MORPHOLOGY AND PATHOMORPHOLOGY

Alterative and Plastic Insufficiency of Cardiomyocytes: Isoproterenol-Induced Damage to Myocardium during Anthracycline Cardiomyopathy

E. L. Lushnikova, L. M. Nepomnyashchikh, and D. E. Semenov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 6, pp. 697-702, June, 2001
Original article submitted February 26, 2001

The development of regenerative and plastic myocardial insufficiency induced by anthracycline antibiotic rubomycin is accompanied by a decrease in cardiomyocyte sensitivity to damage produced by synthetic catecholamine isoproterenol. The incidence and the size of coagulation necrosis foci of cardiomyocytes developed 6 h after isoproterenol injection significantly decreased with increasing in the interval between rubomycin injection and subsequent administration of isoproterenol. In Wistar rats receiving rubomycin 3-5 days prior to isoproterenol and exhibiting signs of regenerative and plastic insufficiency, no cardiomyocyte contracture, intracellular myocytolysis, or lump degradation characteristic of cardiac insufficiency induced by endo- and exogenous catecholamines were found.

Key Words: *alterative myocardial insufficiency; regenerative and plastic myocardial insufficiency; anthracycline cardiomyopathy; cardiomyocytes; isoproterenol damage*

Investigation of structural mechanisms underlying the development of myocardial insufficiency induced by a wide spectrum of damaging factors allows to identify alterative and plastic insufficiency, the two basic types differing in the type of cardiomyocyte (CMC) damage, mechanisms of CMC death and elimination, regenerative potential of parenchymal cells, and the outcome of these damages [2,3,8]. During alterative myocardial insufficiency induced by ischemic and metabolic damages to CMC, the irreversible alterations arrest functional activity of some CMC and cause their death. These focal or microfocal changes are usually accompanied by CMC necrosis and the development of focal cardiosclerosis. Plastic myocardial insufficiency results from disturbances of intracellular CMC regeneration caused by inhibition or suppression of protein synthesis and cardiac decompensation due to imbalance between functional load and plastic processes in CMC. Changes in CMC during plastic insufficiency are diffuse and are manifested in progressive cell atrophy and apoptosis leading to diffuse cardiosclerosis [3-6].

Different mechanisms of alterative and plastic myocardial insufficiency and peculiarities of regeneration suggest that these forms of myocardial insufficiency do not occur simultaneously. However, this point is little studied. It is of theoretical and practical importance to study the possibility of combination of these forms of myocardial insufficiency caused by combined or alternating action of factors inducing alterative and plastic insufficiency of CMC.

Our aim was to study the morphological changes in rat CMC induced by a cardiotoxic dose of isoproterenol administered during anthracycline cardiomyopathy.

Department of General Pathology and Pathomorphology, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** pathol@cyber.nsk.ma.ru. Lushnikova E. L.

MATERIALS AND METHODS

Anthracycline cardiomyopathy was modeled in 35 Wistar rats (body weight 180-200 g) by intraperitoneal injection of rubomycin hydrochloride (30 mg/kg). Isoproterenol (0.8 ml/100 g, 1% solution) was injected subcutaneously 1-24 h or 2-5 days after rubomycin. The control group (n=16) consisted of intact rats injected with isoproterenol in the same dose. The rubomycin-treated rats were decapitated 6 h after isoproterenol injection, while controls were sacrificed 30 min-6 h after injection of isoproterenol.

The hearts of all rats were examined under light and polarization microscopes [7]. The myocardial specimens were fixed in 10% neutral formalin. Deparaffinized sections were stained with hematoxylin and eosin, colloidal iron-PAS-hematoxylin, and by the method of van Gieson. The sections were examined under a Docuval universal light microscope.

RESULTS

In the myocardium of intact rats the toxic dose of exogenous catecholamine isoproterenol induced the formation of multiple necrotic foci. Most of these foci were small and cjnsisted of no more than 2-10 cells. In the period from 30 min to 2 h postinjection, there were acute myocardial hemodynamic disturbances manifested in venous and capillary plethora, pronounced edema in intermuscular connective tissue, and stasis of erythrocytes. On minute 30 postinjection, most myofibrils retained their normal structure. In cells intensively stained with acid dyes, anisotropy of A-disks in polarized light increased, while isotropic disks remained changed or became shorter, which attested to I and II degree contracture (Fig. 1). Two hours after the start of the experiments, myofibrils with III degree contracture damages and lump degradation of myofibrils were more often seen in all rats (Fig. 2, *a*, *c*).

