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Developmental Changes in Isoform Expression of Ca^{2+}/Calmodulin-Dependent Protein Kinase II δ-Subunit in Rat Heart

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Abstract In the heart, Ca^{2+}/calmodulin-dependent protein kinase II is critically involved in the regulation of Ca^{2+} homeostasis. Previously the predominant expression of a subclass of Ca^{2+}/calmodulin-dependent protein kinase II δ-subunit, containing a second variable domain, was demonstrated in cardiac tissue. Here we report on the expression pattern of the non-neuronal members of this δ-subunit subclass, δ_2, δ_3, δ_4, and δ_9 in the developing heart of the rat. By semiquantitative RT-PCR isoform δ_3 was shown to be typically expressed in the heart, whereas δ_4 was expressed in skeletal muscle of adult rat. From embryonic day 14 up to the adult state of rat ventricular muscle, amounts of δ_9 transcripts remained unchanged, transcript levels of isoforms δ_2 and δ_3 were significantly increased, whereas level of δ_4 transcript was significantly decreased. Immunoblotting, using an antibody recognizing specifically those δ-isoforms containing the second variable domain, revealed three separated protein signals at about 59 kDa, 58 kDa, and 56 kDa. The immunoreaction at about 59 kDa, corresponding to the predicted molecular mass of δ_4, was dramatically diminished, whereas a significant increase in the signal at about 58 kDa was assumed to represent an increase in isoform δ_3. The protein signal at about 56 kDa, close to the predicted molecular mass of isoform δ_2, was high in the embryonic heart and significantly decreased after birth. Our data suggest the predominant expression of isoform δ_2 in the embryonic heart, establish δ_3 to be the typical isoform in the adult heart and define the skeletal muscle form δ_4 to be characteristic for fetal and neonatal stages of the heart. J. Cell. Biochem. 74:202–210, 1999. © 1999 Wiley-Liss, Inc.

Key words: rat heart development; ventricular myocardium; gene expression; CaMKII; δ-CaMKII isoforms; semiquantitative RT-PCR; immunoblot

The multifunctional Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) is a ubiquitous mediator of Ca^{2+} signaling in neuronal and non-neuronal eukaryotic tissues. CaMKII is a multimeric holoenzyme consisting of 8–12 subunits encoded by a multigene family containing four distinct genes, α, β, γ, and δ [reviewed in Braun and Schulman, 1995]. The distinct subunit isoform composition locates the CaMKII holoenzyme to different compartments of the cardiomyocyte, thereby achieving specificity of function [Srinivasan et al., 1994; Ramirez et al., 1997]. Whereas the α- and β-CaMKII mainly occur in neuronal tissues, the γ- and δ-subunits are present in many tissues including brain [Tobimatsu and Fujisawa, 1989]. CaMKII is involved in the regulation of numerous cellular functions, such as growth [Masse and Kelly, 1997], differentiation [Wang and Simonson, 1996; Masse and Kelly, 1997], cell cycle [Planas-Silva and Means, 1992], gene expression [Nghiem et al., 1994], and memory formation [Mayford et al., 1995]. CaMKII isoforms and their functions in non-neuronal tissues are less well elucidated. In the heart CaMKII is crucially involved in the regulation of Ca^{2+} homeostasis thereby affecting contraction [for review see Braun and Schulman, 1995]. However, little is known about occurence and specific functions of distinct CaMKII isoforms in the heart. Significant activities of Ca^{2+}/calmodulin-dependent protein kinase were detected in the cytosolic as well as in membraneous compartments of cardiac tissue [Louis and Maffit, 1982; Iwasa et al., 1985]. Biochemical characterization revealed similarities of cardiac Ca^{2+}/calmodulin-dependent protein kinase with the CaMKII class [Jett

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et al., 1987; Gupta and Kranias, 1989]. The abundant expression of the δ-isoforms compared to γ-CaMKII was proposed from studies of mammalian heart [Singer et al., 1997]. In cardiac tissue, previous work by Edman and Schulman [1994], as well as studies by Baltas et al. [1995], suggest the predominant expression of distinct isoforms of a CaMKII δ-subunit subclass characterized by the presence of a second variable domain. Five isoforms have been identified for containing a second variable domain, termed δ_1, δ_2, δ_3, δ_4, and δ_9 [Tobimatsu and Fujisawa, 1989; Schworer et al., 1993; Mayer et al., 1995]. Analyses of various rat tissues revealed evidence that δ_1-CaMKII is absent in the heart, and suggest that δ_1 is expressed only in the brain [Schworer et al., 1993]. Recently, we identified isoforms δ_2, δ_3, δ_4, and δ_9 in the cardiac cell line H9c2 [Hoch et al., 1998]. However, the expression pattern of these isoforms has not been elucidated in the developing heart.

