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Neurally-mediated increase in calcineurin activity regulates cardiac contractile function in absence of hypertrophy

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Abstract

Objective: The calcineurin pathway has been involved in the development of cardiac hypertrophy, yet it remains unknown whether calcineurin activity can be regulated in myocardium independently from hypertrophy and cardiac load. **Methods:** To test that hypothesis, we measured calcineurin activity in a rat model of infrarenal aortic constriction (IR), which affects neurohormonal pathways without increasing cardiac afterload. **Results:** In this model, there was no change in arterial pressure over the 4-week experimental period, and the left ventricle/body weight ratio did not increase. At 2 weeks after IR, calcineurin activity was increased 1.8-fold ($P<0.05$) and remained elevated at 4 weeks (1.7-fold, $P<0.05$). Similarly, the cardiac activity of calcium calmodulin kinase II (CaMKII) was increased significantly after IR, which confirms a regulation of Ca^{2+}-dependent enzymes in this model. In cardiac myocytes, the increased activity of calcineurin was accompanied by a significant decrease in L-type Ca^{2+} channel activity (I_{Ca}) and contraction velocity ($-dL/dt$). Cardiac denervation prevented the activation of calcineurin after IR, which demonstrates that a neurohormonal mechanism is responsible for the changes in enzymatic activity. In addition, cardiac denervation suppressed the effects of IR on I_{Ca} and $-dL/dt$, which shows that calcineurin activation is related to altered contractility. However, action potential duration, the densities of inward rectifier K^+ currents (I_{K1}), and outward K^+ currents (I_{to} and I_K) were not altered in IR myocytes. **Conclusions:** Calcineurin can be activated in the heart through a neural stimulus, which induces alterations in Ca^{2+} currents and contractility. These effects occur in the absence of myocyte hypertrophy, electrophysiological changes in action potential, and K^+ channel currents.

Keywords: Autonomic nervous system; Calcium (cellular); Contractile function; Hypertrophy; Signal transduction

1. Introduction

Cardiac hypertrophy is a fundamental response to pressure overload. For many years, considerable interest focused on the sympathetic and renin–angiotensin systems, with pharmacological antagonists of these pathways playing a key role in regulating hypertension, hypertrophy and their sequelae in the clinical setting. More recent evidence indicates an important role for calcineurin in the regulation of NFAT (Nuclear Factor of Activated Transcription) activity and the GATA4-dependent transcriptional pathway [1–3]. The concept that calcineurin plays a pivotal role in hypertrophy is deduced from studies in transgenic mice and from experiments in which the hypertrophic response is blocked with administration of calcineurin antagonists, such as cyclosporine A [4–10]. However, the relative contribution of calcineurin and sympathetic or renin–angiotensin systems to cardiac hypertrophy remains difficult

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to assess in vivo because all these systems generally are activated simultaneously by the hypertrophic stimulus.

So far, most studies have stressed the importance of calcineurin as a regulator of gene transcription in the hypertrophied heart. In addition, it is unknown whether regulation of calcineurin can occur independently from hypertrophy and whether such regulation would affect contractile function. The extent to which calcineurin affects the function of myocytes, or vice versa, is relatively unexplored, although potentially important, if calcineurin is to be considered a transducer of Ca^{2+} homeostasis in the heart. Because it is a Ca^{2+}-regulated enzyme, calcineurin could act as a Ca^{2+} sensor and affect myocyte function in the normal heart, similarly to Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [11]. Therefore, the goal of this study was to examine the response of cardiac calcineurin activity to infrarenal aortic constriction (IR), which might perturb the neural regulation of the heart without inducing hypertrophy [12–14]. Our data demonstrate that calcineurin activation can be dissociated from changes in heart mass, under the control of neural mechanisms. This enzymatic stimulation, which can be extended to CaMKII, is accompanied by significant changes in L-type Ca^{2+} channel function and contractility in isolated myocytes.

2. Methods

2.1. Animal models

Male Sprague–Dawley rats (200–210 g) were used for infrarenal aortic constriction (IR) and sham-operated control ($n=7$/group). The animals used in this study were maintained in accordance with *The Guide for the Care and Use of Laboratory Animals* (DHHS Publication No. NIH 83-23, revised 1996, Office of Science and Health Reports, Bethesda, MD). All animals were anesthetized with a mixture of 66 mg/kg ketamine, 13 mg/kg xylazine, and 2 mg/kg acepromazine [15]. A midline abdominal incision was used to expose the abdominal aorta. A 0.5-mm hemoclip (Weck Inc.) was placed around the abdominal aorta 2 mm below the left renal artery to induce IR [16,17]. In the sham-operated group, the aorta was dissected without application of the clip. Animals were followed up at 1 week, 2 weeks, or 4 weeks after operation. In the denervation group, the rats underwent bilateral sympathectomy (stellarectomy) as described [18]. Immediately after denervation, the animals were submitted to IR as described above.

