenized in 300  $\mu$ l of buffer A (mM: EDTA, 4;  $\beta$ -mercaptoethanol, 15; pH 7.4) for 3×30 s at 4°C. The homogenate was centrifuged for 20 min at 14 000 *g*. A 10- $\mu$ l aliquot of 2-fold diluted supernatant in buffer A was added in 50  $\mu$ l of assay mixture (with and without 2  $\mu$ M cAMP) which contained 30 mM potassium phosphate buffer (pH 6.8), 8.3 mg/ml histone IIA, 10 mM MgCl<sub>2</sub>, 5 mM aminophylline, 0.5 mM ATP and 5  $\mu$ Ci [ $\gamma$ <sup>32</sup>P]ATP. Incubation was performed at 30°C for 10 min. The reaction was stopped by addition of 50  $\mu$ l of 0.2 M EDTA. An aliquot of 20  $\mu$ l was spotted on 2.5×2.5 cm<sup>2</sup> P81 phosphocellulose papers (Whatman, Maidstone, UK) which were washed three times with 75 mM phosphoric acid. Filters were dried and radioactivity was determined in a liquid scintillation counter. One unit of PKA activity is that amount of enzyme which catalyzes incorporation of 1 pmol of <sup>32</sup>P into histone in 1 min.

Assays of CaM kinase were performed as described previously [22]. The supernatant fraction of human heart tissue homogenates was produced as described in determination of PKA activity. The reaction was started by addition of a 10  $\mu$ l aliquot of 8-fold diluted supernatant in buffer A to 50  $\mu$ l of a assay mixture containing 10 mM MgCl<sub>2</sub>, 20 mM Tris–HCl (pH 7.4), 20  $\mu$ M syntide 2, 1 mg/ml bovine serum albumin, 0.1 mM ATP and 5  $\mu$ Ci [ $\gamma$ <sup>32</sup>P]ATP. CaM kinase activity was measured in presence of 1 mM CaCl<sub>2</sub> and 10  $\mu$ g/ml calmodulin. Ca<sup>2+</sup>-independent protein kinase activity was determined with 5 mM EGTA. In control experiments trifluoperazine (50  $\mu$ M) inhibited CaM kinase activity completely as reported before in other tissues. Incubation was performed at 30°C for 5 min. Determination of  $\gamma$ <sup>32</sup>P exchange was performed as described above for PKA. The CaM kinase activity is expressed as incorporation of pmol of <sup>32</sup>P into syntide 2 per min per mg protein. Protein was measured according to Bradford [23].

## 2.4. Data analysis

Data shown are means±S.E.M. Statistical differences between the different types of myocardium were calculated by ANOVA followed by Bonferroni's *t*-test. Correlations were examined by linear regression analysis. *P*<0.05 was considered significant.

## 2.5. Materials

[ $\gamma$ <sup>32</sup>P]ATP was obtained from Hartmann Analytic (Braunschweig, Germany). Catalytic subunits of PKA, syntide 2, calmodulin and histone IIA were from Sigma (Munich, Germany). Cyclic adenosine 3',5'-monophosphate was obtained from Boehringer Mannheim (Mann-

heim, Germany). All other chemicals were of reagent grade.

### 3. Results

#### 3.1. Characterization of protein kinases by DEAE-Sephacel chromatography

Kinase activity was characterized initially in a homogenate of failing left ventricular tissue by DEAE-Sephacel chromatography (Fig. 1A). The enzyme was identified as