In order to gain further insights into the significance of the CaMKII isoforms δ_2, δ_3, δ_4, and δ_9, containing the second variable domain, for cardiac function, we studied the amounts of their specific transcripts as well as the expression of δ-CaMKII protein during rat heart development. Our results indicate δ_3-CaMKII to be the typical isoform expressed in the adult heart, δ_2 to be predominant in the embryonic heart, and demonstrate the expression of the skeletal muscle form δ_4 in embryonic and neonatal stages of cardiac development.

MATERIALS AND METHODS

Isolation of Total RNA

Ventricular myocardial tissues from embryonic, neonatal and adult rat and tissues from skeletal muscle of adult rat were isolated and homogenized in a denaturing solution containing 4 M guanidine thiocyanate; 25 mM citric acid sodium salt and 0.5 % w/v N-lauroylsarcosine. After sequentially mixing of the homogenate with 1/10 volume 3 M sodium acetate (pH 4.8), water saturated phenol, and finally chloroform, the resulting mixture was centrifuged, yielding an upper aqueous phase containing total RNA. Following precipitation in isopropanol, the RNA pellet was redissolved in denaturation solution, reprecipitated with isopropanol, and washed with 70% ethanol [Kingston et al., 1994]. Digestion of remaining DNA contaminants was performed in 40 mM Tris/HCl pH 7.5; 6 mM $MgCl_2$ and 75 U RNase-free DNaseI (Pharmacia, Freiburg, Germany) for 10 min at 37°C in a total volume of 100μl. Reextraction of RNA was done by using the RNA clean up protocol of the RNeasy system (Qiagen, Hilden, Germany). Complete digestion of genomic DNA was confirmed by reverse transcription coupled polymerase chain reaction (RT-PCR) in the absence of reverse transcriptase (data not shown).

Amplification Primers and Reverse Transcription Coupled Polymerase Chain Reaction (RT-PCR)

Primers (Table I) were designed for specific amplification of δ-CaMKII isoforms (Fig. 1). Total RNA was denatured for reverse transcrip-

TABLE I. CaMKII δ-Isoform- and GAPDH-Specific Primers Used for PCR

Primer[a]	Specificity	Sequence	Amplicon (bp)	Reference/Acc. no.[b]
P5 (antisense)	common δ	TCAGATGTTTTGCCACAAAGAGGTGCCTCCT		J05072
P7 (sense)	δ_2	CCGGATGGGGTAAAGGAGTCAACTGAGAGCT	531 (P5 + P7)	L13406
P6 (sense)	δ_3	AAAAGGAAGTCCAGTTCGAGTGTTCAGATGAT	549 (P5 + P6)	L13407
P30 (sense)	δ_4	CTACCCCGGCGCTGGAGTCAAC	530 (P5 + P30)	Schworer et al., 1993; Hoch et al., 1998
P36 (sense)	δ_9	GTAAAGGAGCCCCAAACTACTGTAA	564 (P5 + P36)	Mayer et al., 1995; Hoch et al., 1998
P28 (sense)	GAPDH	CAGTCCATGCCATCACTGCC	609 (P28 + P29)	M17701
P29 (antisense)	GAPDH	GGGTCTGGGATGGAATTGTG		M17701

[a]All primers were HPLC (high-performance liquid chromatography)-purified and purchased from BioTez (Berlin). bp, base pairs.
[b]GenBank accession number.

