KCNE2 Protein Is Expressed in Ventricles of Different Species, and Changes in Its Expression Contribute to Electrical Remodeling in Diseased Hearts

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Background—Mutations in KCNE2 have been linked to long-QT syndrome (LQT6), yet KCNE2 protein expression in the ventricle and its functional role in native channels are not clear.

Methods and Results—We detected KCNE2 protein in human, dog, and rat ventricles in Western blot experiments. Immunocytochemistry confirmed KCNE2 protein expression in ventricular myocytes. To explore the functional role of KCNE2, we studied how its expression was altered in 2 models of cardiac pathology and whether these alterations could help explain observed changes in the function of native channels, for which KCNE2 is a putative auxiliary (β) subunit. In canine ventricle injured by coronary microembolizations, the rapid delayed rectifier current (I_{Kr}) density was increased. Although the protein level of ERG (I_{Kr} pore-forming, α subunit) was not altered, the KCNE2 protein level was markedly reduced. These data are consistent with the effect of heterologously expressed KCNE2 on ERG and suggest that in canine ventricle, KCNE2 may associate with ERG and suppress its current amplitude. In aging rat ventricle, the pacemaker current (I_{f}) density was increased. There was a significant increase in the KCNE2 protein level, whereas changes in the α-subunit (HCN2) were not significant. These data are consistent with the effect of heterologously expressed KCNE2 on HCN2 and suggest that in aging rat ventricle, KCNE2 may associate with HCN2 and enhance its current amplitude.

Conclusions—KCNE2 protein is expressed in ventricles, and it can play diverse roles in ventricular electrical activity under (patho)physiological conditions. (Circulation. 2004;109:1783-1788.)

Key Words: ion channels ■ electrophysiology ■ hypertrophy

The KCNE gene family encodes small proteins (103 to 170 amino acids) with 1 transmembrane domain. They function as β-subunits of voltage-gated cation channels by interacting with α-subunits and modulating their properties.2-7 Heterologously expressed KCNE1–KCNE3 can interact with multiple target α-subunits.2-5,8-11 Conversely, some α-subunits can interact with multiple KCNE subunits.2,6,7,9,11 Therefore, the relationships between KCNE and α-subunits are complicated.

Among the KCNE subunits, KCNE2 seems to be the most promiscuous one. Heterologously expressed KCNE2 can associate with ERG (I_{Kr} α-subunit),1 Kv4.x (x=2 or 3)4 and Kv3.4 (M. Pourrier, PhD, et al, unpublished data, 2002) (transient outward or I_{to} α-subunit), KCNQ1 (slow delayed or I_{Ks} α-subunit); M. Zhang, PhD, et al, unpublished data, 2003), and HCNx (x=1 or 2, I_{f} α-subunits).10 The importance of KCNE2 in maintaining the ventricular electrical stability is suggested by the linkage between inherited mutations or a polymorphism in KCNE2 and sporadic or acquired long-QT syndrome (LQT6).3,12

Investigators studying the role of KCNE2 in ventricular repolarization and arrhythogenic mechanism(s) of LQT6 face two problems. First, expression of KCNE2 protein in the ventricle has not been established.13 Second, so far, all the experiments studying the functional roles of KCNE2 have been conducted in heterologous expression systems. The exact role of KCNE2 in native channels in the heart requires confirmation.

Here, we first show that 2 antibodies (Abs) (targeting 2 regions of KCNE2 sequence highly homologous among

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Figures I through IV are available in the Data Supplement provided with the online-only version of this article at http://www.circulationaha.org.

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may contribute to electrical remodeling in cardiac pathology.

In all experiments, human KCNE2-α subunits were transfected into 2 animal models of cardiac pathology. We previously described changes in the protein level of KCNE2 and its regulatory subunit Cx43 in ventricles of human, dog, and rat. We then developed an in vitro translation assay that allows the production of full-length α-subunit of KCNE2 in the presence of KCNE2 C-subunit. The assay can readily detect KCNE2 protein in ventricles of human, dog, and rat. We then used Ab1 to detect KCNE2 proteins in Western blots. Using Ab1 to detect KCNE2 proteins in Western blots, we tested Ab1 in Western blots of in vitro translated, 35S-methionine labeled, human KCNE2 (hKCNE2). In vitro translation reaction was carried out without or with hKCNE2 cRNA. Translation products were fractionated with SDS-PAGE. PVDF membrane was probed with Ab1 (top), and gel was exposed to x-ray film for autoradiogram (bottom).

