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Modulation of contractility by myocyte-derived arginase in normal and hypertrophied feline myocardium

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Jung, Albert S., Hajime Kubo, Rachel Wilson, Steven R. Houser, and Kenneth B. Margulies. Modulation of contractility by myocyte-derived arginase in normal and hypertrophied feline myocardium. *Am J Physiol Heart Circ Physiol* 290: H1756–H1762, 2006. First published December 3, 2005; doi:10.1152/ajpheart.01104.2005.—L-Arginine, the sole substrate for the nitric oxide (NO) synthase (NOS) enzyme in producing NO, is also a substrate for arginase. We examined normal feline hearts and hearts with compensated left ventricular (LV) hypertrophy (LVH) produced by ascending aorta banding. Using Western blot analysis, we examined the abundance of arginase isozymes in crude homogenates and isolated cardiac myocytes obtained from the LVs of normal and LVH hearts. We examined the functional significance of myocyte arginase via measurement of shortening and intracellular calcium in isolated myocytes in the presence and absence of boronoethyl chloride (BEC), a specific pharmacological inhibitor of arginase. Both arginase I and II were detected in crude myocardial homogenates, but only arginase I was present in isolated cardiac myocytes. Arginase I was downregulated in LVH compared with normal. Inhibition of arginase with BEC reduced fractional shortening, maximal rate of shortening (+dL/dt) and relengthening (–dL/dt), and the peak of the free cytosolic calcium transient in normal myocytes but did not affect these parameters in LVH myocytes. These negative inotropic actions of arginase inhibition were associated with increases in cGMP generation. These studies indicate that only arginase I is present in cardiac myocytes where it tends to limit NO and cGMP production with the effect of supporting basal contractility. In experimental LVH induced by pressure overload, our studies demonstrate reduced arginase I expression and reduced functional significance, allowing greater arginine substrate availability for NO/cGMP signaling.

arginase; hypertrophy; nitric oxide; guanosine 3',5'-cyclic monophosphate; calcium

AS IN THE VASCULATURE, nitric oxide (NO) synthase (NOS) regulates the generation of NO in cardiac myocytes by deaminating the amino acid substrate L-arginine to produce NO and citrulline. NO modulates cardiac contractility via activation of soluble guanylyl cyclase, inducing increases in cGMP. In turn, cGMP modulates contractility via the activation of certain phosphodiesterases, activation of protein kinase G, and by direct stimulation of calcium cycling proteins. On the other hand, there are reports that NO can alter contractility independent of cGMP by direct nitrosylation or acetylation of various channels and calcium cycling proteins.

In the normal myocardium, the acute effects of increases in NO are bimodal, with a positive inotropic effect at low amounts of NO exposure but a negative one at higher amounts

(28). NO donors have been shown to exhibit weak to moderate negative inotropic effects in nondiseased preparations (8, 25, 34). On the other hand, in pathological states, there is increasing evidence that upregulation of NOS and/or increased production of NO improves left ventricular (LV) performance and myocyte contractility and prevents or delays the onset of LV hypertrophy (LVH) and heart failure (21, 22, 40).

L-Arginine, the sole substrate for NOS in producing NO, is also a substrate for arginase. Thus arginase has a potential role in limiting substrate available for NO production. In other tissues, there are two arginase isozymes, arginase I and arginase II; however, the myocardial expression and the functional role of arginase have not been previously reported. Accordingly, the objectives of this study were to examine the presence of arginase isozymes in both normal and hypertrophied feline cardiac myocytes and to pharmacologically inhibit arginase to examine its functional role in modulating cardiac contractility.

METHODS

Feline model of LVH. Young felines ~1.0 kg were banded as described previously (19). Progressive aortic constriction induced progressive LVH. All animals used in this study had concentric LVH and were without evidence of pleural effusion or ascites at the time of euthanization for tissue sampling and myocyte cell isolation. Animals were handled in accordance with the guidelines of the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication 85–23, revised 1996), and studies were approved by the Temple University Animal Care and Use Committee.

Myocyte isolation and morphological characterization. Feline LV myocytes were isolated as previously described (14, 32, 33). To characterize cardiac myocyte morphology, we utilized isosmotic fixation with 1.5% glutaraldehyde and Ficol gradient enrichment. Median cell volume was determined by Coulter Channelyzer analysis of >10,000 myocytes, as previously described (42).

Assessment of arginase abundance. Protein was isolated from both crude homogenates and isolated myocytes from the LVs of feline hearts. Cell and tissue samples were from normal hearts and LVH hearts as described above. Protein extraction and Western blot analysis were performed as previously described (26). Assays of isolated myocytes and crude homogenates employed identical techniques. For these experiments, we employed specific monoclonal antibodies directed against arginase I and arginase II (Santa Cruz Biotech, Santa Cruz, CA). Antibodies were labeled with horseradish peroxidase by anti-mouse Ig secondary antibody, 1:2,000 dilution (Amersham Life Science). Targeted antigens were visualized with enhanced chemiluminescence assay (NEN Life Science). Films were scanned (Epson Expression 636), and band intensities were quantified with densito-

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metric analysis by use of the NIH Image 1.62f program. Targeted bands were normalized to cardiac actin.

Isolated myocyte physiology. Freshly isolated myocytes were loaded with fluo-3 AM (Molecular Probes, Eugene, Oregon), at 4–10 μM final concentration, and in 1 mM Ca^{2+} . Myocytes were placed in a chamber on the stage of an inverted microscope. The chamber was superfused at 1–2 ml/min with Tyrode solution (150 mM NaCl, 5.4 mM KCl, 1 mM CaCl_2 , 1.2 mM MgCl_2 , 10 mM glucose, 2 mM pyruvate, 5 mM HEPES, pH 7.4, 37 $^\circ\text{C}$). Rod-shaped myocytes were chosen on the basis of their appearance and the absence of irregularities or function disturbances, such as spontaneous contractions. Cytosolic free calcium concentration ($[\text{Ca}^{2+}]_i$) was measured with fluo-3 as described previously (23). The emission at 530 nm was recorded to represent the $[\text{Ca}^{2+}]_i$ transient and used to derive F/F_0 , the pseudoratio of intracellular calcium as previously described (38).