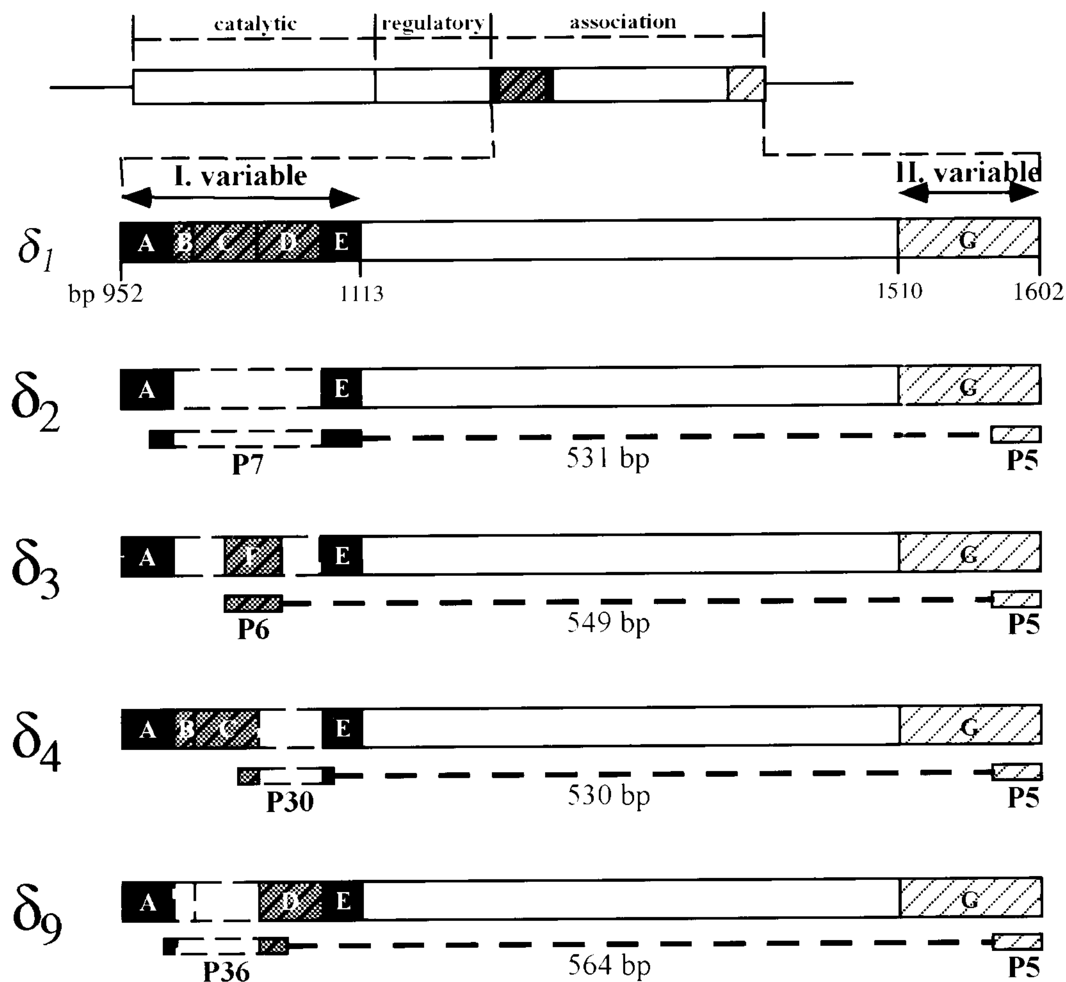

Fig. 1. δ-CaMKII isoform variability and PCR strategy. The cDNA structures of the brain specific δ_1-CaMKII (marked with italic letters) [Tobimatsu and Fujisawa, 1989; Schworer et al., 1993] and the non-neuronal CaMKII isoforms δ_2, δ_3, δ_4, and δ_9 (marked with bold letters) [Mayer et al., 1995] as well as PCR primers (P5, P6, P7, P30, P36) and PCR products are represented schematically (for primer sequences see Table I). Numerical nomenclature of the δ-CaMKII used here is according to Mayer et al. [1995]. Sequences were obtained from rat δ-CaMK II [Tobimatsu and Fujisawa, 1989]. Parts A–E,F of the I. variable domain (I. variable) as well as part G of the II. variable domain (II. variable) are based on the δ_1-CaMKII cDNA structure as well as on δ_2-, δ_3-, δ_4-, and δ_9-CaMKII cDNAs which contain or are void of these parts [Edman and Schulman, 1994; Mayer et al., 1995]. Positions in bp are given for δ_1-CaMKII. In comparison to CaMKII isoforms δ_1, δ_2, δ_4, and δ_9, isoform δ_3 contains an 33 bp insertion (part F) in the I. variable domain, identified as a nuclear localization signal (NLS) [Srinivasan et al., 1994]. Parts A and E in the I. variable domain are darkened to indicate their proposed role as a conserved tether in all δ-isoforms.

tion and immediately chilled on ice. Random primed cDNA synthesis was performed with 3.5μg denatured RNA added to a total volume of 70μl containing 7μl of 10x PCR buffer (Eurogentec, Seraing, Belgium); 7μl 25 mM $MgCl_2$; 3.5μl dNTP (10 mM each nucleotide; United States Biochemical, Cleveland, OH); 1.4μl 37.7 U/μl RNase inhibitor (RNAguard, Pharmacia); 7μl 0.1 M dithiothreitol (DTT; Gibco BRL, Eggenstein, Germany); 1.4μl 62.5 A_{260}/ml hexanucleotide mix (Boehringer Mannheim, Mannheim, Germany); and 3.5μl Superscript™ II RNaseH$^-$ Reverse Transcriptase 200 U/μl (Gibco BRL). The reaction mix was overlaid with 50μl mineral oil (Sigma, Deisenhofen, Germany). Following incubation for 10 min at 25°C, cDNA synthesis was carried out for 50 min at 42°C. For inactivation of reverse transcriptase, the reaction mix was incubated 15 min at 70°C. Each PCR reaction was performed in a total volume of 100μl containing 10μl 10 × PCR buffer; 6μl 25 mM $MgCl_2$; 2μl 10 mM dNTP; 50 pmol of each specific primer; 0.2μl thermostable DNA polymerase 5 U/μl (Goldstar red DNA polymerase, Eurogentec), and an aliquot of the reverse transcription reaction equivalent

