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Isoproterenol Inhibits Transcription of Cardiac Cytokine Genes Induced by Reactive Oxygen Intermediates

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Background: **Cytokines such as tumor necrosis factor α (TNF-α) are produced by the myocardium in heart disease and might be stimulated by reactive oxygen. In some cell types, cyclic adenosine monophosphate (AMP) inhibits TNF-α production. The authors tested the hypothesis that stimulation of cardiac β-adrenergic receptors would inhibit cytokine gene transcription induced by reactive oxygen.**

Methods: **Rat hearts were perfused with buffer containing hypoxanthine. Reactive oxygen intermediates were generated by infusion of xanthine oxidase. Myocardial mRNA encoding 11 cytokines was determined. TNF-α, interleukin-6, and cyclic AMP were measured in the coronary effluent.**

Results: **In control hearts, of the screened RNA, only mRNA encoding interleukin-1β, -4, and -6 was detected. Stimulation with hypoxanthine–xanthine oxidase (HX–XO) induced detectable mRNA for TNF-α and interleukin-5 and increased mRNA band density for interleukin-1β, -4, and -6. Simultaneous infusion of isoproterenol inhibited HX–XO-stimulated cytokine gene expression and caused release of cyclic AMP into the coronary effluent. In control hearts, TNF-α was not detected in the coronary effluent. After HX–XO administration, TNF-α was reliably detected at 60 min and interleukin-6 at 90 min. Simultaneous infusion of isoproterenol inhibited TNF-α and interleukin-6 release. Inclusion of propranolol in the perfusion buffer blocked the isoproterenol-induced inhibition of HX–XO-stimulated TNF-α release and release of cyclic AMP into the coronary effluent. In addition, elevating myocardial cyclic AMP with forskolin also blocked release of TNF-α stimulated by HX–XO. Finally, delaying infusion of isoproterenol until 30 min after HX–XO administration still suppressed release of TNF-α.**

Conclusions: **Reactive oxygen species activate cytokine gene transcription in the myocardium. The sympathetic nervous system, acting through β-receptors to elevate myocardial cyclic AMP, regulates cardiac cytokine production by inhibition of transcription.**

MYOCARDIAL production of inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 appears to be triggered by events associated with myocardial ischemia. For instance, TNF-α is elevated in the plasma of patients with unstable angina and after myocardial infarction. The concentration is correlated with the extent of infarction, with complications such as rhythm disturbances, with the development of heart failure, and with creatinine kinase concentrations.[1–5] Elevated interleukin-6 has been reported in patients with unstable angina and with uncomplicated myocardial infarction.[6–8] Furthermore, both TNF-α and interleukin-6 are expressed in the myocardium and released by the heart after aortic declamping after cardiopulmonary bypass.[9–12] The precise stimulus for production of these cytokines during ischemia and how myocardial production is regulated is currently not understood.

Myocardial infarction, unstable angina, and aortic cross-clamp– declamping are associated with episodes of ischemia–reperfusion during which reactive oxygen intermediates are generated.[13–15] Oxidation of purines by xanthine oxidase (XO) during ischemia–reperfusion can produce superoxide, hydroxyl species, and hydrogen peroxide.[16] In this regard, perfusion of isolated rat hearts with buffer containing hydrogen peroxide induced the expression of TNF-α in myocardial tissue.[17] Furthermore, in rats, myocardial ischemia–reperfusion activated nuclear regulatory factor κB (NF-κB), an immediate response element regulating TNF-α gene transcription,[18] and resuscitation from hemorrhagic shock caused both activation of NF-κB and expression of TNF-α in the heart.[19] In addition, we found that treating coronary smooth muscle cells with the reactive oxygen intermediates generating system, hypoxanthine–XO (HX–XO), activated NF-κB and stimulated release of TNF-α.[20] Thus, reactive oxygen intermediates may initiate myocardial expression of inflammatory cytokines through a mechanism dependent on the activation of NF-κB.