Contraction of myocytes was assessed by edge detection. Myocytes were field stimulated at 1.0 Hz for at least 1 min before steady-state baseline measurements were made. Myocytes were then exposed to boronoethyl chloride (BEC) (10^{-5}M), which is a specific but nonselective (both type I and type II isozymes) arginase inhibitor (9, 11), for 3 min. After steady-state $[\text{Ca}^{2+}]_i$ transient and contractions were recorded, BEC and oxadiazolo quinoxalin-1-one (ODQ, 10^{-5}M , prepared in DMSO), a soluble guanylate cyclase (GC) inhibitor, were administered concomitantly for 3 min before steady-state $[\text{Ca}^{2+}]_i$ transient and contractions were recorded again. Other myocytes were also exposed to ODQ alone without exposure to BEC to observe the effects of GC inhibition alone on contractility and calcium cycling. The $[\text{Ca}^{2+}]_i$ signal and length signals were saved on a computer for later analysis with pCLAMP software (Axon Instruments, CA). Peak F/F_0 was used to quantify peak $[\text{Ca}^{2+}]_i$, and the time to 50% decay (T_{50}) of the $[\text{Ca}^{2+}]_i$ transient was measured. Contractile parameters, including fractional shortening (FS), peak maximal rate of shortening ($+dL/dt$) and peak maximal rate of relengthening ($-dL/dt$), were measured. Under each experimental condition, at least five separate $[\text{Ca}^{2+}]_i$ transients and contractions were measured.

cGMP measurement. Cyclic nucleotides were measured in suspensions of isolated myocytes from both normal ($n = 4$) and from LVH ($n = 3$), both obtained as described above. For each animal, aliquots of cells (at least 10) were taken from the cell suspension media, and the viable cell number was counted with a hemacytometer (Advent Genetics, Ann Arbor, MI). The cell concentration of the suspension was calculated, and aliquots of 1.5 million cells were exposed to either BEC or vehicle for 5 min on a shaker (Biotech, Philadelphia, PA) at 25 $^\circ\text{C}$. The reaction was quenched with 300 μl of 1 N HCl. After 20 min, each sample was neutralized with 300 μl of 1 N NaOH and centrifuged for 3 min at 12,000 rpm and 4 $^\circ\text{C}$. The supernatant was stored at -80°C until analysis. Subsequently, the cGMP concentration was measured in duplicate via a competitive enzyme immunoassay (ELISA, Cayman Chemical, Ann Arbor, MI), as previously described (2, 15).

Statistical analysis. For isolated myocyte physiology measurements, all data were averaged, and results are presented as means \pm SE unless otherwise stated. Statistical significance was determined by one-way, two-way, or repeated-measures two-way ANOVA and a Student-Newman-Keuls post hoc test (Graph Pad InStat and SPSS statistical software). For Western blot and ELISA measurements, an unpaired two-tailed Student's *t*-test was employed to compare normal and LVH samples. Values of $P < 0.05$ were considered significant.

RESULTS

Arginase in normal and hypertrophied myocardium. As shown in Figure 1, *B* and *D*, aortic banding induced cardiomyocyte hypertrophy as evidenced by a significant increase in heart-to-body weight ratio and median cell volume, respectively. As seen in Fig. 1*A*, both arginase I and II were detected in crude LV homogenates obtained from normal and hypertro-

phied feline myocardium. In these preparations, the abundance of both isozymes was significantly reduced in the presence of myocyte hypertrophy. In isolated myocytes (Fig. 1*C*), only arginase I was detected (homogenized kidney tissue; positive control). Here again, a reduced abundance of this isozyme was observed in the presence of myocyte hypertrophy. The lack of arginase II in the myocyte-enriched suspensions suggests that the arginase II detected in crude homogenates was originating in nonmyocytes.

Effect of arginase inhibition on myocyte contractility and calcium cycling. Figure 2*A* and Table 1 present raw data and averaged data from experiments in which the acute effects of arginase inhibition with BEC were evaluated in isolated myocytes. In normal myocytes, arginase inhibition induced striking negative inotropic effects, including significant decreases in peak fractional shortening, $+dL/dt$, and $-dL/dt$. These negative inotropic effects were associated with significant decreases in the peak $[\text{Ca}^{2+}]_i$. However, arginase inhibition did not cause significant changes in the T_{50} of the $[\text{Ca}^{2+}]_i$ transient.

During continued administration of BEC, application of the GC inhibitor ODQ reversed the negative inotropic effects of arginase inhibition. These findings suggest that the negative inotropic actions of BEC were mediated via the NO/cGMP pathway. Experiments in which BEC increased cGMP levels in cardiac myocyte suspensions (Fig. 2*B*) further support the conclusion that arginase inhibition augments NO and cGMP production.

Figure 3*A* and Table 1 present raw data and averaged data from experiments in which the acute effects of arginase inhibition with BEC were evaluated in hypertrophied myocytes from banded animals. Hypertrophied myocytes tended to have lower baseline fractional shortening and peak $[\text{Ca}^{2+}]_i$ levels compared with normal myocytes, but these differences were not statistically significant. More importantly, arginase inhibition with BEC produced little or no changes in myocyte contractile and relaxation parameters and likewise did not affect the peak or decay kinetics of the $[\text{Ca}^{2+}]_i$ transient. In suspensions of isolated LVH myocytes, BEC did not induce significant increases in cGMP concentration (Fig. 3*B*). As highlighted in Fig. 4, the functional responses to arginase inhibition were significantly different in normal and LVH myocytes.