to 500 ng total RNA. PCR reaction mix was overlaid with 50μl mineral oil (Sigma). All amplifications were performed in a TRIO thermocycler (Biometra, Göttingen, Germany) involving an initial step of 3 min at 95°C followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C, and a terminal last delay of 7 min at 72°C. For standardization of individual RT-PCR reactions glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific amplifications in the linear range (19 cycles) were performed in parallel. Aliquots of 10 μl of the PCR reaction mixtures and the ready to load 100 bp DNA standard (Gibco BRL) were loaded onto a 2% agarose gel containing ethidium bromide. Following gel electrophoresis, stained products were visualized by UV light. Background-substracted optical densities from peak areas were obtained by the PCbas 2.09 system (Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany). The authenticity of the amplified products was verified by size by agarose gel electrophoresis with molecular weight markers (Hind III digest of Lambda DNA; United States Biochemical and 100 bp DNA-Ladder; Gibco BRL) and by sequence determination (InViTec, Berlin, Germany).

Immunoblotting

For immunoblotting about 30 mg of frozen heart tissue was homogenized in a 10-fold volume of homogenisation buffer containing 10 mM Hepes pH 7.5; 0.2 mM phenylmethylsulfonylfluoride (PMSF); 0.1 mM dithiothreitol (DTT) and 1μg/ml leupeptin with an Ultra Turrax homogenizer (Janke & Kunkel, Staufen, Germany) at 50 000 rpm 3 times for 10 sec. Aliquots of homogenates containing 10 μg of protein were solubilized with the same volume of 2 × concentrated sodium dodecyl sulfate (SDS) sample buffer and boiled for 2 min prior application to 10% SDS polyacrylamide gels [Laemmli, 1970]. Gels were run with a constant voltage of 100 V. Separated proteins were electrotransferred from the gels onto polyvinylidenedifluoride (PVDF) membranes. Processing for immunoblotting was performed as described [Towbin et al., 1979]. For detection of δ-CaMKII an antibody was used raised against a synthetic peptide corresponding to the C-terminal amino acid sequence unique to a subset of δ-subunit variants [Hoch et al., 1998]. The anti-δ-CaMKII antibody was used in a final concentration of 1μg/ml. For detection as secondary antibody anti-rabbit IgG (Sigma) conjugated with peroxydase was used. The immunoreaction was visualized using the enhanced chemoluminescence kit (Amersham, Braunschweig, Germany) and autoradiography on X-ray films. Densitometric analyses of autoradiograms were performed with the PDI imaging system (PDI, New York, NY).

Statistical Analyses

Results were given as means + standard errors (SEM). After data have been checked for normal distribution, statistical analyses were performed by the unpaired Students *t*-test or Mann-Whitney test. A P value of < 0.05 was considered statistically significant.

RESULTS

δ-CaMKII Isoform Pattern in Striated Muscle Tissues From Adult Rat

To gain insights into tissue specificity of the expression of CaMKII isoforms δ_2, δ_3, δ_4, and δ_9 in adult rat (week 24), the presence of transcripts of these isoforms was compared in cardiac and skeletal muscle. A representative result of RT-PCR with primer pairs specific for the CaMKII isoforms δ_2, δ_3, δ_4, δ_9, and the housekeeping gene GAPDH is shown in Figure 2a. In adult rat heart amplification products for CaMKII isoforms δ_2, δ_3, and δ_9 are detectable in comparable amounts. Isoform δ_4 is not transcribed in adult rat heart (Fig. 2a, upper panel). In skeletal muscle δ_2, δ_4, and δ_9 transcripts are found. Amplification of isoform δ_3 revealed no detectable signal (Fig. 2a, lower panel). Thus, in the adult rat transcription of CaMKII isoform δ_3 is typical for heart tissue, and δ_4 is characteristically transcribed in skeletal muscle.

In order to analyse δ-CaMKII expression on the protein level, an anti-peptide antibody was used, specific for the C-terminal second variable domain of δ-CaMKII. This antibody was employed to determine the δ-CaMKII expression pattern in striated muscle from adult rat (Fig. 2b). In preparations from skeletal and cardiac tissues the most prominent immunoreaction corresponds in size to the molecular mass at about 58 kDa. A signal below this immunoreaction was detected in homogenates of both skeletal and cardiac muscle tissues, corresponding in size to about 56 kDa. Furthermore a faint band above the major signal was observed exclu-