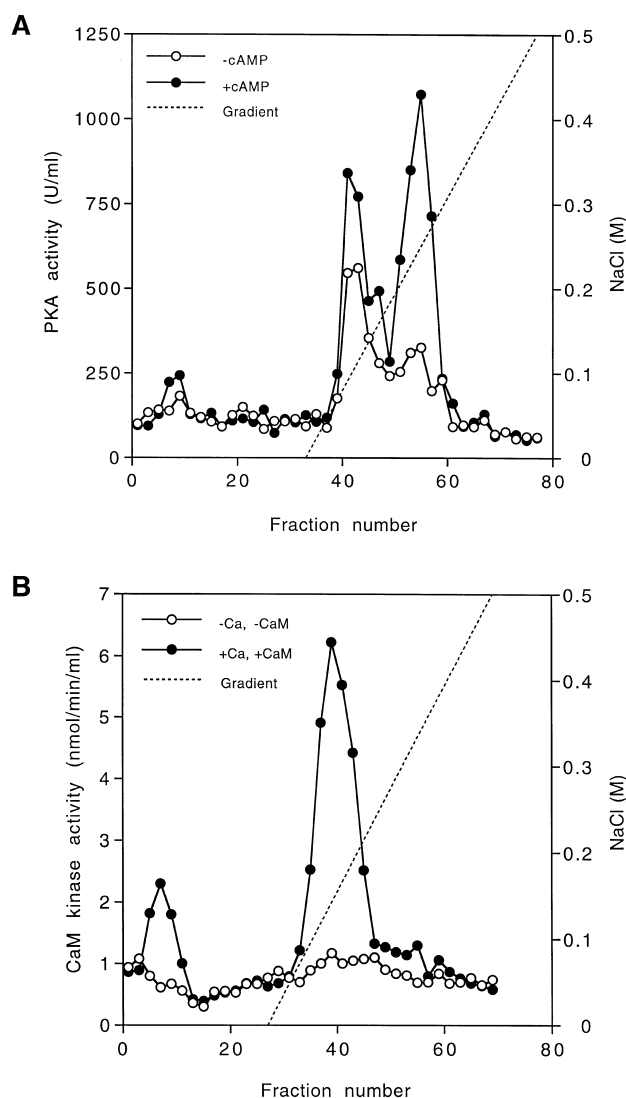


Fig. 1. DEAE-Sephacel chromatography. Crude extracts of human hearts, obtained from 2 g of failing left ventricular tissue, were applied on a DEAE-Sephacel column. After washing the column, a linear gradient (0 to 0.5 mol/l) of NaCl (–) was started. Aliquots (10  $\mu$ l) of every second fraction were then assayed for protein kinase activity. (A) PKA activity was assayed in the presence (●) or absence (○) of cAMP (2  $\mu$ mol/l). (B)  $\text{Ca}^{2+}$ /calmodulin-dependent kinase activity was determined in presence (●) or absence (○) of  $\text{Ca}^{2+}$  (1 mM) and calmodulin (10  $\mu$ g/ml).

PKA by its ability to phosphorylate histone H2A. Furthermore, cAMP was able to stimulate protein kinase activity and the specific inhibitor H-8 (data not shown) attenuated the activity. The column was eluted with a NaCl gradient, and fractions were assayed for PKA activity in the presence and absence of cAMP. Two peaks of PKA activity were observed. The first peak was eluted with 0.07–0.13 M NaCl, and the second peak with 0.2–0.3 M NaCl. The first and second peaks of PKA activity from elution profile are subsequently referred to as types I and II protein kinase, respectively. The proportions of type I and type II protein kinase activity were comparable. The elution profile shown for PKA was highly reproducible. A small peak of activity, which is probably the free catalytic subunit, was observed in the flow-through volume of the DEAE-Sephacel column. The activity of the kinase in fractions 37–49 and 49–61 was increased by cAMP, demonstrating that it was PKA activity. We did not perform a separation of PKA isozymes in nonfailing ventricular myocardium because of lack of material.

Fig. 1B represents the elution profile of a  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase in a crude extract of heart tissue by DEAE-Sephacel chromatography. The specification of CaM kinase is based on: (1) the kinase phosphorylated the specific substrate syntide 2 [22]. Syntide 2 is a synthetic phosphate acceptor peptide of 15 amino acids; (2) In addition, the enzyme activity was stimulated by 1 mM  $\text{Ca}^{2+}$  and 10  $\mu$ g/ml calmodulin and (3) 5 mM EGTA and 50  $\mu$ M trifluoperazine inhibited CaM kinase activity almost completely consistent with characterization of the enzyme by others [22]. CaM kinase activity was measured in eluted fractions in absence and presence of  $\text{Ca}^{2+}$  and calmodulin. One major peak of CaM kinase was observed, and this activity started to elute with 0.09 M NaCl. Under the conditions of our elution procedure we detected a definite but small peak of  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase activity in the flow-through volume of fractions 3–13. This activity might be related to the catalytic domain of CaM kinase.