Hemodynamics were measured under the same anesthesia as described above. Arterial pressure and heart rate were measured by a fluid-filled catheter inserted into the right carotid artery. Femoral arterial pressure was measured simultaneously with another catheter inserted into the left femoral artery. Another group of rats was used to measure arterial blood pressure and heart rate in the conscious state using a DataSciences telemetry implant (PA-C20). The probe was placed into the left carotid artery 3 days before banding under general anesthesia and remained for 31 days after surgery. Signals were acquired using a receiver (RPC-1) connected to a calibrated pressure output adapter. An index of locomotor activity was recorded directly from the receiver output and processed with specific software.

2.2. Enzyme activity

After sacrifice, hearts were weighed, and the left ventricle was separated and homogenized using Polytron (Brinkman PT 3000) in 1 ml lysis buffer (50 mol/l Tris (pH 7.5), 0.1 mmol/l EDTA, 0.1 mmol/l EGTA, 0.2% NP-40, 1 mmol/l DTT, 100 μmol/l PMSF, 5 mmol/l ascorbic acid, and protease inhibitor cocktail). Following sample preparation, calcineurin activity was measured by the dephosphorylation rate of a synthetic phosphopeptide substrate (RII peptide) with a calcineurin assay kit (BioMol) following the manufacturer's instructions [7]. Released free phosphate was detected colorimetrically with the Green reagent (BioMol) based on the classic Malachite green assay [19,20]. CaMKII activity was measured in 20 μg of total homogenate as per the protocol of the manufacturer (Gibco Life Technologies).

2.3. Immunoblotting

Left ventricles were homogenized in lysis buffer (10 mmol/l Tris (pH 7.4), 70 mmol/l NaCl, 25 mmol/l NaF, 20 mmol/l Na-pyrophosphate, 7.5% glycerol, 0.5% NP-40, 0.5% Triton, 0.5 mmol/l $MgCl_2$, 0.5 mmol/l $CaCl_2$, 0.5 mmol/l DTT, 1 mmol/l orthovanadate, 100 μmol/l PMSF, and protease inhibitor cocktail). An equal amount of protein from each sample was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (BioRad), transferred to nitrocellulose membrane (Hybond-C extra, Amersham Pharmacia Biotech), and blocked with 1% BSA in TBST buffer. Membranes were incubated with primary antibodies against calcineurin A (Transduction Laboratories) and CaMKII (Promega). Bands were detected using ECL reagent, NEN Renaissance Western Blot Chemiluminescence Reagent (NEN Life Science), and quantified by Personal Densitometer SI (Molecular Dynamics).

2.4. Quantitative reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA was extracted from the samples using the method of Chomczynski and Sacchi [21]. Quantitative RT–PCR for ANF was performed by TaqMan assay on a 7700 Sequence Detector (Applied Biosystems) [22] with specific primers (forward: 5′-GTGTACAGTGCGGTGTCCAA-3′, reverse: 5′-ACCTCATCTTCTACC-

GGCATC-3′) and a fluorescent probe (5′FAM-TGATGGATTTCAAGAACCTGCTAGACCACC - 3′TAMRA). The quantitative 2-step PCR included a 10-s step at 95 °C and a 1-min step at 60 °C for 40 cycles. Internal RNA standards were prepared from the PCR-amplified cDNA after ligation of the T7 promoter using the MegaShortScript kit (Ambion, Austin, TX) [23]. Because of possible variations in sample-to-sample loading conditions, the values of the transcript were normalized to the transcript level of cyclophilin measured in each sample. The PCR data therefore are reported as the number of transcripts per number of cyclophilin transcripts.

2.5. Histology

Left ventricles were separated and fixed in 10% (w/v) phosphate-buffered formalin. Myocyte cross-sectional area was measured using the MetaMorph image system software (Universal Imaging, West Chester, PA). Myocyte outlines were apparent after silver-staining methacrylate embedded sections (1 μm thick), which were obtained midway between the LV base and apex. Suitable cross sections were defined as having nearly circular capillary profiles and circular-to-oval myocyte sections. Traces of 100–200 myocyte outlines were obtained in the left ventricle of each animal.

2.6. Electrophysiology and myocyte contractility measurements

Left ventricular (LV) myocytes were isolated from control and IR rats as described [24,25]. Myocyte length was measured using a video motion edge detector at 32±2 °C [25,26], and $-dL/dt$, the velocity of myocyte contraction, was calculated from the length data. Whole-cell Ca^{2+} currents were recorded in myocytes from control and IR rats using patch-clamp techniques as previously described [27,28]. Cell capacitance was measured using voltage ramps of 0.8 V/s from a holding potential of −50 mV. External solution was (mmol/l): $CaCl_2$, 2; $MgCl_2$, 1; tetraethyl ammonium chloride, 135; 4-aminopyridine, 5; glucose, 10; HEPES, 10 (pH 7.3). The pipette solution contained (mmol/l): Cs-aspartate, 100; CsCl, 20; $MgCl_2$, 1; MgATP, 2; GTP, 0.5; EGTA, 5 and HEPES, 5 (pH 7.3). These solutions provided isolation of Ca^{2+} currents from other membrane currents and Na^+/Ca^{2+} exchanger.