As seen in Fig. 5*A*, GC inhibition alone produced marked positive inotropy as seen by both FS and peak $[\text{Ca}^{2+}]_i$ levels in normal cells. Pilot studies with LVH myocytes also suggested marked positive inotropy with GC inhibition as seen in Fig. 5*B*, but the magnitude of this effect appears less than in normal myocytes. However, because of the small number of cells, we cannot be certain that an intergroup difference exists.

DISCUSSION

Although both arginase I and II are expressed in the heart, the main new finding of these studies is that only arginase I is present in cardiac myocytes where it tends to limit NO and cGMP production with the effect of supporting basal contractility. In experimental LVH induced by pressure overload, our studies demonstrate reduced arginase I expression and reduced functional significance, allowing greater arginine substrate availability for NO/cGMP signaling. Recently, Berkowitz et al. (5) showed that arginase inhibitors increase NOS enzyme

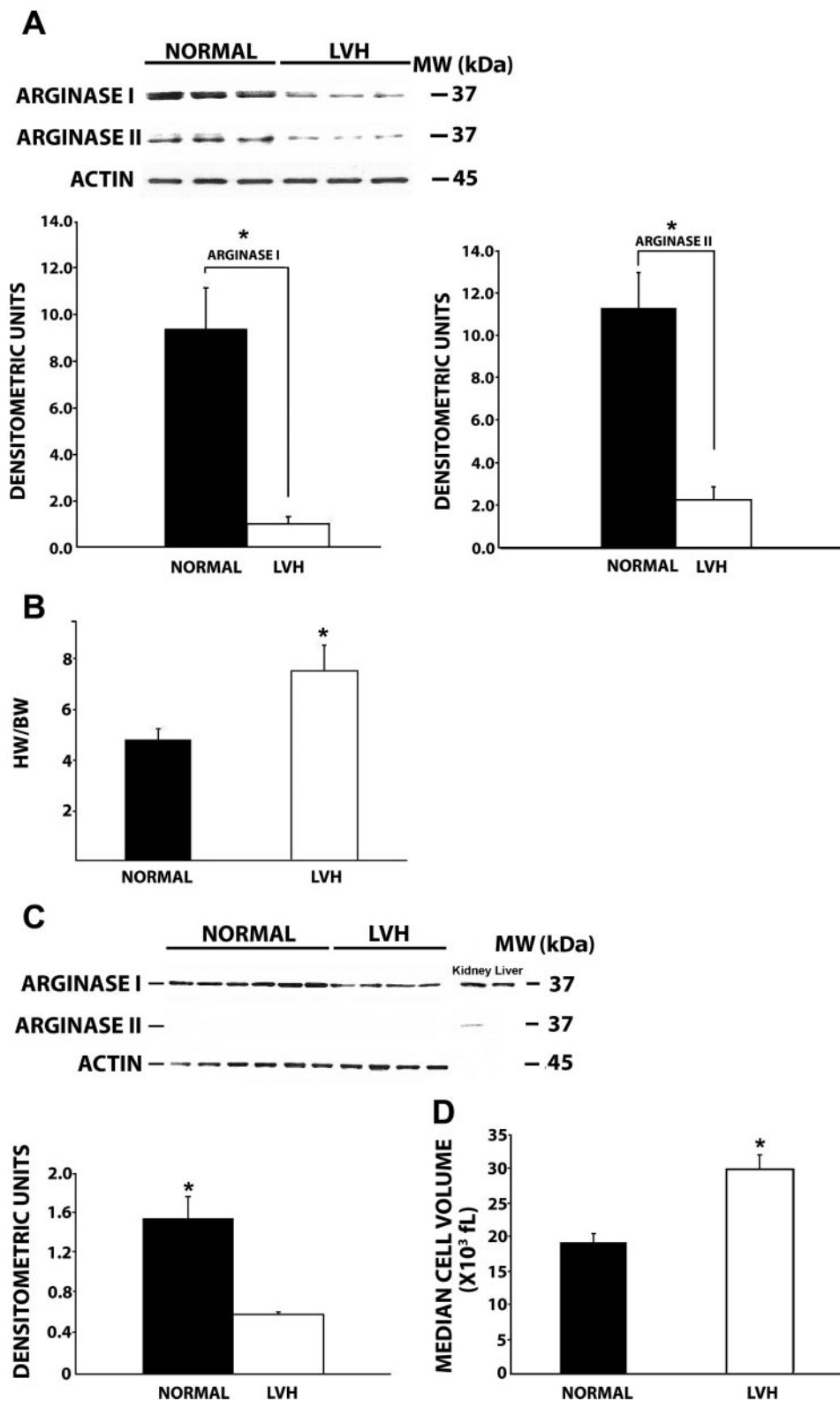


Fig. 1. Expression of arginase isozymes in left ventricular (LV) crude homogenates. *A*: arginase I and II were both present in homogenates from normal and LV hypertrophy (LVH) tissue. The abundance of both isozymes was reduced in LVH. *B*: HW/BW, ratio of heart weight to body weight. *C*: only arginase I was present in myocyte-enriched suspensions from normal and LVH tissue. *D*: median myocyte volume derived from Coulter counter analysis (see METHODS). For all analyses: **P* < 0.05 for normal vs. LVH.

activity, cGMP production, and vascular relaxation, suggesting that arginase competitively regulates L-arginine bioavailability for endothelial NOS (eNOS) in rat aortic vasculature. To our knowledge, the present study is the first demonstrating the functional significance of arginase in cardiac myocytes.