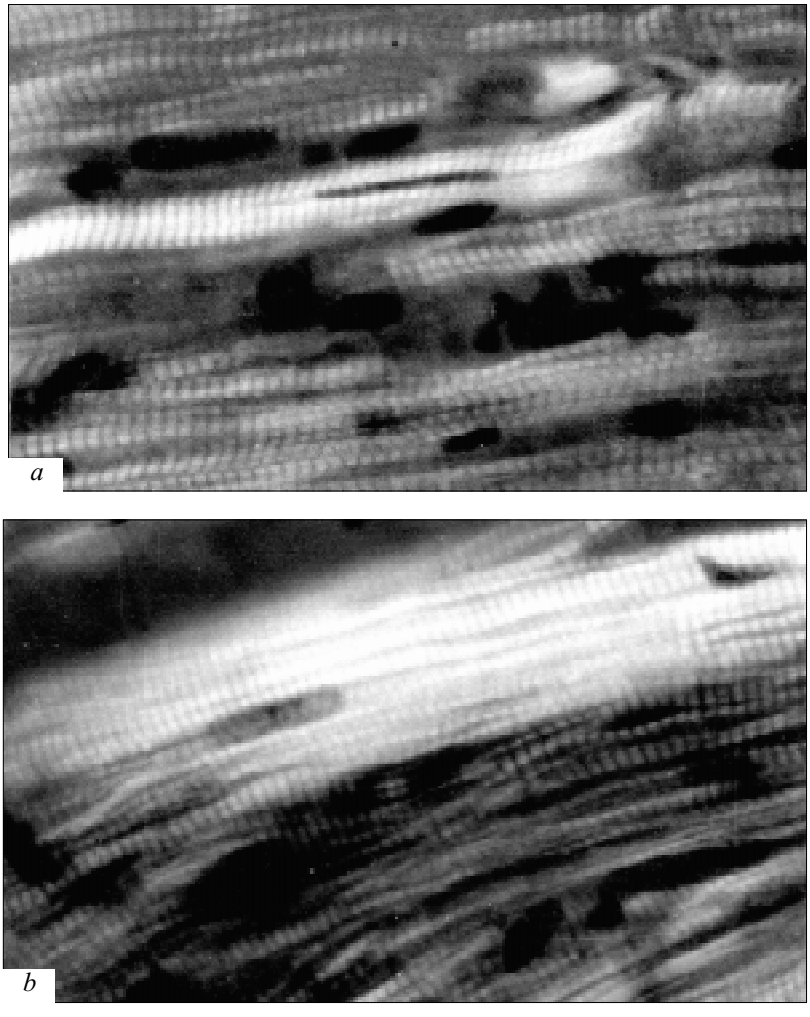

Fig. 1. Isoproterenol-induced damage to rat myocardium 30 min postinjection. Polarization microscopy, ×800. *a*) cardiomyocyte (in the center) with I degree contracture: enhanced anisotropy of A-disks, isotropic disks are unchanged; *b*) II degree contracture in damaged cell: enhanced anisotropy of A-disks, and shortening of isotropic disks.