Immunocytocchemistry
COS-7 cells were transfected with c-myc–tagged hKCNE2. One day after transfection, cells were fixed by 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. Cells were incubated with mouse anti–c-myc monoclonal Ab (mAb) and rabbit anti-KCNE2 polyclonal Ab (Ab1) simultaneously. Immunoreactivity was detected by labeling cells simultaneously with 2 secondary Abs: Alexa 488–conjugated goat anti-mouse IgG and Alexa 568–conjugated goat anti-rabbit IgG (Molecular Probes). Canine ventricular myocytes were processed similarly but probed with Ab1 and anti-rabbit secondary Ab only. Immunofluorescence was viewed with a confocal laser scanning microscope (Olympus, Fluoview FV300).

Immunoprecipitation
Membrane proteins were prepared as described above. Protein preparations (300 μg each) were precleared by incubating with washed Protein-G beads (Pierce) at 4°C for 1 hour. The beads were removed by centrifugation, and the precleared protein preparations were incubated with KCNE2 Ab2 (50-fold dilution) at 4°C for 1 hour. The mix was then incubated with Protein-G beads (coated with 1% BSA overnight) and then washed) on a rotation platform at 4°C for 1 hour, followed by centrifugation. The supernatant fractions were dried, boiled in SDS sample buffer, and loaded in the “Sup” lanes (see Figure 5). The beads were washed, and bound proteins were eluted by boiling in SDS sample buffer and loaded in the “IP” lanes. For comparison, the original protein preparations were loaded in the “Tot” lane (60 μg/lane).

Ab Production and Sources
The following Abs were used: KCNE2 Ab1 (raised against a peptide corresponding to the hKCNE2 sequence shown in Figure 1C), ERG (anti-C), KCNE2 Ab2 and HCN2 (Alomone), and c-myc mAb (Oncogene).

Single-Cell Preparation and Patch-Clamp Experiments
Single myocytes were isolated from the midmyocardial region of canine left ventricle (LV). Whole-cell currents were recorded with AxoPatch 200 (Axon Instruments). Data acquisition/analysis was performed with pClamp suite software as described previously.

Results
Ab1 Detected Core and Glycosylated KCNE2 Proteins in Western Blot Experiments
We tested Ab1 in Western blots of in vitro translated, 35S-methionine–labeled, hKCNE2 protein. The radioactivity served as an independent test for the band size(s) of translation products. Translation primed by hKCNE2 cRNA generated 3 radioactive bands at 25, 20, and 15-kDa (Figure 1A,
There was no radioactive band if cRNA was omitted (-cRNA). Therefore, all 3 bands in the "+cRNA" lane derived from newly translated hKCNE2 proteins. The corresponding immunoblot (Figure 1A, top) shows that in the +cRNA lane, Ab1 could recognize 3 strong bands of the same sizes as seen in the autoradiogram. Similar observations were obtained in 5 other experiments.

The expected size of core hKCNE2 is 15 kDa. There are 2 consensus N-glycosylation sites: N6 and N29. Therefore, the banding pattern in Figure 1A suggested that in vitro translation of hKCNE2 under our experimental conditions yielded the core (15-kDa), singly glycosylated (20-kDa), and doubly glycosylated (25-kDa) forms. Indeed, treatment with N-glycosidase F collapsed the 25- and 20-kDa bands into the 15-kDa band (Figure 1B; similar observations in another experiment).

Ab1 also detected a 20-kDa band in the -cRNA lane (Figure 1A, top). This is probably dog KCNE2 protein from the canine pancreatic microsomes present in the translation reaction, because (1) KCNE2 transcript has been detected in pancreas,18 (2) a 20-kDa band was consistently detected by both KCNE2 Abs (Ab1 and Ab2) in membrane proteins from dog hearts (Figures 1D and 2A; see also Figures 4A and 5), and (3) after N-glycosidase F treatment, this band was not detectable, consistent with the notion that it too was collapsed by the enzyme into a smaller band (Figure 1B).

Ab1 Detected Native KCNE2 Proteins in Ventricles

In membrane fractions from human, dog, and rat LV, Ab1 could detect protein bands with sizes suggestive of the expression of differentially glycosylated KCNE2 isoforms: 25 kDa in human and rat (with a 20- or 15-kDa band in some but not all human samples) and 20 kDa in dog (Figure 1, C through E). To verify these observations, we used an independent Ab (Ab2) that should also recognize KCNE2 isoforms from all 3 species (Figure I-C in the Data Supplement).

