Chronic β-Adrenergic Stimulation Induces Myocardial Proinflammatory Cytokine Expression

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**Background**—The sympathetic nervous system and proinflammatory cytokines are believed to play key roles in the pathophysiology of congestive heart failure. To evaluate a possible relationship between these neurohormonal systems, we studied the effects of chronic β-adrenergic stimulation on the myocardial and systemic elaboration of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.

**Methods and Results**—Male rats received either L-isoproterenol (2.4 mg·kg⁻¹·d⁻¹, n=8) or saline (n=7) via miniosmotic pumps for 7 days. Myocardial cytokine expression was analyzed by both Northern and Western blotting and localized in the tissue using immunohistochemistry. ELISA was performed to measure circulating levels of cytokines. In myocardium from control animals, neither TNF-α nor IL-1β were detected, whereas IL-6 was present at very low levels. Isoproterenol led to a significant (P<0.01) increase in mRNA and protein expression of all 3 cytokines. Immunohistochemistry did not detect immunoreactivity for either cytokine in myocardium from controls; however, all 3 cytokines were readily detected (P<0.05) throughout the myocardium, localized to resident cells and vessels, in animals treated with isoproterenol. Neither treatment group had detectable levels of cytokines in the serum.

**Conclusions**—Chronic β-adrenergic stimulation induces myocardial, but not systemic, elaboration of TNF-α, IL-1β, and IL-6. (Circulation. 2000;101:2338-2341.)

**Key Words:** isoproterenol ■ tumor necrosis factor-α ■ interleukins ■ sympathetic nervous system ■ myocardium
ing cDNA (American Type Culture Collection) and oligonucleotide probes were used: hTNF-α, mIL-1β, mIL-6. h28S rRNA (40 base single stranded oligo; Oncogene Science) was used as an internal control, with results expressed as a ratio of the specific gene to the corresponding 28S rRNA. For Western blots, rabbit anti–rat IL-1β, IL-6, and TNF-α antibodies (Biosource International) were used at a concentration of 3 (IL-1β, TNF-α) and 5 μg/mL (IL-6). Prestained protein molecular weight standards (Broad range; Bio-Rad Labs) were run simultaneously with protein homogenates.

**Localization of Proinflammatory Cytokines by IHC**

Five-micrometer-thick cryosections were used for immunostaining using a commercially available kit (Vectastain ABC Elite Kit, Vector Laboratories). IHC was performed as described earlier. Omission of primary antibody and rabbit preimmune serum in place of primary antibody served as controls. For IHC, rabbit anti–rat IL-1β and IL-6 (as described above) and anti–TNF-α antibodies (Serotec) were used at a concentration of 2 (TNF-α) or 3 (IL-1β, IL-6) μg/mL. IHC staining intensity was evaluated in a blinded manner by light microscopy and graded on a semiquantitative scale from 0 to 3 (0 indicates none; 1, weak; 2, intermediate; 3, strong).

**Serum Cytokine Levels**

Serum TNF-α (sensitivity <0.7 ng/mL), IL-1β (sensitivity <3.0 pg/mL), and IL-6 (sensitivity <8.0 pg/mL) levels were measured by ELISA using commercially available kits (Biosource International). Studies were performed as per the manufacturer’s instructions. Interpretation was done by an observer blinded to treatment group.

**Statistical Analysis**

Data shown are mean±SD. Data were subjected to ANOVA with Student t test for significance. Correction for multiple comparisons were made using the Bonferroni factor. Probability values of 0.05 or less were considered significant.

**Results**

**Effects of β-Adrenergic Stimulation on Hemodynamics and Heart Weights**

Although isoproterenol did not affect systolic or diastolic blood pressure at either 3 or 7 days of treatment, heart rate increased significantly (Table 1). Isoproterenol-treated animals exhibited significantly greater heart weights, as well as increased heart/body weight ratios at the time of euthanasia (Table 2). Isoproterenol did not influence body weight over the 7 days of treatment (data not shown).

**Histology and Histomorphometry**

Hematoxylin-eosin staining was used to evaluate the extent of myocardial inflammation. Isoproterenol induced myocardial inflammatory cell infiltration, predominantly in the subendocardium. Mononuclear cell infiltrates ranged from isolated

| TABLE 1. Influence of Isoproterenol on Blood Pressure and Heart Rate |
|------------------------|------------------------|------------------------|
|                        | Control                | Isoproterenol          |
|                        | Baseline               | Day 3                  | Day 7                  |
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| BPsyst                 | 126.4±28.2             | 132.9±21.6             | 134.2±14.2             |
| BPsyst                 | 73.5±21.1              | 62.7±14.0              | 68.7±14.5              |
| HR                     | 386.7±31.1             | 413.9±50.5             | 439.4±34.4*            |

*P<0.005, †P<0.0001 vs respective baseline; ‡P<0.01 vs respective control.

| TABLE 2. Impact of Isoproterenol on Heart Weight |
|------------------------|------------------------|
|                        | Control                | Isoproterenol          |
| Heart wt               | 1.19±0.14              | 1.41±0.13†             |
| LV wt                  | 0.48±0.13              | 0.68±0.16*             |
| RV wt                  | 0.22±0.03              | 0.27±0.04*             |
| Heart/body             | 0.32±0.04              | 0.39±0.02†             |

LV indicates left ventricle; RV, right ventricle; wt, weight. Ratios reflect respective weights.

*P<0.01, †P<0.005 vs respective control.
and focal in some areas to confluent in others. Inflammation was not observed in saline controls (data not shown).