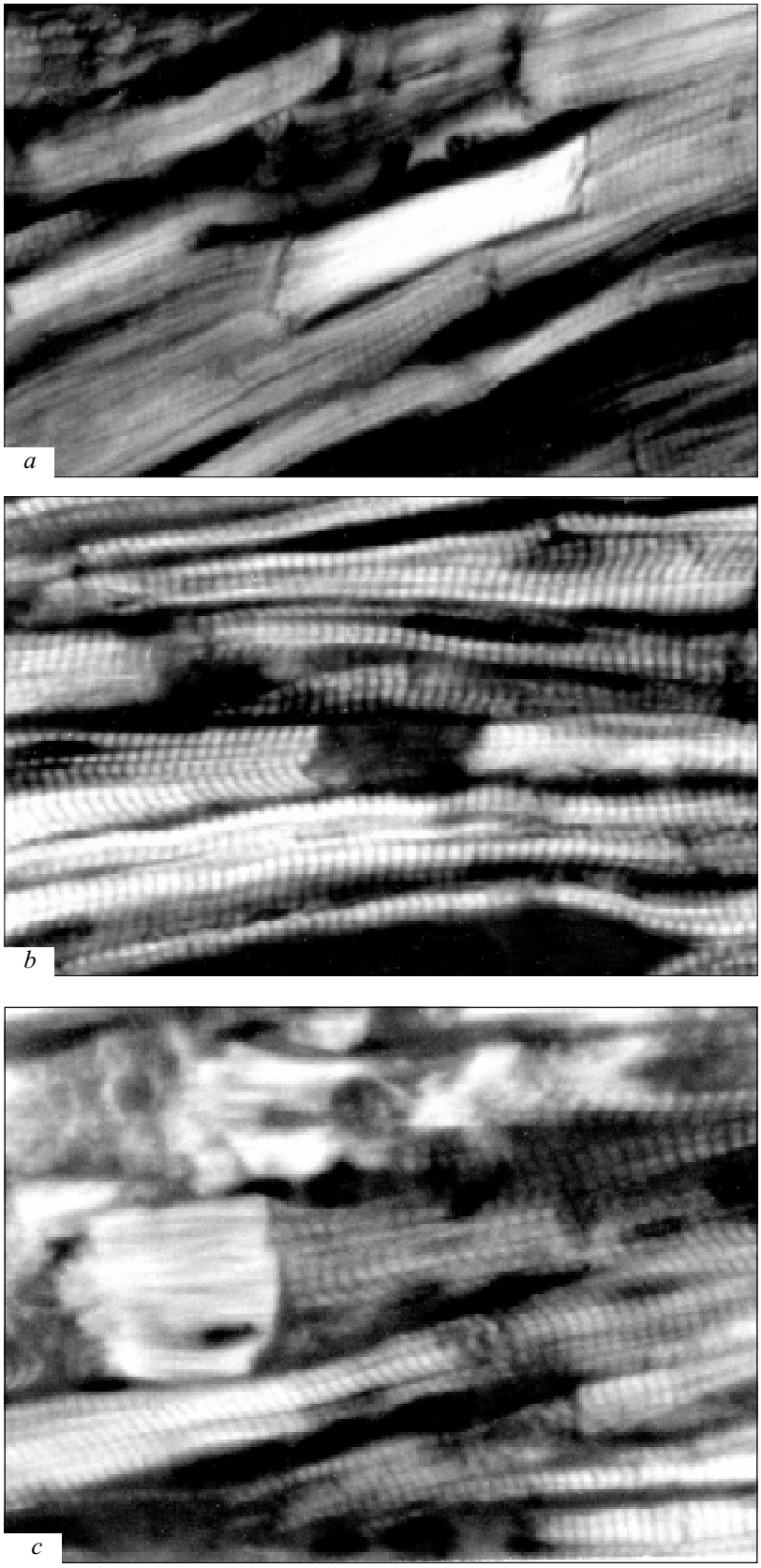

Fig. 2. Isoproterenol-induced damage to rat myocardium 2 h postinjection. Polarization microscopy, ×800. *a*) III degree contracture: increased anisotropy in damaged cardiomyocyte is accompanied by complete disappearance of isotropic disks; *b*) intracellular myocytolysis: the damage is surrounded by A-disks of intact myofibrils; *c*) III degree contracture and lump degradation of myofibrils in damaged cardiomyocytes.