Ab2 Detected the Same Major Bands in Native Proteins as Ab1

Figure 2A shows that indeed, Ab2 detected a major 25-kDa band in rat LV and a major 20-kDa band in dog LV. Both bands were abolished if Ab2 was preincubated with excess Ag (Figure 2A, right). Figure 2B shows that Ab2 could detect a strong 25-kDa band in membrane fraction from human LV of 2 nonfailing and 2 failing hearts (different hearts than those used in Figure 1C). Therefore, Ab1 and Ab2 generated similar banding patterns in membrane proteins from human, dog, and rat hearts. Neither Ab1 nor Ab2 cross-reacted with KCNE1 protein (Figure I in the Data Supplement).
Ab1 Detected KCNE2 Protein in Canine Ventricular Myocytes

Ventricular myocardium contained both myocyte and nonmyocyte elements. To test whether KCNE2 protein is indeed expressed in the myocytes, we applied immunocytochemistry and confocal microscopy to myocytes isolated from canine LV. The suitability of Ab1 in this type of experiment was confirmed by data presented in Figure 3A. COS-7 cells were transfected with an hKCNE2 construct that had an extracellular c-myc tag inserted between amino acids 19 and 20. The c-myc tag did not interfere with trafficking of hKCNE2 within cells or its interaction with target α-subunits in cell membrane. Therefore, it served as an independent test for the expression and localization of hKCNE2 protein. The left and right panels of Figure 3A show immunofluorescence signals from the same field of confluent COS-7 cells originating from c-myc mAb and from Ab1, respectively. The two had very similar distribution patterns (superposition in the right panel), indicating that Ab1 could specifically detect KCNE2 in immunocytochemistry.

Figure 3B shows that Ab1 generated clear immunofluorescence signals in a canine ventricular myocyte. Similar observations were obtained from 6 cells from 2 animals. If Ab1 was omitted from the incubation buffer, only autofluorescence was detected using a strong laser intensity (Figure 3B, right). Ab1 immunofluorescence in canine ventricular myocytes had a preferential cell surface localization, with some clustering in the t-tubules. This pattern of subcellular distribution appears to be similar to that described for ERG and Kv4.3.

We then studied whether there are changes in the KCNE2 protein level, alone or with target α-subunits, in the ventricle under pathological conditions and whether these changes could explain changes in the function of putative target channels (on the basis of heterologous expression experiments, Figure 1-B). We used Ab2 because it gave stronger and cleaner bands than Ab1.

KCNE2 Downregulation Accompanied an Increase in \( I_{Kr} \) in Microembolized Canine Ventricle

A canine model of ischemic cardiomyopathy was produced by repetitive coronary microembolizations. Three months after the last microembolization (LV ejection fraction dropped from 60±5% to 28±7%), hearts were harvested (n=5). In all hearts, there was a widespread microinfarction in the anterior and lateral wall of LV as well as in the apex. Midmyocardial myocytes were isolated from the affected regions of LV free wall and subjected to patch-clamp recordings. There was severe cellular hypertrophy (cell capacitance increased from 188±9 to 258±8 pF; n=67 and 72, respectively, \( P<0.001 \)). Action potential duration was prolonged (33°C with 1 Hz stimulation, action potential duration measured when repolarization reached \(-20 \text{ mV} \) and normalized by cell capacitance. Numbers in parentheses are numbers of animals (A) or cells (B). *\( P<0.05 \) control vs microembolized by unpaired \( t \) test.

Figure 4. A, Left, Western blots of membrane fractions from LV apex of control and microembolized dog hearts probed with ERG Ab (top) or KCNE2 Ab (Ab2, middle). Animals are marked at top. Bottom, CB stain of ERG gel showing 45-kDa α-actin band (Figure IV in the Data Supplement) used for correcting loading variations. Note that for Figures 4 and 6, each immunoblot had its own CB stain for data normalization, although only representative CB stains are shown. Band sizes are marked at right. Right, Summary of densitometry data based on same Western blots. Intensities of ERG or KCNE2 bands were divided by 45-kDa CB stain of respective lanes and normalized by mean intensity of samples from control dogs. B, Left, Current traces from LV myocytes of control and microembolized hearts. Current traces were recorded before and after application of 1 μmol/L dofetilide. Right, Summary of \( I_{Kr} \) current densities from myocytes from control and microembolized hearts. \( I_{Kr} \) was measured as dofetilide-sensitive current at \(-20 \text{ mV} \) and normalized by cell capacitance. Numbers in parentheses are numbers of animals (A) or cells (B). *\( P<0.05 \) control vs microembolized by unpaired \( t \) test.
However, $I_{Kr}$ (when detectable as a dofetilide-sensitive time-dependent outward current at $-20 \text{ mV}$) had a higher current density in myocytes from microembolized hearts than in control myocytes ($0.57 \pm 0.11 \text{ pA/pF}$ versus $0.25 \pm 0.04 \text{ pA/pF}$, $n=11$ and 7, respectively, $P<0.05$, Figure 4B). There was no significant difference in $I_{Kr}$ gating kinetics between these 2 groups of myocytes (Figure III in the Data Supplement).