Action potentials were recorded using perforated patch technique. For action potential and K^+ current recordings, myocytes were bathed with Tyrode solution (mmol/l): NaCl, 135; $CaCl_2$, 1.8; $MgCl_2$, 1; KCl, 5.4; glucose, 10; HEPES, 10 (pH 7.3). The pipette solution for action potential recordings contained amphotericin B 200 μg/ml and (mmol/l): KCl, 140; $MgCl_2$, 2; NaCl, 10; ATP, 2; HEPES, 5 (pH 7.3). To record K^+ currents, nifedipine (10 μmol/l) was added to block I_{Ca}. Patch pipette solution contained (mmol/l): K aspartate, 110; KCl, 20; $MgCl_2$, 2; ATP, 2; GTP, 0.5; EGTA, 5; HEPES, 5 (pH 7.3).

All experiments were performed at room temperature (20–22 °C).

2.7. Statistics

All data were presented as mean±S.E. and were analyzed by analysis of variance with post hoc Tukey correction. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of IR aortic banding on parameters of myocardial hypertrophy

Because the aim of this study is to test whether cardiac calcineurin activity can be stimulated by paracrine mechanisms independently of hypertrophy, our first goal was to exclude by physiological, morphological, and molecular parameters the possibility that IR triggers hypertrophy. The procedure of IR did not affect systolic or mean arterial pressure during the entire protocol (Fig. 1a). No significant pressure gradient was observed between the carotid and femoral arteries after banding. Similarly, IR did not affect body weight, heart weight, and LV weight (Fig. 1b). LV weight related to tibial length was also not different. Myocyte cross-sectional area and ANF gene expression measured by quantitative PCR were similar in both groups (Fig. 1c,d).

3.2. Activation of calcineurin and CaMKII following infrarenal aortic banding

Having shown that IR was not accompanied by hypertrophy, we determined next whether it affects the activity of the Ca^{2+}-regulated enzymes, calcineurin and CaMK II. As shown in Fig. 2a, calcineurin activity increased 1.8-fold 2 weeks after IR, compared to sham-operated animals, and this increase was maintained at 4 weeks. In contrast, calcineurin activity in the spleen was not statistically different between groups 4 weeks after IR (not shown). A 1.5-fold increase in total protein level of calcineurin was found by immunoblot in banded animals compared to controls after 4 weeks (Fig. 2b). Therefore, the increased activity corresponds mostly to an increase in calcineurin protein content. CaMKII activity was also measured to test whether other Ca^{2+}-regulated enzymes are affected by IR. As shown in Fig. 3, a significant increase in the phospho-/total protein ratio of CaMKII was found by immunoblotting in the IR group 2 weeks after banding (Fig. 3a), which was accompanied by a significant increase in enzyme activation in this group (Fig. 3b).