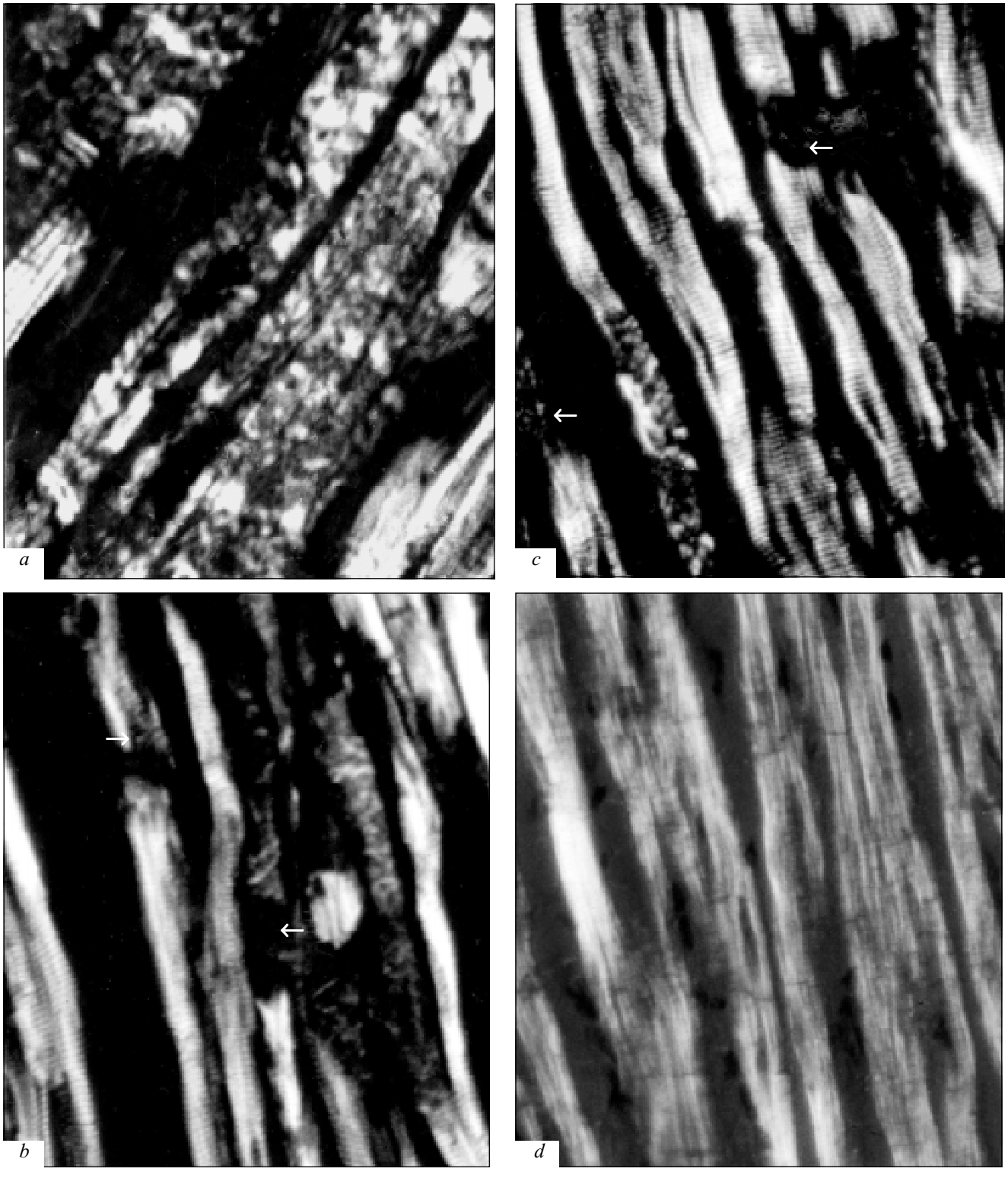

Fig. 3. Isoproterenol-induced damage to rat myocardium during anthracycline cardiomyopathy. Polarization microscopy, ×800. *a*) multiple foci of lump degradation of myofibrils (coagulation necrosis) 6 h after isoproterenol injection; *b*) middle-size foci of coagulation necrosis and individual cells with myocytolysis (arrows) 30 h after injection of rubomycin (30 mg/kg) and 6 h after injection of isoproterenol; *c*) shrinkage of coagulation necrosis foci with signs of myocytolysis (arrows) 54 and 6 h after injections of rubomycin and isoproterenol, respectively; *d*) the absence of acute focal alterations in cardiomyocytes 78 and 6 h after injection of rubomycin and isoproterenol, respectively.

In the last case, diffuse PAS-positive reaction was revealed in the myofibrils, which could not be prevented by amylase treatment. In the same period, individual CMC with intracellular myocytolysis were observed. This is a specific type of acute pathology characterized by local myofibril lysis. In addition, the large foci of intracellular myocytolysis had no anisotropic structures, and these foci were empty in polarized light (Fig. 2, *b*).

Six hours after isoproterenol injection all rats had small coagulation necrotic foci of CMC. In polarized light, myofibrils of damaged CMC had multiple light-polarizing lumps attesting to lump degradation (Fig. 3, *a*). Sarcoplasm of these CMC was intensively stained during PAS-reaction, which attested to penetration of extracellular proteins into the cells (plasma impregnation). In the same term, the myocardium of some rats demonstrated foci of intracellular myocytolysis apart from lump degradation. Therefore, the cardiotoxic doses of isoproterenol induced damage to CMC characteristic of alterative myocardial insufficiency.

The incidence and size of coagulation necrosis foci developed 6 h after isoproterenol injection markedly decreased with increasing the interval between rubomycin and isoproterenol injections (*i.e.*, with progression of regenerative and plastic myocardial insufficiency). This decrease was most pronounced on days 1-2 after rubomycin injection (Fig. 3, *b*), while on days 3-5 postinjection most rats had no necrotic and myocytolysis foci (Fig. 3, *c*, *d*). In other words, the development of plastic insufficiency in CMC was accompanied by pronounced decrease of CMC vulnerability to damages produced by exogenous synthetic catecholamine isoproterenol.