It has been suggested that KCNE2 associates with ERG to form native $I_{Kr}$ channels in the heart. Indeed, ERG protein could be coimmunoprecipitated with KCNE2 using Ab2 from membrane proteins prepared from both control and microembolized ventricles (Figure 5). We used Western blots to quantify the protein level of KCNE2 and ERG in membrane proteins from the LV apical region of microembolized hearts as well as control hearts ($n=4$ and 5, respectively). Figure 4A shows that the average level of ERG protein was not altered in microembolized hearts, whereas the KCNE2 protein was markedly reduced.

**KNCE2 Upregulation Accompanied an Increase in Pacemaker Current in Aging Rat Ventricle**

It has been well documented that $I_{f}$ density is increased in LV myocytes from aging (18- to 24-month-old) versus young (2- to 4-month-old) WKY and SHR rats. Between aging WKY and SHR ventricles, $I_{f}$ density is higher in the latter. The increase in $I_{f}$ density is not accompanied by any change in the voltage-dependence of activation, ruling out an age-related change in cAMP modulation of $I_{f}$ channels as a possible cause. The main $\alpha$-subunit of $I_{f}$ channels in ventricular myocardium is HCN2. It has been shown that KCNE2 can associate with HCN2 in oocytes and increase its current amplitude without altering the voltage-dependence of activation. However, it is not clear whether KCNE2 plays any role in the increase in $I_{f}$ density in aging WKY and SHR ventricles. We used Western blots to quantify the protein level of KCNE2 and HCN2 in membrane fractions prepared from the right ventricles of WKY and SHR rats of 3 age groups: young (4 to 5 months), middle-aged (9 to 11 months), and old (18 to 22 months).

The KCNE2 protein level showed a clear age-related increase in SHR ventricles ($P<0.05$, old versus young or middle-aged). A similar trend was seen in WKY, but the change was not statistically significant. Compared between WKY and SHR, the KCNE2 protein level appeared to be

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**Figure 5.** KCNE2 Ab2 coimmunoprecipitated ERG with KCNE2 from membrane proteins prepared from control and microembolized canine ventricles. Lanes Tot, Sup, IP, and _ represent total membrane protein, supernatant, immunoprecipitate, and immunoprecipitate in absence of Ab2, respectively. After electrophoresis in 6% homogeneous gels (top) or 4% to 20% gradient gels (bottom), proteins were blotted to membranes. Membranes were probed with ERG Ab (anti-C, top) or KCNE2 Ab2 (bottom). Size marker locations are marked on left. ERG band size (155 kDa) and KCNE2 band sizes (20 kDa as major band, with a peak at 28 kDa band in Tot and IP lanes) are equivalent to 25-kDa band seen in 14% homogeneous gel in Figure 2A).

**Figure 6.** A, Western blots of membrane fractions from right ventricular myocardium of WKY and SHR of 3 age groups (marked at top) probed with Ab2 (KCNE2, top) or HCN2 Ab (middle). Bottom, CB stain of 45-kDa bands from HCN2 gel. B, Summary of densitometry data from 4 KCNE2 and 4 HCN2 Western blots (including those in A). Each Western blot contained samples from a separate group of animals loaded in same order as shown in A. KCNE2 or HCN2 band intensities were divided by 45-kDa CB stain of respective lanes and normalized by that of “young WKY” lane in same blot. C, Heart-to-body weight (HW:BW) ratios. Data in B and C were analyzed by 1-way ANOVA: $P=0.028$ (KCNE2), $P=0.551$ (HCN2), and $P<0.001$ (HW:BW). This was followed by Fisher’s least significant difference method (KCNE2) or Dunn’s method (HW:BW) for all pairwise comparisons. *, # $P<0.05$ for indicated comparisons.
lower in SHR in the young and middle-aged groups (when there was only mild hypertrophy in SHR versus WKY, Figure 6C) but not in the old-age group (when SHR animals began to develop severe hypertrophy, Figure 6C).