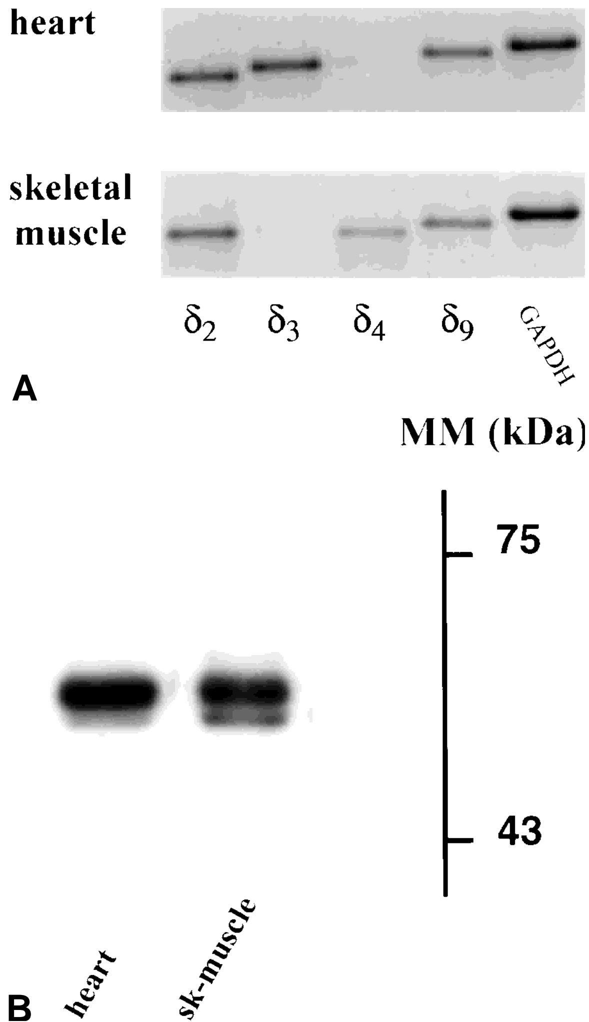

Fig. 2. δ-CaMKII isoforms in heart and skeletal muscle of the adult rat. **A:** Amounts of transcripts of isoforms δ_2, δ_3, δ_4, and δ_9 in adult rat heart and skeletal muscle. Representative RT-PCR products that encompass both variable domains (Fig. 1) of the indicated δ-CaMKII isoforms were amplified from the indicated tissues. **B:** Immunoblot of δ-CaMKII subunit in the indicated tissues (sk-muscle, skeletal muscle). For immunodetection an antibody was used raised against a synthetic peptide corresponding to the C-terminal amino acid sequence unique to a subset of δ-subunit isoforms. MM, molecular mass. For experimental details see Materials and Methods.

sively in skeletal muscle derived homogenate corresponding to the molecular mass at about 59 kDa.

δ-CaMKII Isoform Pattern in the Developing Rat Heart

In order to elucidate the expression pattern of δ-CaMKII isoforms during cardiac development, rat hearts at embryonic day 14, postnatal day 1, 5, 20, and adult hearts (week 24) were analyzed. Representative RT-PCRs for isoforms δ_2, δ_3, δ_4 and δ_9 are shown in Figure 3a. For each time point RT-PCR experiments were performed from three to five independent heart preparations to describe semiquantitatively differences in the transcript levels (Fig. 3b). The amount of transcripts for isoform δ_9 was found to be unchanged in the various investigated developmental states. The amount of transcripts for isoform δ_2 is elevated significantly from 104.1± 7.9% at embryonic day 14 to 172.3 ± 16.5% (n = 3–5, $P = 0.03$) at neonatal day 5 and remains at this level up to the adult state. Amounts of transcripts of isoforms δ_3 and δ_4 change dramatically during development of the myocardium. From the embryonic state to the adult the transcript levels of δ_3-CaMKII accumulate significantly from 92.0 ± 11.1% to 231.9 ± 8.9% (n = 3–5, $P < 0.0001$) at day 20 and 225.2 ± 20.9% (n = 3–4, $P = 0.004$) in the adult state. In contrast, the amount of transcripts of δ_4-CaMKII declines continously from 106.3 ± 7.6% to 75.1 ± 7.3% (n = 3–4, $P = 0.03$) at day 5 to 44.6 ± 8.6% (n = 3–5, $P = 0.003$) at day 20 and 35.1 ± 9.9% (n = 3–5, $P = 0.003$) in the adult stage (Fig. 3b).

δ-CaMKII protein expression in the developing heart was analysed using the δ-CaMKII-anti-peptide antibody described above. Three clearly separated bands were obtained by immunoblotting corresponding to molecular masses at about 59 kDa, 58 kDa, and 56 kDa (Fig. 4). In neonatal and postnatal states the strongest signal corresponds in size to the molecular mass at about 58 kDa (Fig. 4, open bars). This signal increases significantly 7.3 fold (n = 3, P = 0.0002) from embryonic day 14 to neonatal day 1, and up to 15.6 fold (n = 3, $P < 0.0001$) in the adult heart. In contrast, the 59 kDa protein drops dramatically during the postnatal phase and is no longer detectable at least at day 20 after birth (Fig. 4, black bars). At embryonic day 14 the strongest detectable signal corresponds in size to about 56 kDa (Fig. 4, grey bars). This signal decreases significantly 3.4-fold (n = 3, $P = 0.005$) at neonatal day 1 and remains at this level in the following developmental states.