**β-Adrenergic Stimulation Induces Proinflammatory Cytokine Expression**

The results of Northern and Western blotting are shown in Figure 1. In control myocardium, TNF-α and IL-1β were not detected, and IL-6 was only detected at low levels (densitometry; mRNA, 0.18±0.021; protein, 50±2). Isoproterenol infusion significantly increased expression of all 3 cytokines (mRNA: TNF-α, 0.89±0.46, P<0.01; IL-1β, 0.42±0.026, P<0.01; IL-6, 0.64±0.14, P<0.0001) (protein: TNF-α, 301±60.6, P<0.01; IL-1β, 152±45.3, P<0.01; IL-6, 225±60.6, P<0.0001), indicating that chronic β-adrenergic stimulation was associated with proinflammatory cytokine expression.

**Figure 2.** A representative photomicrograph showing localization of IL-1β, IL-6, and TNF-α immunoreactivity in myocardium. Brown stain indicates positive immunoreactivity. Counterstain was performed by methyl green. No positive immunoreactivity was detected for either IL-1β (A), IL-6 (B), or TNF-α (C) in saline-injected animals. However, intense immunoreactivity was detected in isoproterenol administered animals for all 3 cytokines (D, IL-1β; E, IL-6; F, TNF-α). Omission of primary antibody, rabbit preimmune serum in place of primary antibody, and primary antibody after its neutralization with respective peptide antigen served as controls and demonstrated no positive immunoreactivity for either cytokine in myocardium from isoproterenol administered animals (data not shown). Magnification ×250.
IHC revealed no positive immunoreactivity for either cytokine in control myocardium (Figure 2). However, in isoproterenol-treated animals, all 3 cytokines were readily detected and were localized to the cytoplasm of myocardial cells and blood vessels in a diffuse manner (immunoreactivity scores: TNF-α, 2.0±0.88; IL-1β, 1.875±0.79; IL-6, 2.44±0.62; all p<0.05). Importantly, IHC staining was not noted in areas of inflammatory cell infiltration but rather in the myocardial tissue distinct from these areas.

In contrast, ELISA revealed no detectable amounts of either cytokine in serum in both saline and isoproterenol-treated animals at any timepoint.

Discussion

The results of this study demonstrate for the first time that chronic β-adrenergic stimulation with isoproterenol leads to myocardial gene expression and protein production of TNF-α, IL-1β, and IL-6. This appears to be a local effect, as there was no identifiable spillover of cytokines into the systemic circulation. The lack of systemic spillover is consistent with clinical studies that have indicated a lack of correlation between circulating TNF-α and norepinephrine levels in HF. Additionally, our results confirm the findings of previous studies showing that isoproterenol promotes myocardial hypertrophy while producing infarcitke lesions characterized by myocyte necrosis, myofibrillar degeneration, and leukocyte infiltration.

The pathogenesis of catecholamine-induced cardiac toxicity has yet to be fully defined. Mechanisms previously proposed include the following: myocardial necrosis and apoptosis, ischemia-reperfusion injury and free radical generation, and cAMP-dependent calcium overload of the cardiac myocyte. Whether β-adrenergic activation induces myocardial expression of TNF-α, IL-1β, and IL-6 directly via cAMP generation or indirectly via increased heart rate, myocardial ischemia, hypertrophy, free radical generation, or calcium overload is not clear. Regardless, the interplay between catecholamine stimulation and proinflammatory cytokines may have implications in regards to adverse chamber remodeling after myocardial injury. Our laboratory has recently shown that the salutary effects of β-adrenergic blockade on left ventricular dilation and hypertrophy after myocardial infarction were accompanied by selective reductions in myocardial expression of TNF-α and IL-1β, cytokines known to impair contractile function while promoting chamber enlargement. Thus, proinflammatory cytokines may play a significant role in the development of β-adrenergic cardiac toxicity.

Given that cytokines such as TNF-α can stimulate neutrophil migration, myocyte adherence, free radical production, and phagocytosis, we cannot determine from our study whether inflammatory cell infiltration is a cause or an effect of cytokine production. However, TNF-α, IL-1β, and IL-6 immunoreactivity after isoproterenol was not confined to regions of inflammatory cell infiltration but rather found diffusely in myocardial tissue throughout the ventricle. This suggests either direct expression of cytokines by myocytes and blood vessels or paracrine effects. The lack of change in systemic blood pressure during isoproterenol infusion suggests that changes in wall stress per se did not play a major role in inducing myocardial cytokine expression. However, as we did not measure chamber size in this study, this cannot be excluded as a contributing factor.

In summary, our study confirms that constant β-adrenergic stimulation serves as a stimulus for local myocardial expression of TNF-α, IL-1β, and IL-6 unaccompanied by systemic elaboration. Although this was associated with myocardial inflammatory cell infiltration, generation of these cytokines occurs diffusely in myocardium, including areas remote from inflammation. Thus biological “cross-talk” exists between these 2 key neurohormonal systems in myocardium, and this interplay may play an important role in the pathogenesis of HF. Further studies need to be performed to delineate the mechanism of isoproterenol-induced myocardial proinflammatory cytokine gene expression.

Acknowledgments

This work was supported by a Grant-in-Aid (D.R.M) and an Established Investigator grant (S.D.P) from the American Heart Association and the Research Service of the Department of Veterans Affairs (S.D.P). The authors gratefully acknowledge the excellent technical assistance of Teri Frosto.

References

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Circulation. 2000;101:2338-2341
doi: 10.1161/01.CIR.101.20.2338

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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