The predicted size of rat HCN2 protein is 97 kDa. The HCN2 Ab consistently detected a single 67-kDa band in rat ventricles. The signal could be greatly attenuated if the Ab was preincubated with excess Ag (data not shown). Similar findings were reported for HCN2 Western blots in canine ventricles. It is possible that the 67-kDa band reflected a splice variant of HCN2 in the heart, or it could represent the major proteolytic product of HCN2 protein in the membrane protein preparations despite the inclusion of protease inhibitor cocktail in the buffers. The average Western blot data (Figure 6B, bottom) suggested that the HCN2 protein in both WKY and SHR ventricles showed a similar biphasic change as the animals aged: HCN2 protein appeared to decrease from young to middle age but appeared to increase again in the old animals. Probably because of the biphasic age-related change, the HCN2 protein level in the old WKY and SHR ventricles showed marked variations: Of 4 Western blots, 2 showed a marked increase in the old age group (one shown in Figure 6A), whereas 2 others showed a modest decrease. Therefore, the HCN2 protein levels in the old animals did not reach statistically significant changes either between WKY and SHR or when compared with other age groups.

Discussion

Our major findings can be summarized as follows: (1) KCNE2 protein is readily detectable in the ventricles of human, dog, and rat. Both Ab1 and Ab2 recognized a major 25-kDa band in human and rat ventricles and a major 20-kDa band in dog ventricle that might represent doubly and singly glycosylated KCNE2 isoforms, respectively. (2) KCNE2 protein was detected in canine ventricular myocytes, and its subcellular distribution appeared to be similar to that of ERG and Kv4.3, 2 of its putative target α-subunits. (3) KCNE2 protein level was markedly reduced in microembolized canine ventricles but increased in aging rat ventricles.

Data shown in Figure 4 are consistent with the notion that in LV myocytes from normal canine hearts, many or most of the ERG channels are associated with KCNE2, which exerts a suppressing effect on the \( I_K \) current amplitude. After microembolizations, the KCNE2 protein level was severely downregulated. This might relieve the ERG channels of the suppressing effect of KCNE2, leading to an increase in the \( I_K \) current density. However, the molecular mechanism of KCNE2–ERG interaction is not entirely clear, and KCNE2 might also interact with \( I_K \) (M. Zhang et al, unpublished data, 2003) in the canine ventricle. Therefore, the impact of changes in the KCNE2 protein level on ventricular repolarization and risk for arrhythmias in diseased heart is complicated and requires further investigation.

Figure 6 shows that in SHR ventricles from aging versus young or middle-aged animals, there was a significant increase in the KCNE2 protein level, whereas changes in the HCN2 protein level were variable and not statistically significant. These observations are consistent with the notion that KCNE2 can associate with HCN2 in aging rat ventricle and increase its current amplitude. The role of HCN2 requires further investigation but may contribute to the more pronounced increase in \( I_K \) in aging SHR versus WKY.