The question of whether NO signaling affects cardiac myocyte contractility under basal conditions in the normal heart has been the subject of considerable debate. Several early studies reported that activation of NOS with increased NO production tends to decrease contractility via cGMP-dependent or cGMP-

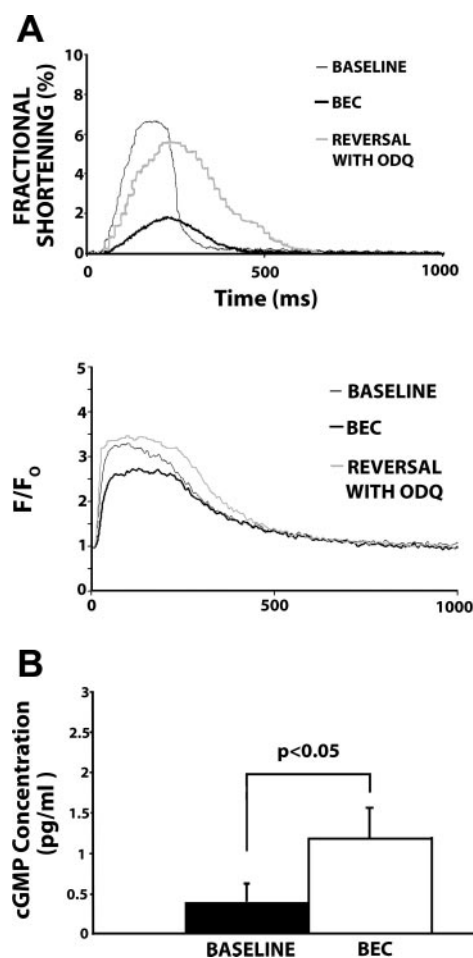


Fig. 2. Representative shortening and free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) transients from normal feline myocytes. *A*: arginase inhibition with the specific arginase inhibitor boronoethyl chloride (BEC) causes significant decrease in both peak fractional shortening (FS) and $[\text{Ca}^{2+}]_i$, which is reversed by administration of oxadiazolo quinoxalin-1-one (ODQ) during continued BEC. F/F_0 , pseudorange of intracellular Ca^{2+} (see METHODS). *B*: arginase inhibition with BEC caused a significant increase in cGMP concentration in suspensions of normal myocytes.

independent mechanisms (3, 6, 7, 12, 17, 43). Moreover, as recently reviewed, analysis of the phenotypes of mice genetically deficient in one or more isoforms of NOS further supports the conclusion that NO signaling tends to attenuate contractility under basal conditions (28). Nevertheless, because administration of NOS inhibitors to normal cardiac myocytes alters

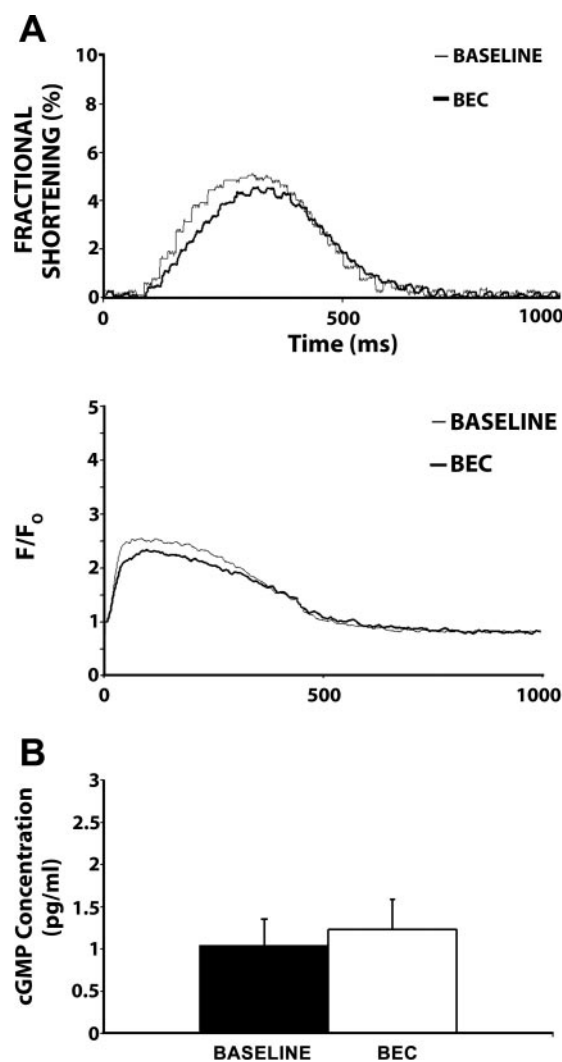


Fig. 3. Representative shortening and $[\text{Ca}^{2+}]_i$ transients from hypertrophied feline myocytes. *A*: arginase inhibition with BEC did not significantly alter either shortening or $[\text{Ca}^{2+}]_i$ transients. *B*: arginase inhibition with BEC did not significantly increase cGMP concentration in suspensions of LVH myocytes.

basal contractility minimally (18, 28), most investigators have drawn the conclusion that NO-dependent signaling has a minor role in altering basal contractility. In this context, our findings of significant myocyte arginase I expression and decreased contractility with arginase inhibition raise the possibility that

Table 1. Myocyte contractions and Ca^{2+} transients

	Peak FS, %	+dL/dt, $\mu\text{m/s}$	-dL/dt, $\mu\text{m/s}$	Peak $[\text{Ca}^{2+}]_i$, F/F_0	T ₅₀ $[\text{Ca}^{2+}]_i$, ms
Normal					
Baseline	6.1 ± 0.8	30.1 ± 3.2	-33.1 ± 2.2	2.7 ± 0.3	170 ± 15
BEC	2.9 ± 0.4*	18.1 ± 2.2*	-23.3 ± 4.2*	1.8 ± 0.2*	162 ± 11
BEC and ODQ	6.8 ± 0.7†	24.3 ± 2.1†	-28.8 ± 3.2†	3.0 ± 0.3†	186 ± 10†
LVH					
Baseline	5.3 ± 0.9	27.1 ± 0.8	-27.4 ± 2.7	2.4 ± 0.4	168 ± 4.2
BEC	5.2 ± 0.4‡	24.2 ± 2.1‡	-29.6 ± 1.9‡	2.3 ± 0.3‡	169 ± 5.8