#### 3.2. Protein kinase activity in failing and nonfailing hearts

Total PKA activity was measured in homogenized extracts of left ventricular tissue from failing and nonfailing hearts. Basal PKA activity was comparable in all groups:  $60.5 \pm 5.6$ ,  $68.0 \pm 6.2$  and  $51.7 \pm 4.3$  U/mg in NF ( $n=8$ ), IDC ( $n=10$ ) and IHD ( $n=10$ ), respectively. We determined CaM kinase activity in homogenates of left ventricular tissue from failing and nonfailing hearts (Fig. 2). Maximal stimulation of CaM kinase was achieved by addition of 1 mM  $\text{CaCl}_2$  and 10  $\mu$ g/ml calmodulin.  $\text{Ca}^{2+}$ -independent protein kinase activity was measured in presence of 5 mM EGTA. At this concentration EGTA inhibits CaM kinase activity completely due to binding of

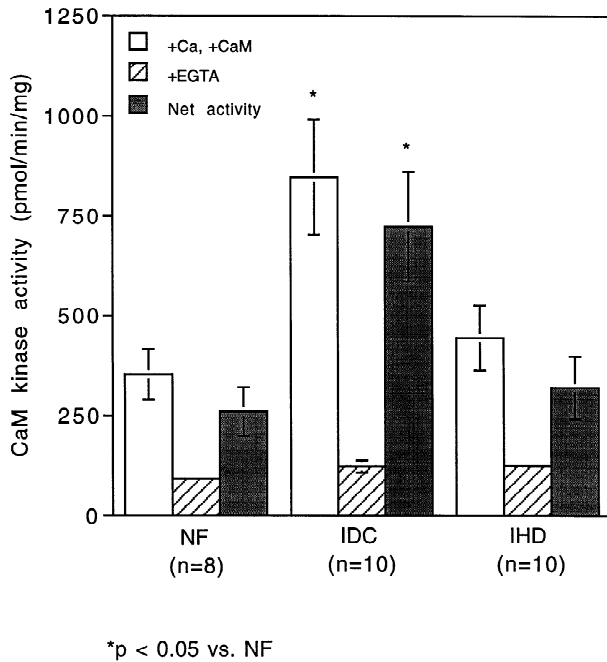


Fig. 2. CaM kinase activity in homogenized extracts from failing (idiopathic dilated cardiomyopathy, IDC; ischemic heart disease, IHD) and nonfailing (NF) left ventricular myocardium. Extracts were prepared and protein kinase activity was measured as described in Section 2.  $\text{Ca}^{2+}$ /calmodulin-dependent kinase activity was measured with syntide 2 as substrate in the presence of  $\text{Ca}^{2+}$  and calmodulin (open bars).  $\text{Ca}^{2+}$ -independent protein kinase activity was determined with EGTA (hatched bars). Net activity depicts the difference between both activities (solid bars). Number of hearts ( $n$ ) which were studied are shown below. Data were compared using one-way analysis of variance indicating significant differences between groups. Significance of differences between pairs was tested by Bonferroni  $t$ -test. ☆=a significant difference versus NF.

intracellular  $\text{Ca}^{2+}$ . Net activity depicts the difference between stimulated and  $\text{Ca}^{2+}$ -independent protein kinase activity. Net activity of CaM kinase in IDC was increased by almost 3-fold compared to NF. There are no significant changes in the CaM kinase activity between NF and hearts suffering from IHD.

### 3.3. Relation between myocardial function and CaM kinase activity

Hemodynamical data were not significantly different between IDC and IHD (Table 2). CaM kinase activities were plotted versus the hemodynamic data from patients with end-stage heart failure due to both IDC and IHD. There was a close positive correlation between either the left ventricular ejection fraction (Fig. 3A) or the cardiac index (Fig. 3B) and CaM kinase activity in failing hearts with IDC. We found no significant correlation between the increase in CaM kinase activity and the pulmonary capillary wedge pressure in IDC. In addition, the CaM kinase activities in IHD did not correlate with the hemodynamic data from patients suffering from IHD.