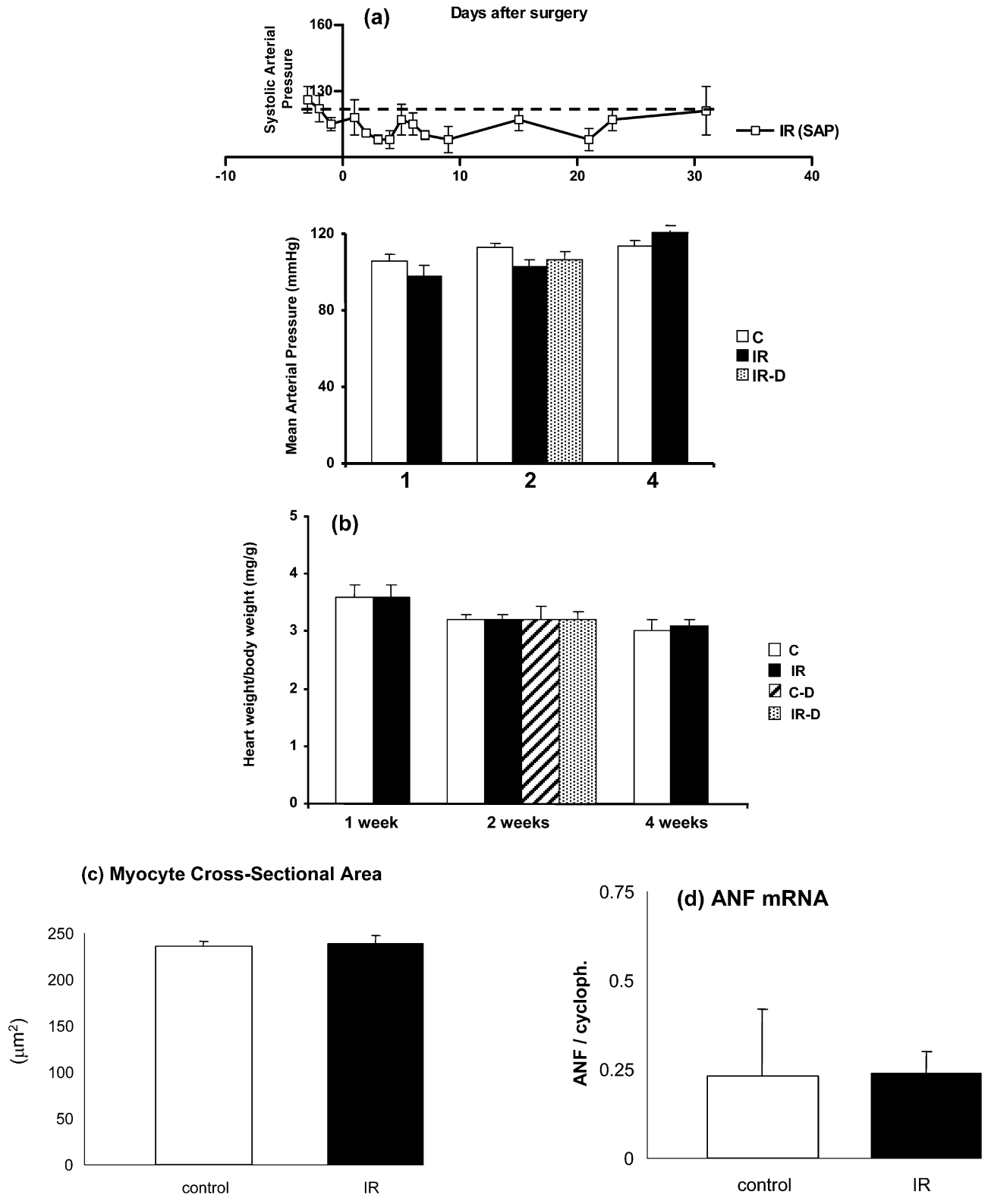

Fig. 1. Effects of IR aortic banding on arterial pressure, HW/BW ratio, myocyte cross-sectional area, and ANF gene expression. (a) Systolic arterial pressure measured directly in conscious rats showed no change over a 34-day (3 days pre- and 31 days post-operation) observation period (top). Mean arterial pressure was not different from control (C) rats at 1, 2, or 4 weeks after IR banding (IR); mean arterial pressure was also not changed in IR rats with chronic cardiac denervation (IR-D) after 2 weeks (bottom). (b) Heart weight to body weight ratio did not change 1, 2, or 4 weeks after IR banding or 2 weeks after cardiac denervation with IR banding and control group. (c) Myocyte cross-sectional area and (d) ANF mRNA production were comparable between groups.

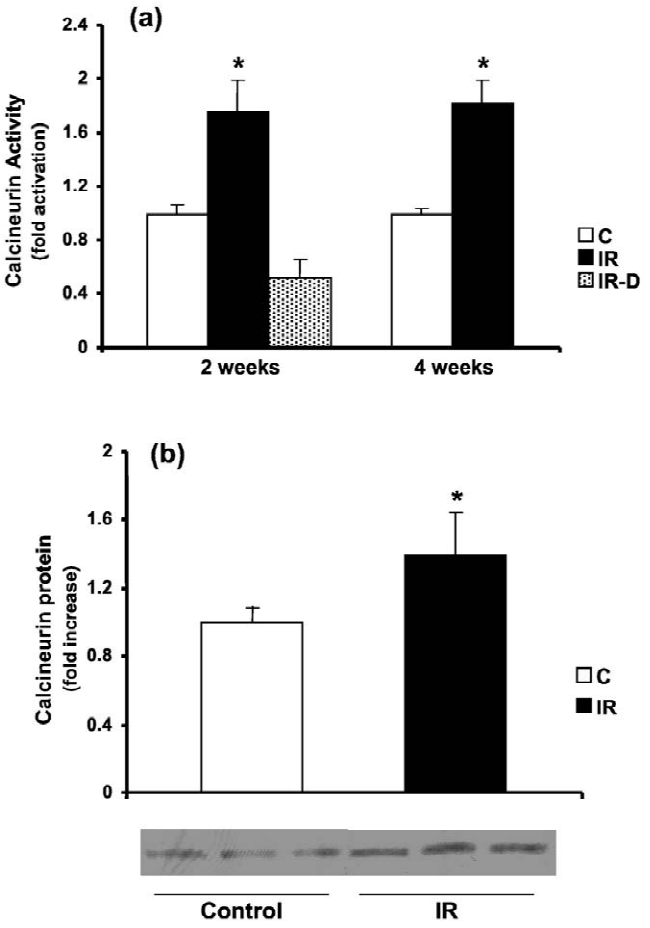

Fig. 2. Effects of IR aortic banding on calcineurin activity in left ventricle. (a) Calcineurin enzymatic activity was measured in the left ventricle at 2 weeks (C, $n=16$; IR, $n=17$; IR-D, $n=5$) and 4 weeks (C, $n=15$; IR, $n=13$) after operation. The asterisks denote significant difference, $P<0.05$, compared with the control group. (b) Quantitative immunoblotting of calcineurin protein in the left ventricle 4 weeks after IR banding.