DISCUSSION

To gain insights into the significance of the CaMKII isoforms δ_2, δ_3, δ_4, and δ_9 in the heart, we compared their expression in adult cardiac with skeletal muscle tissue and studied the

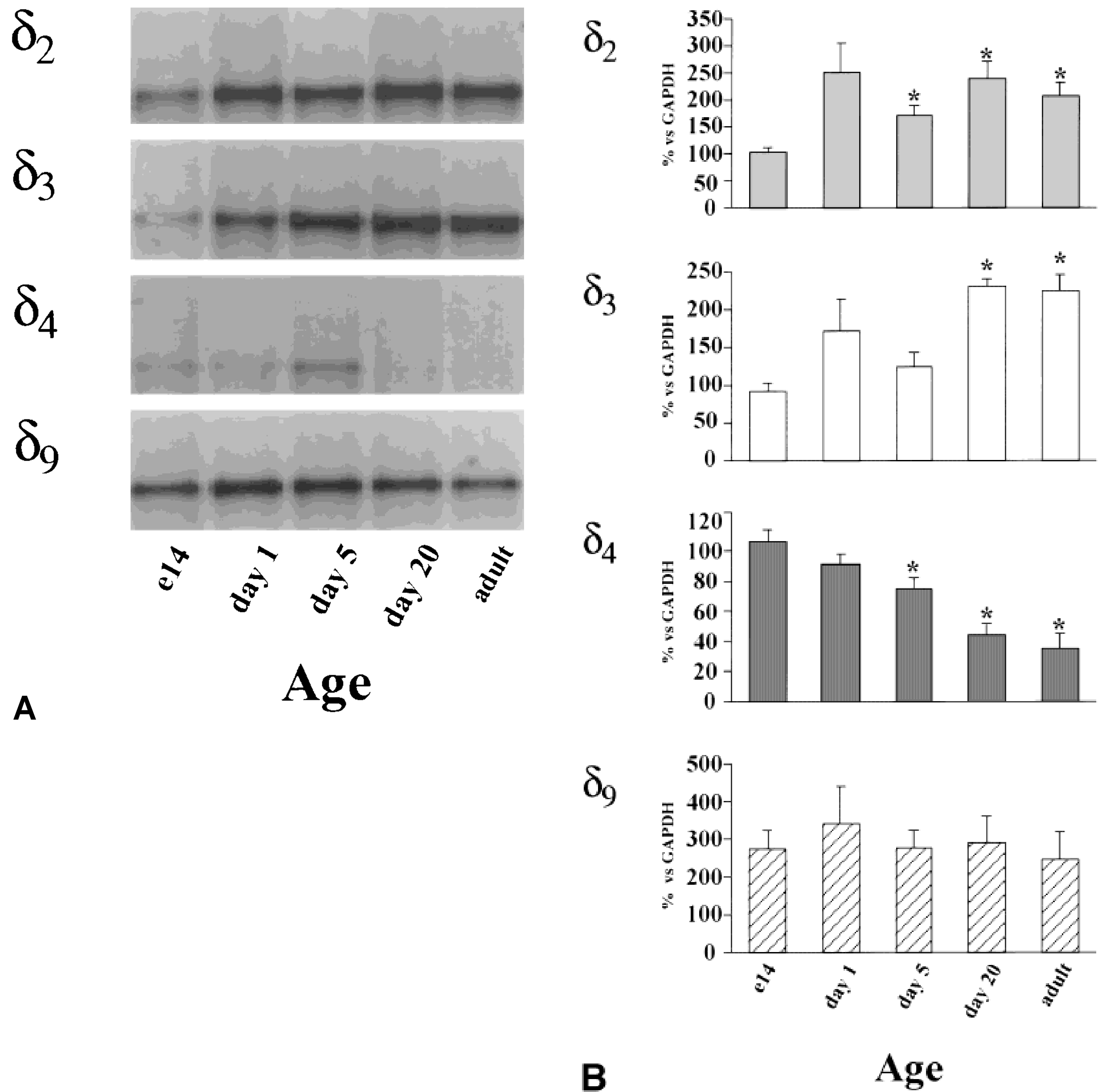

Fig. 3. Amounts of transcripts for δ-CaMKII isoforms in the developing rat heart. **A:** Representative RT-PCR products that encompass both variable domains of the indicated δ-CaMKII isoforms were amplified from embryonic day 14 (e14), neonatal day 1, 5, 20, and the adult state (week 24). For experimental details see Materials and Methods. **B:** Data of semiquantitative RT-PCR analyses from three to five independent heart preparations as shown in Figure 3a. δ-CaMKII transcript levels were normalized to GAPDH expression and given relative to GAPDH at embryonic day 14 (e14). Asterisks indicate statistical significant changes vs. δ-CaMKII expression at embryonic day 14.

expression pattern in the developing ventricular myocardium of rat.