Values are means ± SE. LVH, left ventricular hypertrophy; FS, fractional shortening; +dL/dt, maximal rate of shortening; -dL/dt, maximal rate of relengthening; $[\text{Ca}^{2+}]_i$, free cytosolic calcium concentration; T₅₀ $[\text{Ca}^{2+}]_i$, time to 50% decay of the $[\text{Ca}^{2+}]_i$ from its peak; BEC, boronoethyl chloride (a specific arginase inhibitor); ODQ, oxadiazolo quinoxalin-1-one (a soluble guanylate cyclase inhibitor). * $P < 0.05$ for BEC vs. respective baseline; † $P < 0.05$ for BEC/ODQ vs. BEC alone; ‡ $P < 0.05$ between groups under analogous conditions.

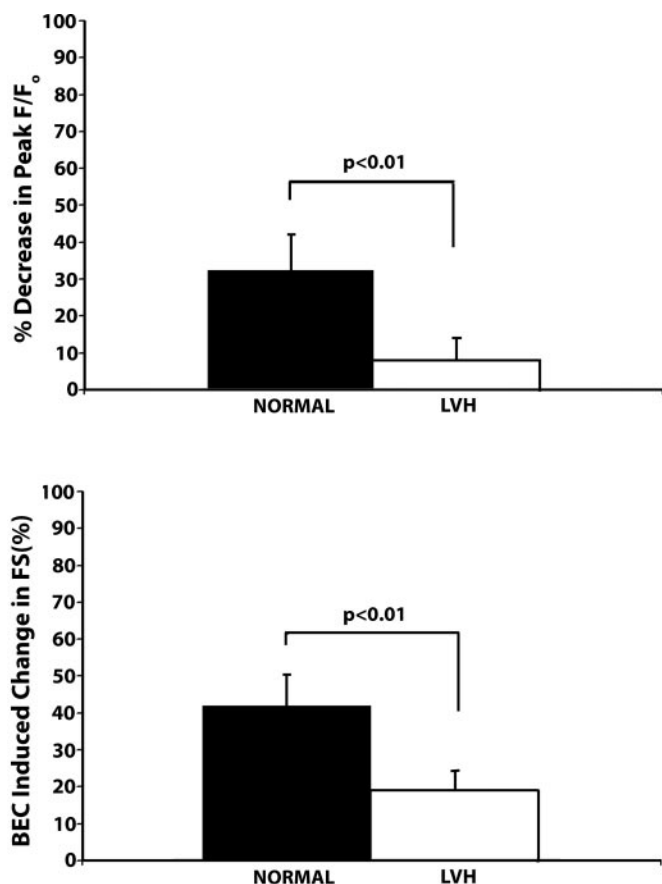


Fig. 4. Difference in effect of arginase inhibition in normal vs. hypertrophied myocytes.

the minimal effects of NOS inhibition in the basal state are due to the fact that NOS/NO/cGMP signaling is already being inhibited by a substrate limitation imposed by endogenous arginase. In this construct, decreases in contractility observed during further NOS/NO activation reflect a shift in a dynamic equilibrium between arginase and NOS rather than activation of dormant NOS/NO signaling per se.

Our findings of decreased expression of arginase I and diminished effects of arginase inhibition on basal contractility in LVH compared with normal is strong evidence that NO signaling is increased in LVH, at least in part, via decreased competition by arginase. Indeed, these findings of decreased arginase complement other studies indicating increased NOS activity in LVH (31, 35). Other things being equal, these coordinated changes should serve to additively enhance NO-dependent signaling in the compensated phase of pathological hypertrophy. Interestingly, basal cGMP in LVH tended to be higher than in normal. This raises the question of whether enhanced cGMP levels in LVH might have a significant role in