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Fig. 1 (A) Transmembrane topology of pore-forming (α) subunits of voltage-gated cation channels, KCNE auxiliary (β) subunits, and a cytoplasmic KChIP subunit. (B) Effects of KCNE2 on cardiac voltage-gated cation channels (major α- and β-subunits indicated in parentheses). ‘~’ little or no effect, ‘?’ not known. Right column lists references corresponding to those in the paper (* Pourrier M, PhD, et al., unpublished data, 2002, # Zhang M, PhD, et al., unpublished data, 2003). (C) Alignment of partial amino acid (aa) sequences of KCNE2 from human and 6 animal species (GP = guinea pig). ‘.’ aa identical to human sequence. Transmembrane domain (TMD) is boxed. Epitope sequences for antibodies 1 and 2 (Ab1 and Ab2) are underlined.
**Fig. II** No cross reactivity between KCNE2 Abs with KCNE1 protein. KCNE1 and KCNE2 proteins (encoded by human isoforms, hKCNE1 and hKCNE2, respectively) were made by *in vitro* translation, with no cRNA (-cRNA) as negative control (marked on top of each panel). The translation products were run on SDS-PAGE, blotted to membranes, and probed with KCNE2 Ab2 (A), KCNE2 Ab1 (B), or KCNE1 Ab (from Alomone, C). * Bands specifically recognized by Ab, with band sizes (in kDa) marked on the right. The ‘-cRNA’ lanes showed a band of 15 kDa (and a faint 25 kDa band after prolonged exposure, as in C). KCNE2 Ab2 and Ab1 both detected 3 strong bands in the ‘KCNE2’ lanes: 25, 20 and 15 kDa (the latter had a much higher intensity than that in the –cRNA lanes). However, neither KCNE2 Ab could detect any signal in the KCNE1 lanes (i.e. signals not different from those in the –cRNA lanes). The success of KCNE1 protein translation was verified in C: KCNE1 Ab could detect 2 faint bands of 22 and ~18 kDa (in addition to bands similar to those seen in the –cRNA lane). The predicted size of core hKCNE1 protein is 14.6 kDa, and there are 2 N-glycosylation sites. This KCNE1 Ab could not detect any specific signals in the KCNE2 lane (other than those seen in the –cRNA lane). Therefore, KCNE2 Ab1 and Ab2 do not cross react with KCNE1 protein and KCNE1 Ab does not cross react with KCNE2 protein.
Fig. III Kinetic properties of $I_{Kr}$ in canine ventricular myocytes from control and microembolized hearts. Myocytes were isolated from the midmyocardium of left ventricular free wall (where microinfarctions were observed in microembolized hearts), and whole-cell variant of patch clamp recordings were performed under the following conditions: (a) bath solution contained (mM) choline Cl 146, KCl 4, MgCl$_2$ 2.5, HEPES 5, dextrose 5.5, pH 7.3, (b) pipette solution contained (mM) K-aspartate 125, KCl 20,
EGTA 10, HEPES 10, MgCl₂ 1, ATP(K) 10, pH 7.3, and (c) temperature was 33±1°C. Delayed rectifier currents (I_{Kr} + I_{Ks}) were isolated from other interfering current by (a) nominally Na- and Ca-free bath solution (removing Na, Ca and Na/Ca currents), and (b) a holding voltage of −50 mV (inactivating I_{to}). Delayed rectifier currents were activated by 5-s depolarization pulses from a holding voltage of −50 mV to test voltages (V_t) of −40 to +60 mV in 10 mV increments once every 30 s. I_{Kr} was further isolated from I_{Ks} by measuring dofetilide (DOF, 1 uM)-sensitive currents. To minimize the problem of current ‘run-down’, time lapse between control current recording and after DOF application was limited to 5-8 min (during which DOF was washed in while membrane currents at −10 mV and tail currents at −50 mV were continuously monitored to be sure that drug effects reached a steady-state). (A) Original DOF-sensitive (∆I) tail currents from a control and a microembolized myocyte (calibrations shown in the right panel applied to both myocytes). The voltage clamp protocol is diagrammed in the inset. (B) Original tail current traces before and after DOF application (- and + DOF, respectively) using the voltage clamp protocol diagrammed in the inset. Also shown are ∆I (shown as dots) and 1-exponential curve fit. (C) Original test pulse currents recorded before and after DOF application using the voltage clamp protocol diagrammed in the inset. Shown on the bottom are ∆I (dots) superimposed on 1-exponential curve fit. (D) Voltage-dependence of I_{Kr} activation. DOF-sensitive tail currents (as those shown in A) were normalized by the currents induced by V_t to +20 mV, and the ‘normalized tail current-V_t’ relationship was fit with a simple Boltzmann function to estimate the half-maximum activation voltage (V_{0.5}) and slope factor (k): normalized tail = 1/(1+exp((V_{0.5}-V_t)/k)). The V_{0.5} and k values for control myocytes were −15.7±3.6 and 8.7±0.4 mV. The values for microembolized myocytes were −19.1±3.2 and 8.9±0.4 mV (p > 0.1 for both). (E) Time constants (τ) of I_{Kr} deactivation and activation. Deactivation τ values were estimated from 1-exponential fit to DOF-sensitive tail currents as those shown in (B). Activation τ values were estimated from 1-exponential fit to DOF-sensitive test pulse currents as those shown in (C). Note that the τ values are plotted on a logarithmic scale. For all τ values, p > 0.1 for control vs microembolized myocytes.
Fig. IV  A monoclonal Ab against α-actin (Sigma AC-40) specifically recognized a 45 kDa band in membrane proteins from 4 different canine ventricles. This mAb cross reacts with α-actin from different species including rat. The observation supports the notion that the strong 45 kDa band seen in coomassie blue stain of membrane proteins from dog and rat ventricles (Figs. 4 & 6 of the paper) derived from α-actin, which has been shown to be a reliable internal control for immunoblots (J Biol Chem 2003;278:25558-25567). Size marker positions are marked on the left.