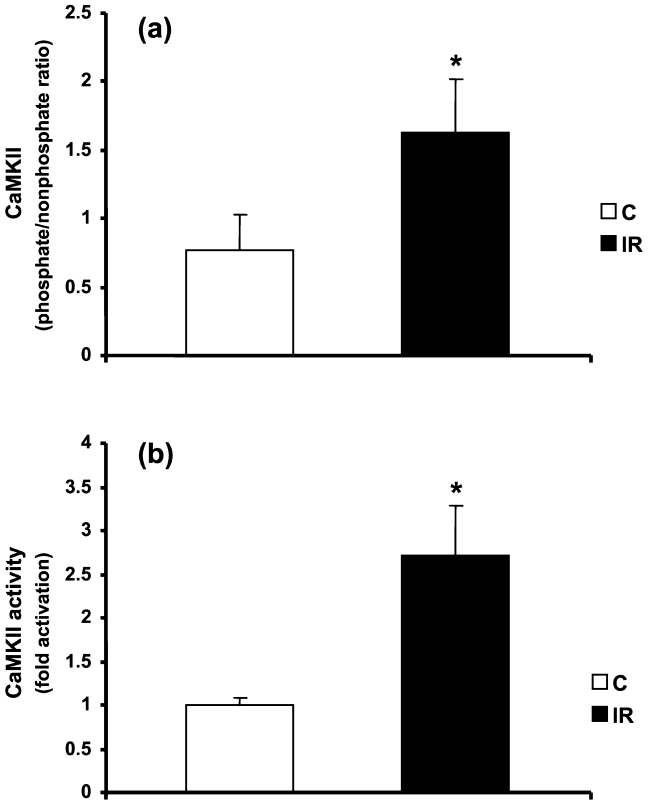

Fig. 3. Effects of IR aortic banding on CaMKII enzyme activity. (a) Phosphate/total ratio. (b) CaMKII activity. Both were significantly ($P<0.05$, $n=5$) increased 2 weeks after IR banding compared with the control group.

3.3. Effects of cardiac denervation

To determine whether a paracrine stimulus activated by IR is responsible for the activation of Ca^{2+}-regulated enzymes in myocardium, additional animals were submitted to cardiac denervation prior to IR. Cardiac denervation was followed by a 90% decrease in tissue norepinephrine (93 vs. 934 pg/mg). In addition, intracardiac administration of veratrine (5 μg/kg) failed to induce brachycardia in the denervated group (not shown). Cardiac denervation had no effect on heart rate, heart weight/body weight ratio, and blood pressure compared with the IR group or with controls (Fig. 1a,b). However, the activation of calcineurin that was found after IR was abolished in the denervated group (Fig. 2a). These results confirm our hypothesis that a paracrine stimulus activated by IR can stimulate cardiac calcineurin independently of triggering cardiac hypertrophy.

3.4. Alteration of Ca^{2+} currents

A physiological effect of calcineurin independent from hypertrophy is the regulation of L-type Ca^{2+} channel currents (I_{Ca}) [29,30]. The tracings in Fig. 4a illustrate representative I_{Ca} from control and IR myocytes also obtained 4 weeks after banding. At that time-point, IR myocytes had significantly decreased I_{Ca} compared with control (Fig. 4a). As shown in Fig. 4a, bottom trace, there was no significant change in current–voltage relationships. At the maximum potential (+10 mV), I_{Ca} inactivated rapidly during maintained depolarization. However, the time to half-decay ($T_{1/2}$) of the current in IR was prolonged. $T_{1/2}$ for IR and for control were 23.3±1.3 ms ($n=53$) and 18.0±0.7 ms ($n=92$; $P<0.01$), respectively. These differences were abolished by denervation, as there was no difference in I_{Ca} between denervated hearts alone and denervated hearts after IR (8.2±0.5 vs. 8.5±0.6 pA/pF, respectively). There was no change in the current–voltage relationships between the two groups (not shown). LV myocyte size as estimated by cell capacitance was not altered in IR myocytes compared to control myocytes (Fig. 4b), consistent with the data showing that cardiac mass is normal.