Transcripts for isoform δ_2 were detectable in both heart and skeletal muscle tissue from adult rat confirming our assumptions based on studies in the cardiac cell line H9c2, which suggest a ubiquitous expression of this isoform [Hoch et al., 1998]. For isoform δ_3 and δ_4 our transcript data establish previous results describing the cardiac (δ_3) and skeletal muscle specific (δ_4) expression of these isoforms in striated muscle tissue from adult rat [Schworer et al., 1993; Edman and Schulman, 1994; Hoch et al., 1998]. Mayer et al. [1995] report on the heart specific expression of CaMKII isoform δ_9. As shown here, we clearly detected transcripts of isoform

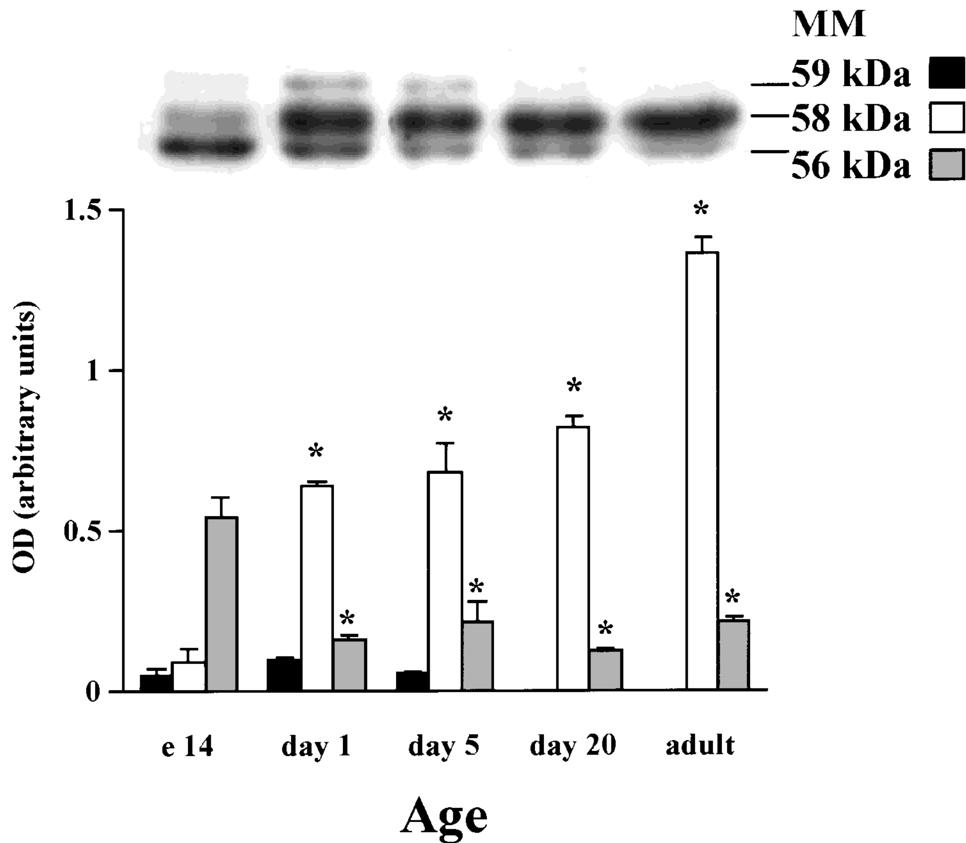

Fig. 4. Amounts of δ-CaMKII proteins in the developing rat heart. Asterisks indicate statistical significant changes vs. δ-CaMKII expression at embryonic day 14 (e14). The adult state corresponds to postnatal week 24. Immunoblots of δ-CaMKII were performed as described in Figure 2b (MM, molecular mass; open bars, signal at about 58 kDa; grey bars, signal about at 56 kDa; black bars, signal at about 59 kDa). For experimental details see Materials and Methods.

δ_9 also in skeletal muscle. This finding is in line with previous data by Hoch et al. [1998], suggesting that the expression of δ_9 does not distinguish between heart and skeletal muscle, and probably accompanies differentiation in excitable cells.