This parallelism between the development of plastic insufficiency and reduced (up to complete disappearance) sensitivity to catecholamines in cardiotoxic doses suggests that inhibition of protein synthesis is accompanied by changes in either β-adrenoceptors system (specific target for isoproterenol) or in G-protein-adenylate cyclase complex, which transmits the signals inside the cell. According to current views, the interaction between isoproterenol and β-receptor on cell membrane activates adenylate cyclase, thereby increasing intracellular cAMP level. cAMP-dependent protein kinase A activates L-type Ca^{2+} channels, which leads to accumulation of Ca^{2+} in the cytosol [11,14, 15]. Catecholamines in high doses increase CMC membrane permeability, which results in the development of coagulation necrosis [1,8].

Under conditions of anthracycline cardiomyopathy, high doses of isoproterenol moderately stimulated contractile activity of the myocardium. This was evidenced by improvement of general physiological state of experimental animals and a decrease of microscopic signs of circulatory disturbances in rats decapitated 6 h after isoproterenol injection. There is evidence that during acute insufficiency the baseline and stimulated levels of adenylate cyclase in the left and right ventricles markedly decreased in comparison with the control [16]. However, under these conditions the density of β-adrenoceptors in CMC and their affinity do not vary significantly. In addition, during the development of acute myocardial insufficiency induced by isoproterenol, contractility of trabeculae in the right ventricle also considerably decreased. All these data indicate that acute myocardial insufficiency is accompanied by desensitization of β-adrenoceptors due to uncoupling in the signal transduction chain. During chronic myocardial insufficiency (for example, during idiopathic dilatation cardiomyopathy) similar disturbances in coupling between β-adrenoceptors with G-proteins [9] do not occur in CMC. By contrast, the major role in the genesis of chronic myocardial insufficiency is played by growth of sympathetic activity and CMC apoptosis mediated via β-adrenoceptor pathway [10,12,13].

Our findings do not attest to specific tolerance of CMC to damaging effects of catecholamines during anthracycline cardiomyopathy. Probably, these data reflect a general biological relationship. Parallelism probably exists between CMC sensitivity to catecholamines and the level of protein synthesis in these cells. Long-term suppression of protein synthesis in CMC can protect the heart against acute damage caused by endogenous catecholamines, in particular, during stress.

REFERENCES

1. L. M. Nepomnyashchikh, *Morphogenesis of Major Pathological Processes in the Heart* [in Russian], Novosibirsk (1991).
2. L. M. Nepomnyashchikh, *Alterative Insufficiency of Cardiomyocytes during Metabolic and Ischemic Damages* [in Russian], Moscow (1998).
3. L. M. Nepomnyashchikh, *Regenerative and Plastic Insufficiency of Cardiomyocytes during Impaired Protein Synthesis* [in Russian], Moscow (1998).
4. L. M. Nepomnyashchikh, E. L. Lushnikova, and D. E. Semenov, *Byull. Eksp. Biol. Med.,* **130**, No. 10, 463-468 (2000).
5. L. M. Nepomnyashchikh and D. E. Semenov, *Ibid.,* No. 9, 336-341.
6. D. E. Semenov, L. A. Semenova, L. M. Nepomnyashchikh, and Yu. G. Tsellarius, *Ibid*., **97**, No. 5, 629-633 (1984).
7. L. A. Semenova, L. M. Nepomnyashchikh, and D. E. Semenov, *Morphology of Plastic Cardiomyocyte Insufficiency* [in Russian], Novosibirsk (1985).
8. Yu. G. Tsellarius and L. A. Semenova, *Histopathology of Focal Metabolic Damage to Myocardium* [in Russian], Novosibirsk (1972).

9. M. R. Bristow, F. L. Anderson, J. D. Port, *et al., Circulation,* **84**, 1024-1039 (1991).
10. W. S. Colucci, *Clin. Cardiol.,* **21**, 120-124 (1998).
11. C. Communal, K. Singh, D. B. Sawyer, and W. S. Colucci, *Circulation*, **100**, 2210-2212 (1999).
12. S. Engelhardt, L. Hein, F. Wiesmann, and M. J. Lohse, *Proc. Natl. Acad. Sci. USA,* **96**, 7059-7064 (1999).
13. J. D. Port, H. D. Weinberger, J. D. Bisognano, *et al., Am. J. Coll. Cardiol.*, **31**, 177A (1998).
14. S. Saito, Y. Hiroi, Y. Zou, *et al., J. Biol. Chem.*, **275**, 34528-34533 (2000).
15. K. Singh, C. Communal, D. B. Sawyer, and W. S. Colucci, *Cardiovasc. Res.,* **45**, 713-719 (2000).
16. M. White, R. J. Wiechmann, R. L. Roden, *et al., Circulation*, **92**, 2183-2189 (1995).