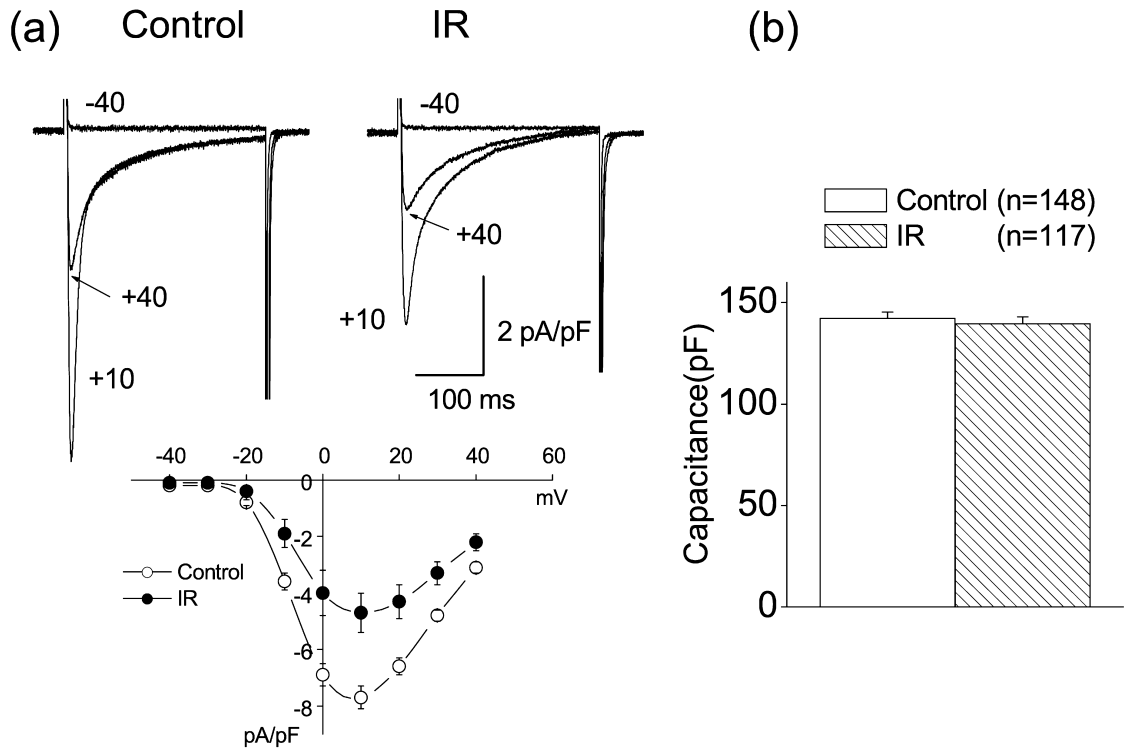

Fig. 4. Effects of IR aortic banding on I_{Ca} in isolated ventricular myocytes. (a) Whole-cell I_{Ca} recorded in control and IR myocytes 4 weeks after IR. Traces show currents elicited from a holding potential of −50 mV to the indicated test potentials at 0.1 Hz. (b) There was no difference in cell capacity between groups, which is consistent with the absence of hypertrophy after IR.

3.5. Alterations of contractility

We also determined whether the inhibitory effects of calcineurin activation on I_{Ca} were correlated with a change in contractile function. In myocytes from IR rats, the rate of contraction ($-dL/dt$) was decreased significantly compared to control myocytes (168±8 μm/s versus 205±5 μm/s, $P<0.01$). Remarkably, the $-dL/dt$ was similar to control values in IR rats after denervation (209±12 μm/s). These data show that the reciprocal effects of IR and denervation on Ca^{2+} channel are mirrored by similar changes in contractility.

3.6. Action potential and K^+ channel currents

The most prominent alteration in hypertrophied myocardium is a prolongation of the action potential. Data from calcineurin-overexpressing mice indicate an action potential prolongation caused by decreased K^+ channel currents [31]. To determine whether similar changes are observed in our model, we measured the action potential and K^+ channel currents.

Action potentials (Fig. 5a) were recorded in control and IR ventricular myocytes in Tyrode solution with 'physiological' pipette solution. There was no significant change in action potential. The action potential duration quantified at 50% and 90% repolarization (APD_{50} and APD_{90}) was similar between the two groups (Fig. 5b). There was no significant difference in the resting membrane potentials between control and IR myocytes (70.3±1.5 vs. 70.0±2.1 mV).

It is important to note that action potential shape and duration are species-specific due to differences in the underlying inward and outward currents. In adult rat ventricular myocytes, the 4-aminopyridine (4-AP) sensitive (I_{to}) and insensitive K^+ currents (I_K) have been described to play an important role in the early and middle phase of action potential [32]. Accordingly, we compared each of these currents (Fig. 6). Fig. 6a and b show typical outward K^+ currents before and after the application of 4-AP. There was no difference in the current–voltage relation in the absence of 4-AP and response to 4-AP (Fig. 6c).

The inward rectifier K^+ currents (I_{K1}) are also important in maintaining the resting membrane potential and terminal phase of the action potential. To activate I_{K1}, hyperpolarizing pulses were applied from a holding potential of −40 mV to test potentials between −50 and −100 mV. There was no significant change in the amplitude or current–voltage relationships between the two groups. The mean current amplitudes at −100 mV in control and IR