In this study we identified alterations in the expression pattern of transcripts for CaMKII isofoms δ_2, $\delta_{3,}$ and δ_4 in the developing rat heart. A complete new picture arises for CaMKII isoform δ_4, originally identified to be characteristic for skeletal muscle [Schworer et al., 1993]. Amounts of δ_4 transcripts, shown here for the first time to be present in the heart, decreases significantly during cardiac development from embryonic and neonatal states up to the adult, defining δ_4 to be a fetal and neonatal cardiac CaMKII isoform, not detectable in the adult heart. Our previous work demonstrated a higher amount of δ_9 transcript in adult rat heart compared to H9c2 myoblasts, a cell line derived from embryonic ventricular tissue [Hoch et al., 1998]. In the developing heart, as shown here, there were no changes in δ_9 transcript levels between embryonic day 14, the earliest state investigated, and the adult, indicating that δ_9 expression reaches its final level already during embryogenesis. In contrast, transcript levels of CaMKII isoforms δ_2 and δ_3 of ventricular myocardium were shown to significantly increase from fetal up to the adult state.

Although, due to its specificity, the antibody used for immunoblotting does not differentiate between the individual CaMKII δ-isoforms containing the second variable domain, three protein signals clearly separated by size could be detected. In addition to its predicted molecular mass [Edman and Schulman, 1994], recent studies of our group on cardiac sarcoplasmic reticulum vesicles and on the cardiac cell line H9c2 suggest the signal detected at about 56 kDa to be CaMKII isoform δ_2 [Baltas et al., 1995; Hoch et al., 1998]. The obtained protein signals at about 59 kDa and 58 kDa, according to their

predicted molecular masses, calculated from their amino acid sequences, may represent isoforms δ_4 [Schworer et al., 1993; Mayer et al., 1995] and δ_3 together with δ_9 [Edman and Schulman, 1994; Mayer et al., 1995], respectively. In heart and skeletal muscle tissue homogenates of adult rat, the immunoreactions at about 59 kDa and 56 kDa correspond to the predicted molecular masses of CaMKII isoforms δ_4 and δ_2, respectively, which is in line with our transcriptional data indicating the ubiquitous expression of $\delta_{2,}$ and the specific expression of δ_4 in skeletal muscle. Based on the transcriptional data and the predicted molecular masses the most prominent immunoreaction at about 58 kDa in the adult heart may be interpreted as resulting from the translation of isoforms δ_3 and δ_9, whereas in skeletal muscle, with regard to the absence of δ_3 transcripts, it most likely represents solely isoform δ_9.

In the developing heart from embryonic day 14 up to the adult the major reaction at about 58 kDa, supposed to be isoforms δ_3 and $\delta_{9,}$ was significantly increased. Since there is an increase of δ_3 transcripts but no change of transcript levels of isoform δ_9, it therefore seems to be plausible to account an increased level of δ_3 protein for the augmentation of this signal. The increased expression of isoform δ_3 during cardiac development indicates its rising importance in the adult compared to the embryonic heart. The immunoreactive band at about 59 kDa corresponding to the predicted molecular mass of CaMKII isoform δ_4, changes in a very similar manner as described for δ_4 transcript levels, suggesting that it may represent δ_4-protein. Thus, the present data establish δ_4 to be a fetal and neonatal cardiac CaMKII isoform. The immunoreaction at about 56 kDa, proposed to be isoform δ_2, was prominent at embryonic day 14 and significantly decreased in neonatal and postnatal states. This is in contrast to our transcript data for δ_2 during cardiac development, may be explainable by a posttranscriptional control mechanism leading to the prominent expression of δ_2-protein in the fetal heart.

Combining the three protein signals specifically recognized by the used antibody there is a 2.3-fold increase in total δ-CaMKII protein, containing the second variable domain, during cardiac development from the fetal up to the adult state.

Xu et al. [1997] described a declination of the overall CaMKII activity in the sarcoplasmic membrane preparations of postnatal rabbit heart. In preliminary studies we obtained similar results with sarcoplasmic reticulum vesicles prepared from rat heart (data not shown). Our data strongly suggest changes in the CaMKII holoenzyme composition during cardiac development. This may shift the intracellular location of holoenzyme populations from sarcoplasmic reticulum to other cellular compartments.

Summarizing our present data, we report on the tissue-specific expression of CaMKII isoforms δ_3 and δ_4 in heart and skeletal muscle of adult rat, respectively, as well as on the developmentally-controled expression of isoforms δ_2, $\delta_{3,}$ and δ_4 in rat cardiac tissue. Whereas δ_2 is suggested to be predominant in the fetal and δ_3 established to be typical in the late neonatal and adult heart, we defined δ_4 as a fetal and early neonatal cardiac isoform. We propose that the controlled expression of individual δ-isoforms results in a determinated CaMKII holoenzyme composition defining its intracellular localization and thus providing specific functions to the multifunctional CaMKII to fit the demands at distinct stages of cardiac development.

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