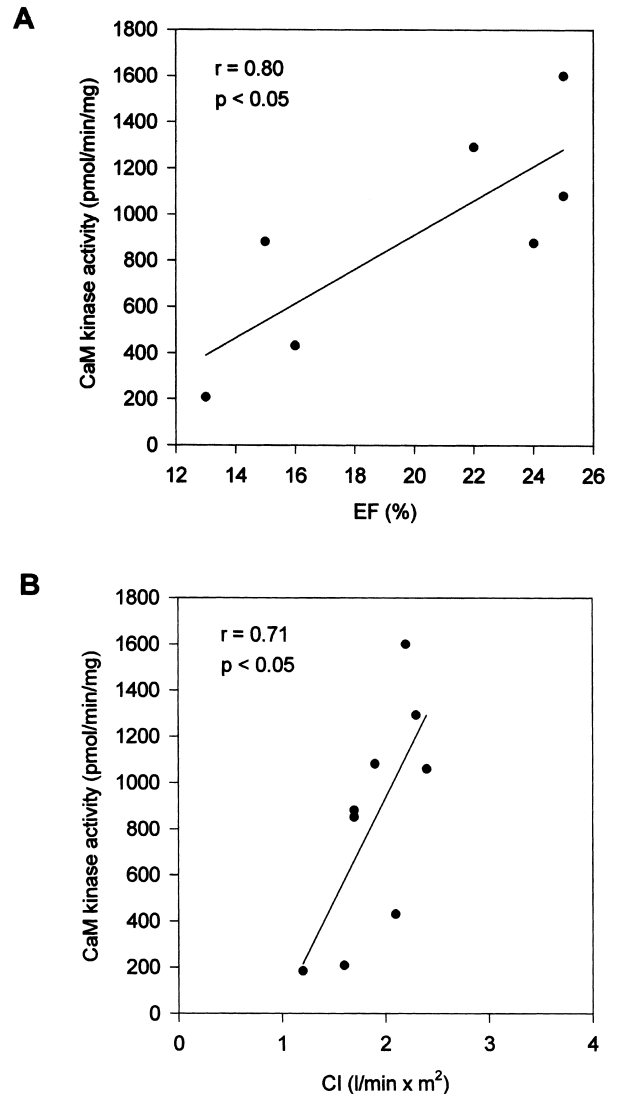


Fig. 3. Graphs showing the relation between either the radionuclide-determined left ventricular ejection fraction (A) or the cardiac index (B) and the  $\text{Ca}^{2+}$ /calmodulin-dependent kinase activity in failing hearts with IDC.

## 4. Discussion

### 4.1. PKA activity in heart failure

The existence of PKA isozymes in human myocardium has been described first by Corbin and Keely [24]. They reported about equal amounts of type I and type II protein kinase in human right atrium. Here, we report for the first time the separation of PKA isozymes in human ventricular tissue. Since it is known that the ratio of PKA isozymes could have functional consequences [25], it is conceivable that the proportions of isozymes in human ventricle could affect the PKA-mediated phosphorylation of cardiac regulatory proteins. Under the conditions of this work, almost equal levels of type I and type II PKA were detected in failing ventricular myocardium.

A number of studies have implicated an altered  $\beta$ -adrenoceptor signalling pathway as an important factor in the initiation and progression of cardiac failure. Despite many alterations in the  $\beta$ -adrenoceptor signalling pathway [2–5,26–28] in our study the basal PKA activity was unchanged between failing and nonfailing hearts. This is in agreement with results of another group [29]. We conclude that the PKA cannot account for the pathogenesis of cardiac failure.

#### 4.2. CaM kinase in the heart

In order to determine the contribution of the  $\text{Ca}^{2+}$ -dependent second messenger system in the dysregulation of the intracellular  $\text{Ca}^{2+}$  content in heart failure, we have characterized the CaM kinase. The existence of a myocardial  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase activity was first observed by Le Peuch et al. [30] and was specified as CaM kinase. Kloepper and Landt [31] identified two  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase activities in bovine heart, whereas Gupta and Kranias [20] showed that canine myocardium contains only one type of purified membrane-bound  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase. Our study shows that DEAE-Sepharose chromatography led, under the conditions described, to the elution of one peak activity, indicating that human ventricular myocardium contains one type of  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase.

#### 4.3. Possible physiological role of cardiac CaM kinase

It is established that, in cardiac muscle, CaM kinase phosphorylates the inhibitory subunit of troponin (TnI), PLB and RyR proteins involved in regulation of cardiac contractility [20,30,32,33]. Controversy exists as to whether or not SERCA2a is a substrate for CaM kinase in vitro and in vivo [34–36]. At present the role of CaM kinase in modulating the  $\text{Ca}^{2+}$  handling is poorly understood. Interestingly, we observed an increased CaM kinase activity in left ventricular tissue suffering from IDC. Our data suggest that increased CaM kinase activity in IDC correlates positively with the hemodynamical values, especially EF and CI. In other words, the increase in CaM kinase activity could represent an early adaptive process of the impaired relaxation in human heart failure due to IDC. The relaxation abnormalities are associated with an increased diastolic  $\text{Ca}^{2+}$  content and a reduced systolic  $\text{Ca}^{2+}$  content under basal conditions or  $\beta$ -adrenergic stimulation [6,37]. The observed alterations are accompanied by reduced  $\beta$ -adrenoceptor agonist-stimulated cAMP levels in failing human myocardium [5]. In addition, the type 1 phosphatase activity is elevated in failing human hearts suffering from IDC [38]. It is conceivable that all these alterations may cause a diminished cAMP-dependent phosphorylation of cardiac regulatory proteins which was shown Bartel et al. [39]. However, at present unknown