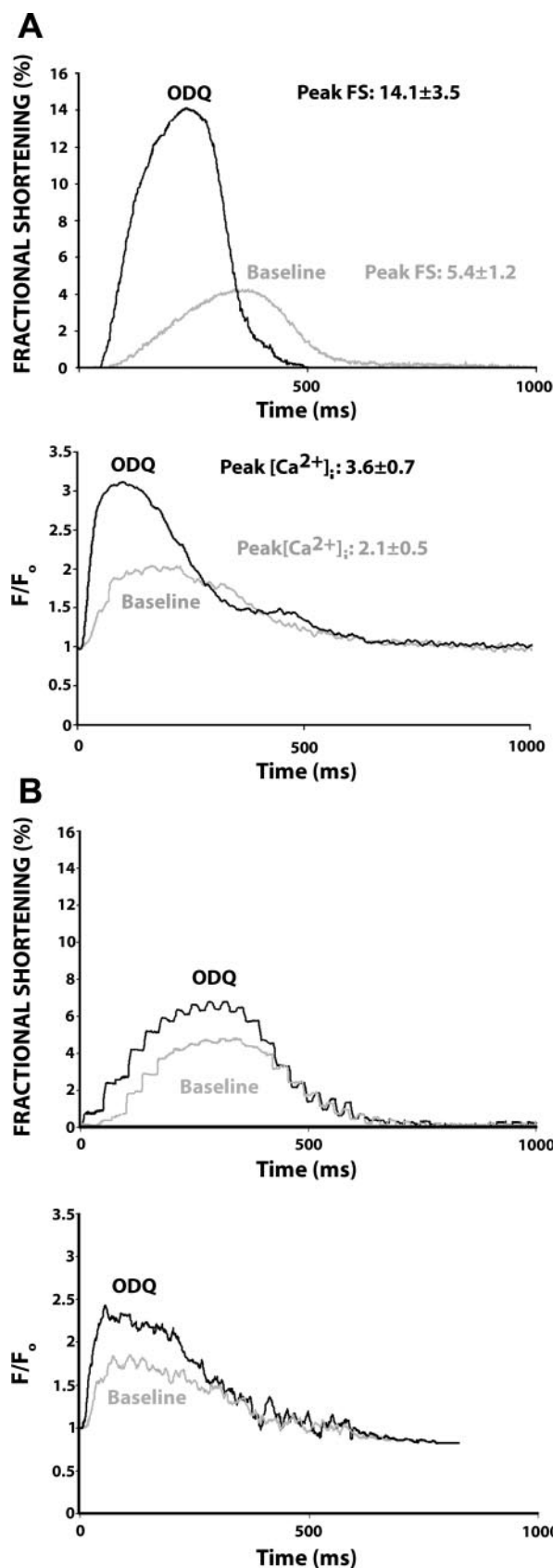


Fig. 5. Representative shortening and $[Ca^{2+}]_i$ transients of normal and LVH myocytes in response to acute guanylate cyclase (GC) inhibition with ODQ. A: GC inhibition with ODQ significantly increases shortening and peak $[Ca^{2+}]_i$ from baseline in normal myocytes ($n = 27$ cells from 10 animals) B: ODQ also augment shortening and peak $[Ca^{2+}]_i$ from baseline in LVH myocytes ($n = 5$ cells from 2 animals), but the magnitude of this effect tends to be attenuated in these pilot studies.

altering basal contractility. GC inhibition alone would reveal the basal inotropic consequence of cGMP. In fact, treating normal myocytes with ODQ alone increased myocyte shortening and peak $[Ca^{2+}]_i$, suggesting a suppressive effect of cGMP on contractility in normal myocytes. In LVH we expected the effect of GC inhibition to be even larger because of the supposed enhancement of NO via arginase inhibition. However, the few LVH myocytes we have treated with ODQ alone have shown similar or lesser effects of ODQ on basal contractility. More studies are required to allow definitive conclusions, but our preliminary findings support cGMP-mediated control of basal contractility.

Whether increases in NO-dependent signaling in LVH are adaptive or maladaptive is still controversial. Previous studies have already demonstrated that arginase dysregulation can modulate NO-dependent signaling outside the vasculature. Arginase II expression was demonstrated in human corpus cavernosa where it is upregulated in diabetes, a disease process associated with erectile dysfunction (24). In the airway, arginase inhibition attenuates methacholine-induced airway constriction by increasing NO production (29). In this broader context, downregulation of myocyte arginase I in the hypertrophied myocardium could represent an adaptation to remodeling that contributes to contractile dysfunction and heart failure. Alternatively, several studies suggest that the initial increase of NO in the course of cardiac disease is protective and acts to decrease free radical formation, oxygen consumption, and calcium overload (21, 40). In addition, Aoki et al. (1) showed that arginase activity in neonatal mouse cardiac myocytes had anti-apoptotic activity.

We observed no significant changes in rates of relaxation or the decay of the $[Ca^{2+}]_i$ transient after arginase inhibition with BEC in myocytes from normal or LVH myocardium. There is still controversy about the effect of endogenous NO on diastolic properties at the myocyte level. Some investigators have shown an increase in diastolic fiber length and relaxation by NO via desensitization of cardiac myofilaments (27). In papillary muscle and in vivo studies, NO donors have been shown to stimulate NO release and selectively affect myocardial relaxation (16, 30, 37). However, other have shown no effect and even a slowing of diastolic calcium decay (36). Further, NOS inhibitors have shown unremarkable findings (10) and the eNOS $-/-$ knockout mouse showed no alteration in diastolic properties (4), suggesting a limited functional role of NO in the regulation of cellular relaxation. Thus the existing literature is generally consistent with our findings that augmentation of NO-mediated effects via arginase inhibition has minimal impact on cellular relaxation, even in normal myocardium, where negative inotropic effects are observed. Of course, the lack of a net effect on relaxation does not preclude the possibility of separate molecular effects that serve to counterregulate one another.

Limitations. There may be concern as to whether BEC, a boronic acid analog of L-arginine, may directly activate NO synthesis, but prior studies have shown that BEC is a high-affinity, selective arginase inhibitor that binds as a tetrahedral boronate anion, which attains a transition state that is fundamentally incompatible with the substrate site of NOS (9, 13). Also, we cannot ignore the possibility that arginase activity and the production of ornithine and urea within the myocyte may have an effect on myocyte function directly. It is well known

that ornithine is the precursor for the synthesis of polyamines such as spermine, spermidine, and putrescine. There have been reports that polyamine concentration alters K^+ channel current in myocytes (41), can decrease inotropy by superfusion of exogenous polyamines (39), and can decrease calcium sensitivity (20). However, these studies show the effects of direct application of high-concentration exogenous polyamines, which is likely distinct from the smaller changes in endogenous ornithine that result from modulation of arginase activity. Another limitation of these studies is the inability to define the subcellular localization of arginase with the methods we employ. It is commonly accepted that the cardiac myocyte has two constitutive NOS isoforms, eNOS and neuronal NOS, localizing to the sarcolemma and sarcoplasmic reticulum, respectively. Whether arginase and arginase inhibition are able to modulate L-arginine substrate availability for both myocyte NOS isoforms remains undetermined. In this regard, both endothelial cells and macrophages have been shown to have L-arginine pools that are localized and not freely exchangeable with the extracellular space, which may bias availability to one NOS isoform over another.

In summary, the present findings demonstrate arginase expression in cardiac myocytes and support its role in limiting NO production. In a feline model of LVH, the abundance and function of arginase decreases and likely contributes to enhanced NO production. Further studies will be required to determine whether arginase manipulation represents an appropriate therapeutic target in cardiac hypertrophy or failure.