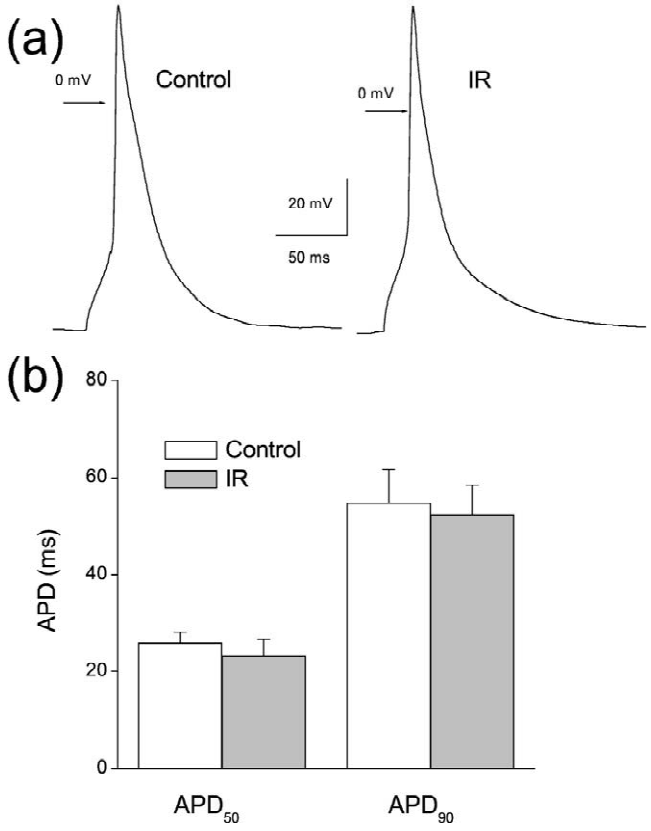

Fig. 5. Effects of IR aortic banding on action potential in isolated ventricular myocytes. (a) Representative action potential recorded in control and IR myocytes 4 weeks after IR. (b) Mean action potential duration (APD) evaluated at 50% and 90% (APD_{50} and APD_{90}). Data are mean±S.E. of control (n=21) and IR (n=18) myocytes from six control and four IR rats.

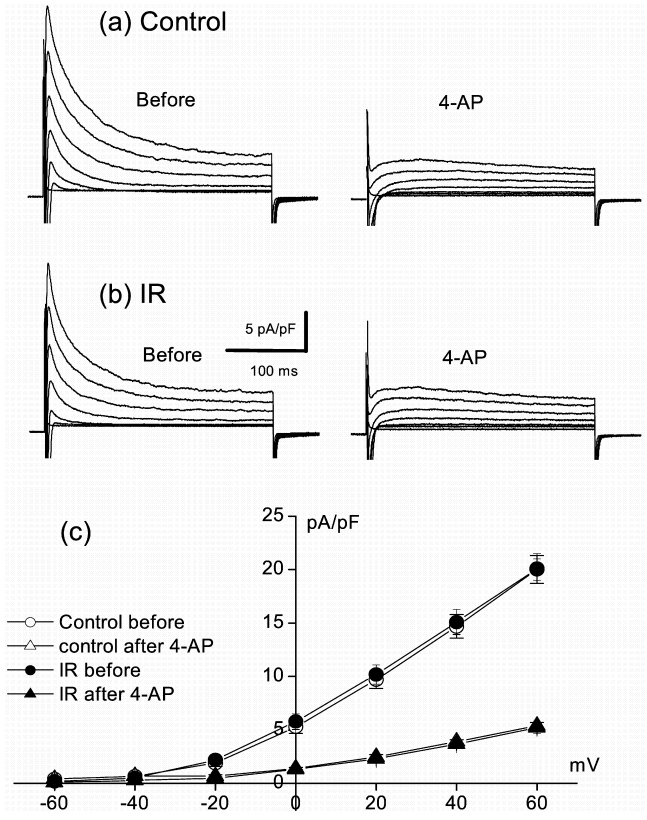

Fig. 6. Effects of IR aortic banding on 4-AP-sensitive K^+ current (I_{to}) and 4-AP-insensitive K^+ current (I_K) in isolated ventricular myocytes. (A) Representative families of currents recorded in control (a) and IR (b). Currents were elicited by voltage steps from −60 to +60 mV in 20-mV increments from a holding potential of −80 mV. (c) current–voltage relation before and after the addition of 4-AP (10 mM) in control and IR. Data are mean±S.E. of control (n=20) and IR (n=19) myocytes from six control and four IR rats.

myocytes were −10.3±0.4 (n=17) and −10.8±1.1 pA/pF (n=19), respectively.

4. Discussion

The transcriptional role of the calcineurin–NFAT pathway in the development of cardiac hypertrophy, particularly when induced by pressure overload, has been examined extensively in the last few years, with controversial results [4–10]. However, it is unknown whether the effects of increased calcineurin activity in the heart can be dissociated from cardiac hypertrophy.

The role of calcineurin in mediating cardiac hypertrophy was first established by Molkentin et al. in 1998 [1], who later engineered a transgenic mouse overexpressing this phosphatase. The mouse rapidly developed a significant increase in heart mass followed by heart failure [1]. The interpretation is that, by dephosphorylating NF-AT3, the latter can interact with the transcription factor GATA4 and thereby activate the transcriptional profile of cardiac hypertrophy. However, one problem with transgenic studies is that the overexpression can be far greater than the increase occurring in pathophysiological conditions. In the present study, the calcineurin activity was increased 2-fold, which is less than in the transgenic model and is probably closer to the physiological range of activation. Indeed, a 2- to 3-fold increase in calcineurin activity has been reported after banding [33] or after exercise-induced hypertrophy [34], which is in a similar range to the activation reported in the present study.