mechanisms might compensatively trigger an enhanced activation of the alternative  $\text{Ca}^{2+}$ /calmodulin-dependent pathway. Therefore, an increased CaM kinase-dependent phosphorylation of PLB may compensate in part the diastolic  $\text{Ca}^{2+}$  accumulation. An enhanced CaM kinase-dependent phosphorylation of TnI could promote myocardial relaxation by decreasing the affinity of troponin C for  $\text{Ca}^{2+}$  ions. A potential phosphorylation of SERCA2a could hasten the rate of relaxation by stimulating  $\text{Ca}^{2+}$  uptake into the SR. Furthermore, it is conceivable that an increased phosphorylation of RyR may release more  $\text{Ca}^{2+}$  ions from the SR during the next systolic contraction, thus compensating the reduced systolic  $\text{Ca}^{2+}$  content in IDC reported.

#### 4.4. Possible pathophysiological role of CaM kinase

We suggest here that the increased CaM kinase activity in IDC leads to enhanced CaM kinase-dependent phosphorylation states of regulatory proteins which tries to compensate impaired relaxation in initially developing heart failure due to IDC (Fig. 3A and B). In addition, purified myosin light-chain kinase (MLCK) has been shown to be phosphorylated by CaM kinase [40]. Persechini et al. [41] have demonstrated that the phosphorylation of skeletal myosin P-light chain by MLCK increases the isometric tension. Hence, it is conceivable that CaM kinase phosphorylates MLCK more in IDC than in IHD. This would be an additional compensatory mechanism to sustain cardiac output in IDC. The generation of high-energy phosphates (e.g. ATP) declines during severe ischemia and is a major reason for contractile dysfunction [42]. If one assumes a higher ATP/ADP ratio in IDC compared to IHD it is likely that this higher ratio and the increased CaM kinase activity result in an increase in the phosphorylation of regulatory proteins in IDC versus IHD. Thus, contractility and performance should better correlate to CaM kinase activity in IDC than in IHD, which we observed.

#### 4.5. Limitations

It could be argued that CaM kinase activation is an epiphenomenon of cardiac hypertrophy. However, this is rather unlikely because it is only increased in certain kinds of hypertrophy. Whereas an increase was detected in IDC no alteration was noted in IHD. Moreover, a previous study by Bokník et al. [43] showed that CaM kinase activity is unchanged in a rat model of  $\beta$ -adrenoceptor-stimulation-induced heart hypertrophy. There is precedent for specific biochemical differences between IDC and IHD [27,38,44]. It is very unlikely that the medication accounts for the differences which we noted in CaM kinase activity between IDC and IHD because in our study almost all patients regardless of etiology (IDC/IHD) received drugs like glycosides, diuretics and ACE inhibitors.

#### 4.6. Perspectives

One approach for elucidating the question as to whether the increased CaM kinase activity in IDC could be an epiphenomenon of the disturbed  $\text{Ca}^{2+}$  handling and the subsequent myocardial dysfunction would be the generation of transgenic mice that overexpress target substrates and enzymes of the  $\text{Ca}^{2+}$ -dependent second messenger pathway. In a transgenic mice model overexpressing calmodulin, hypertrophy of cardiomyocytes occurred [45]. Thus, the development of mice that overexpress CaM kinase might directly tell whether elevated levels of CaM kinase per se can induce cardiomyocyte hypertrophy or dysfunction. In addition, further studies will have to demonstrate whether or not the increased CaM kinase activity in IDC corresponds to increased protein and mRNA levels of the enzyme. At present no specific antibody raised against the human cardiac  $\delta$ -isoform of CaM kinase is available. In our hands, the CaM kinase antibody used by Singer et al. [46] did not cross-react with the human heart tissue in Western blots. Furthermore, one aim of subsequent studies would comprise the generation of highly specific DNA-probes for Northern analysis in human cardiac tissue.

In addition to the central role of the impaired  $\text{Ca}^{2+}$  cycling a variety of mechanisms have been suggested to contribute to the progression of end-stage heart failure [8,9,47–49]. Our data suggest that the increase of CaM kinase activity in IDC may contribute to the compensation in developing heart failure.

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