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REFERENCES

1. Aoki MP, Guinazu NL, Pellegrini AV, Gotoh T, Masih DT, and Gea S. Cruzipain, a major *Trypanosoma cruzi* antigen, promotes arginase-2 expression and survival of neonatal mouse cardiomyocytes. *Am J Physiol Cell Physiol* 286: C206–C212, 2004.
2. Arima T, Ohshima Y, Mizuno T, Kitamura Y, Segawa T, and Nomura Y. Cyclic GMP elevation by 5-hydroxytryptamine is due to nitric oxide derived from endogenous nitrosothiol in NG108–15 cells. *Biochem Biophys Res Commun* 227: 473–478, 1996.
3. Balligand JL, Kelly RA, Marsden PA, Smith TW, and Michel T. Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. *Proc Natl Acad Sci USA* 90: 347–351, 1993.
4. Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, Hobai IA, Lemmon CA, Burnett AL, O'Rourke B, Rodriguez ER, Huang PL, Lima JA, Berkowitz DE, and Hare JM. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature* 416: 337–339, 2002.
5. Berkowitz DE, White R, Li D, Minhas KM, Cernetich A, Kim S, Burke S, Shoukas AA, Nyhan D, Champion HC, and Hare JM. Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation* 108: 2000–2006, 2003.
6. Brady AJ, Poole-Wilson PA, Harding SE, and Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. *Am J Physiol Heart Circ Physiol* 263: H1963–H1966, 1992.
7. Brady AJ, Warren JB, Poole-Wilson PA, Williams TJ, and Harding SE. Nitric oxide attenuates cardiac myocyte contraction. *Am J Physiol Heart Circ Physiol* 265: H176–H182, 1993.