To determine whether calcineurin activation automatically induces cardiac hypertrophy, we utilized an IR model, which does not alter arterial pressure and cardiac load [13,14]. Over the 4-week observation period, the arterial pressure, heart weight, LV weight, heart weight/body weight ratio, and LV weight/tibial length did not increase due to the IR aortic constriction. ANF gene expression, a marker for hypertrophy, also remained unchanged in this model. Interestingly, however, calcineurin activity was increased 1.8-fold 2 weeks after IR

banding and remained elevated until after 4 weeks. This was accompanied by an increase in CaMKII activity. Thus, calcineurin activity was increased without accompanying cardiac hypertrophy. Calcineurin activation and the effects on Ca^{2+} channel function and myocyte function were abolished by cardiac denervation, which supports the concept that a neural regulatory pathway is involved. It is known that calcineurin activity can be increased through reflex pathways [35–37], such as the sympathetic activation which follows abdominal aortic constriction [38]. However, without an activation of the renin–angiotensin system and without an elevation of arterial pressure, this stimulus might not be sufficient to induce a hypertrophic response.

Our results show that enhanced calcineurin activity in the heart regulates myocyte Ca^{2+} flux and function even in the absence of cardiac hypertrophy. Despite the absence of hypertrophy, the increased calcineurin activity was followed by a decrease in Ca^{2+} current density, which was accompanied by a decrease in the rate of contraction ($-dL/dt$) of myocytes. These results are quite different from what has been published previously in the field. For example most studies have shown that increased calcineurin, overexpressed in transgenic mice, resulted in enhanced myocyte contraction, action potential prolongation, and increased Ca^{2+} current [31,28,39,40]. However, it is important to note that these prior studies were conducted in transgenic mice with overexpressed calcineurin; these transgenic mice exhibited considerable hypertrophy and premature mortality. It is conceivable that the effects of calcineurin differ in transgenic mice because of the presence of concomitant hypertrophy.

Calcineurin is a Ca^{2+}/calmodulin dependent-phosphatase, and it is activated by prolonged low-amplitude influx of Ca^{2+} [41,42]. The actual source of Ca^{2+} that activates calcineurin or other Ca^{2+}/calmodulin signaling molecules such as CaMKII is not completely understood. The two major possibilities are the involvement of the L-type Ca^{2+} channels and the SR Ca^{2+} handling [28,43,44]. In a feedback mechanism, the Ca^{2+}-activated calcineurin regulates L-type Ca^{2+} channel currents [29,30]. The prolongation of the time of half-decay in hearts with calcineurin activation (Fig. 4a) reflects differences in Ca^{2+} influx, rather than any change in Ca^{2+} release from the SR [28]. Most importantly, we show that this effect on I_{Ca} is accompanied by a decrease in myocyte contractility. Again, both effects are reversed by denervation, which links the functional changes to calcineurin activation. Although the molecular mechanism of this effect is still speculative, both protein phosphatases (PP) 1 and 2A dephosphorylate the L-type Ca^{2+} channel [45]. For example, the PP1/2A inhibitor calyculin A enhances L-type Ca^{2+} channel currents without affecting the SR Ca^{2+} uptake [46]. In turn, calcineurin has been shown to activate PP1 [47]. Therefore, the decreased Ca^{2+} current density could be due to a dephosphorylation of the L-type Ca^{2+} channel mediated indirectly, or perhaps directly, by calcineurin. However, it has been shown that hypertrophy in itself can increase L-type Ca^{2+} channel activity even if calcineurin is activated [28]. Therefore the regulation of the L-type Ca^{2+} channel involves multiple signaling pathways, and the final effect on I_{Ca} will depend on whether hypertrophy occurs or not, despite an activation of calcineurin. Similar observations were made for other currents, such as K^+ channels [31], which seem more dependent on the hypertrophy process than on calcineurin activity. These data can explain why no change in action potential was found in the present study, considering that the different phases of the action potential are altered by the various molecular adaptations that accompany hypertrophy, instead of calcineurin activity [28,40].

In conclusion, our data demonstrate that calcineurin can be activated in the heart independently from a hypertrophic stimulus, through a neural mechanism that is abolished by denervation. The precise neurohormonal pattern is not known but would most likely involve the release of a mediator that could inhibit cardiac contraction. This could be related to an imbalance between norepinephrine and acetylcholine, or to production of other neural mediators, such as nitric oxide [48,49] or the calcitonin gene-related peptide [50]. Regardless of the mediator, dissociating calcineurin activation from cardiac hypertrophy sheds more light on the function of this important molecule and may have future therapeutic implications, where calcineurin inhibition may be considered for treatment of hypertrophy and/or heart failure.

Acknowledgements

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