8. Chesnais JM, Fischmeister R, and Mery PF. Positive and negative inotropic effects of NO donors in atrial and ventricular fibres of the frog heart. *J Physiol* 518: 449–461, 1999.
9. Colleluori DM and Ash DE. Classical and slow-binding inhibitors of human type II arginase. *Biochemistry* 40: 9356–9362, 2001.
10. Cotton JM, Kearney MT, MacCarthy PA, Grocott-Mason RM, McClean DR, Heymes C, Richardson PJ, and Shah AM. Effects of nitric oxide synthase inhibition on basal function and the force-frequency relationship in the normal and failing human heart in vivo. *Circulation* 104: 2318–2323, 2001.
11. Cox JD, Kim NN, Traish AM, and Christianson DW. Arginase-boronic acid complex highlights a physiological role in erectile function. *Nat Struct Biol* 6: 1043–1047, 1999.
12. Dedkova EN, Wang YG, Blatter LA, and Lipsius SL. Nitric oxide signalling by selective β_2 -adrenoceptor stimulation prevents ACh-induced inhibition of β_2 -stimulated Ca^{2+} current in cat atrial myocytes. *J Physiol* 542: 711–723, 2002.
13. Di Costanzo L, Sabio G, Mora A, Rodriguez PC, Ochoa AC, Centeno F, and Christianson DW. Crystal structure of human arginase I at 1.29-Å resolution and exploration of inhibition in the immune response. *Proc Natl Acad Sci USA* 102: 13058–13063, 2005.
14. duBell WH and Houser SR. Voltage and beat dependence of Ca^{2+} transient in feline ventricular myocytes. *Am J Physiol Heart Circ Physiol* 257: H746–H759, 1989.
15. Elalamy I, Said FA, Singer M, Couetil JP, and Hatmi M. Inhibition by extracellular cAMP of phorbol 12-myristate 13-acetate-induced prostaglandin H synthase-2 expression in human pulmonary microvascular endothelial cells. Involvement of an ecto-protein kinase A activity. *J Biol Chem* 275: 13662–13667, 2000.
16. Flesch M, Kilter H, Cremers B, Lenz O, Sudkamp M, Kuhn-Regnier F, and Bohm M. Acute effects of nitric oxide and cyclic GMP on human myocardial contractility. *J Pharmacol Exp Ther* 281: 1340–1349, 1997.
17. Han X, Kubota I, Feron O, Opel DJ, Arstall MA, Zhao YY, Huang P, Fishman MC, Michel T, and Kelly RA. Muscarinic cholinergic regulation of cardiac myocyte I_{Ca-L} is absent in mice with targeted disruption of endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 95: 6510–6515, 1998.
18. Hare JM, Lofthouse RA, Juang GJ, Colman L, Ricker KM, Kim B, Senzaki H, Cao S, Tunin RS, and Kass DA. Contribution of caveolin protein abundance to augmented nitric oxide signaling in conscious dogs with pacing-induced heart failure. *Circ Res* 86: 1085–1092, 2000.
19. Harris DM, Mills GD, Chen X, Kubo H, Berretta RM, Votaw VS, Santana LF, and Houser SR. Alterations in early action potential repolarization causes localized failure of sarcoplasmic reticulum Ca^{2+} release. *Circ Res* 96: 543–550, 2005.
20. Harris SP, Patel JR, Marton LJ, and Moss RL. Polyamines decrease Ca^{2+} sensitivity of tension and increase rates of activation in skinned cardiac myocytes. *Am J Physiol Heart Circ Physiol* 279: H1383–H1391, 2000.
21. Ichinose F, Bloch KD, Wu JC, Hataishi R, Aretz HT, Picard MH, and Scherrer-Crosbie M. Pressure overload-induced LV hypertrophy and dysfunction in mice are exacerbated by congenital NOS3 deficiency. *Am J Physiol Heart Circ Physiol* 286: H1070–H1075, 2004.
22. Janssens S, Pokreisz P, Schoonjans L, Pellens M, Vermeersch P, Tjwa M, Jans P, Scherrer-Crosbie M, Picard MH, Szeldi Z, Gillijns H, Van de Werf F, Collen D, and Bloch KD. Cardiomyocyte-specific overexpression of nitric oxide synthase 3 improves left ventricular performance and reduces compensatory hypertrophy after myocardial infarction. *Circ Res* 94: 1256–1262, 2004.
23. Jung AS, Quaile MP, Mills GD, Bednarik DP, Houser SR, and Margulies KB. Pharmacological effects of ATI22–107 [2-(2-[2-(2-chloro-4-(6-oxo-1,4,5,6-tetrahydro-pyridazin-3-yl)-phenoxy]-acetylamino)-ethoxymethyl)-4-(2-chloro-phenyl)-6-methyl-1,4-dihydro-pyridine -3,5-dicarboxylic acid dimethyl ester)], a novel dual pharmacophore, on myocyte calcium cycling and contractility. *J Pharmacol Exp Ther* 312: 517–524, 2005.
24. Kim NN, Cox JD, Baggio RF, Emig FA, Mistry SK, Harper SL, Speicher DW, Morris SM Jr, Ash DE, Traish A, and Christianson DW. Probing erectile function: S-(2-boronoethyl)-L-cysteine binds to arginase as a transition state analogue and enhances smooth muscle relaxation in human penile corpus cavernosum. *Biochemistry* 40: 2678–2688, 2001.
25. Kojda G, Brixius K, Kottenberg K, Nix P, Schluter KD, Piper HM, and Noack E. The new NO donor SPM3672 increases cGMP and improves contraction in rat cardiomyocytes and isolated heart. *Eur J Pharmacol* 284: 315–319, 1995.
26. Kubo H, Margulies KB, Piacentino V III, Gaughan JP, and Houser SR. Patients with end-stage congestive heart failure treated with β -adrenergic receptor antagonists have improved ventricular myocyte calcium regulatory protein abundance. *Circulation* 104: 1012–1018, 2001.
27. Layland J, Li JM, and Shah AM. Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes. *J Physiol* 540: 457–467, 2002.
28. Massion PB, Feron O, Dessy C, and Balligand JL. Nitric oxide and cardiac function: ten years after, and continuing. *Circ Res* 93: 388–398, 2003.
29. Meurs H, Hamer MA, Pethe S, Vadon-Le Goff S, Boucher JL, and Zaagsma J. Modulation of cholinergic airway reactivity and nitric oxide production by endogenous arginase activity. *Br J Pharmacol* 130: 1793–1798, 2000.
30. Mohan P, Brutsaert DL, Paulus WJ, and Sys SU. Myocardial contractile response to nitric oxide and cGMP. *Circulation* 93: 1223–1229, 1996.
31. Nava E, Farre AL, Moreno C, Casado S, Moreau P, Cosentino F, and Luscher TF. Alterations to the nitric oxide pathway in the spontaneously hypertensive rat. *J Hypertens* 16: 609–615, 1998.
32. Nuss HB and Houser SR. Sodium-calcium exchange-mediated contractions in feline ventricular myocytes. *Am J Physiol Heart Circ Physiol* 263: H1161–H1169, 1992.
33. Nuss HB and Houser SR. Voltage dependence of contraction and calcium current in severely hypertrophied feline ventricular myocytes. *J Mol Cell Cardiol* 23: 717–726, 1991.
34. Paolucci N, Ekelund UE, Isoda T, Ozaki M, Vandegaer K, Georgakopoulos D, Harrison RW, Kass DA, and Hare JM. cGMP-independent inotropic effects of nitric oxide and peroxynitrite donors: potential role for nitrosylation. *Am J Physiol Heart Circ Physiol* 279: H1982–H1988, 2000.
35. Piech A, Dessy C, Havaux X, Feron O, and Balligand JL. Differential regulation of nitric oxide synthases and their allosteric regulators in heart and vessels of hypertensive rats. *Cardiovasc Res* 57: 456–467, 2003.
36. Sears CE, Bryant SM, Ashley EA, Lygate CA, Rakovic S, Wallis HL, Neubauer S, Terrar DA, and Casadei B. Cardiac neuronal nitric oxide synthase isoform regulates myocardial contraction and calcium handling. *Circ Res* 92: e52–e59, 2003.
37. Smith JA, Shah AM, and Lewis MJ. Factors released from endocardium of the ferret and pig modulate myocardial contraction. *J Physiol* 439: 1–14, 1991.
38. Takahashi A, Camacho P, Lechleiter JD, and Herman B. Measurement of intracellular calcium. *Physiol Rev* 79: 1089–1125, 1999.
39. Ventura C, Ferroni C, Flamigni F, Stefanelli C, and Capogrossi MC. Polyamine effects on $[Ca^{2+}]_i$, homeostasis and contractility in isolated rat ventricular cardiomyocytes. *Am J Physiol Heart Circ Physiol* 267: H587–H592, 1994.
40. Wollert KC and Drexler H. Regulation of cardiac remodeling by nitric oxide: focus on cardiac myocyte hypertrophy and apoptosis. *Heart Fail Rev* 7: 317–325, 2002.
41. Yan DH, Nishimura K, Yoshida K, Nakahira K, Ehara T, Igarashi K, and Ishihara K. Different intracellular polyamine concentrations underlie the difference in the inward rectifier K^+ currents in atria and ventricles of the guinea-pig heart. *J Physiol* 563: 713–724, 2005.
42. Zafeiridis A, Jeevanandam V, Houser SR, and Margulies KB. Regression of cellular hypertrophy after left ventricular assist device support. *Circulation* 98: 656–662, 1998.
43. Ziolo MT, Katoh H, and Bers DM. Expression of inducible nitric oxide synthase depresses beta-adrenergic-stimulated calcium release from the sarcoplasmic reticulum in intact ventricular myocytes. *Circulation* 104: 2961–2966, 2001.