Studies of the regulation of TNF expression in macrophages and monocytes indicate that increased intracellular cyclic adenosine monophosphate (AMP) decreased bacterial lipopolysaccharide-stimulated TNF production.[21,22] In minced ventricular tissue from rat hearts, in papillary muscle, and in isolated myocytes, agents that increase cyclic AMP, such as phosphodiesterase inhibitors, adenosine, and forskolin, have also been shown to inhibit TNF-α release.[23–26] We previously reported that lipopolysaccharide stimulated release of TNF-α from human atria and rat heart, and that this release could be inhibited by treatment with isoproterenol.[27,28] Therefore, it seems possible that TNF-α production by the heart might be regulated, at least in part, by sympathetic

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nervous stimulation of cardiac β-adrenergic receptors and the consequent increase in myocardial cyclic AMP. In the current study, we determined if reactive oxygen intermediates would stimulate expression of inflammatory cytokines in the myocardium and tested the hypothesis that stimulating cardiac β-adrenergic receptors with isoproterenol would inhibit this expression.

Materials and Methods

Materials

Rat recombinant TNF-α was purchased from Genzyme Diagnostics (Cambridge, MA). XO, hypoxanthine, and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Murine fibroblast L929 cells were purchased from American Type Culture Collection (Rockville, MD). Cell culture plastic ware was obtained from Costar (Cambridge, MA).

Isolated Rat Heart

After obtaining approval from the Institutional Animal Care and Use Committee of Mercer University School of Medicine, male Sprague-Dawley rats (weight, 275–445 g) were anesthetized with pentobarbital. The hearts were removed and perfused Langendorf style at a constant pressure of 90 cm H_2O with Krebs-Henseleit buffer at 37°C and gassed with 95% O_2–5% CO_2, pH = 7.4. The content of the buffer was 118 mM NaCl, 4.7 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, and 2 mM $CaCl_2$. All hearts were equilibrated on the perfusion apparatus for 30 min. For the generation of reactive oxygen species, 1 mM hypoxanthine was added to the buffer and, using a syringe pump, XO was infused to produce a final concentration in the perfusing buffer of 0.004 units/ml. In other experiments, isoproterenol (1 μM for 60 min) was either simultaneously infused with the XO or started 30 min after the initiation of the XO infusion (fig. 1). After equilibration, timed samples of coronary effluent were collected immediately before beginning infusion (time 0) and again at 30, 60, 90, 120, and 150 min for measurement of coronary flow and assay for cyclic AMP, TNF-α, and interleukin-6. In addition, to determine the role of cyclic AMP in cytokine release, the β-adrenergic receptor blocker propranolol (0.1 μM) was added to the perfusion buffer containing hypoxanthine, and then XO and isoproterenol were infused. Finally, to elevate myocardial cyclic AMP by a mechanism different from activation of the β-adrenergic receptor, forskolin (10 μM) was infused, and the effect on HX–XO-stimulated cyclic AMP and TNF-α release was determined.

Assay for Tumor Necrosis Factor-α

Biologically active TNF-α in the coronary effluent was measured by a cell cytotoxicity assay using murine fibroblast L929 cells as described previously.[29] L929 cells were seeded into 96-well microtiter plates at an initial density of 5.0×10^4 cells per well in 100 μl of Dulbecco modified Eagle medium plus 5% fetal bovine serum and antibiotics and incubated for 24 h at 37°C in 5% CO_2. After incubation, the medium was removed, and 50 μl of Dulbecco modified Eagle medium with 5% fetal bovine serum containing 20 μg/ml actinomycin D (final concentration, 5 μg/ml) was added to all wells. In each plate, a standard curve was determined with serial dilutions of rat recombinant TNF-α. Test coronary effluent (150 μl) was added to the remaining wells in each 96-well plate. The plates were incubated for 18 h, and the medium was decanted. The remaining viable L929 cells were stained with 50 μl per well of 0.1% crystal violet in 20% ethanol, rinsed with phosphate-buffered saline, and air-dried. Methanol (100 μl) was then added to each well 5 min before reading to solubilize the dye. The optical density of each well was then read on an automated microplate reader Elx 800 (Bio-Tek Instrument, Inc., Winooski, VT) at 595 nm. TNF-α data are expressed as release in picograms per minute, determined as the concentration of TNF-α in the coronary effluent multiplied by the flow (picograms per minute

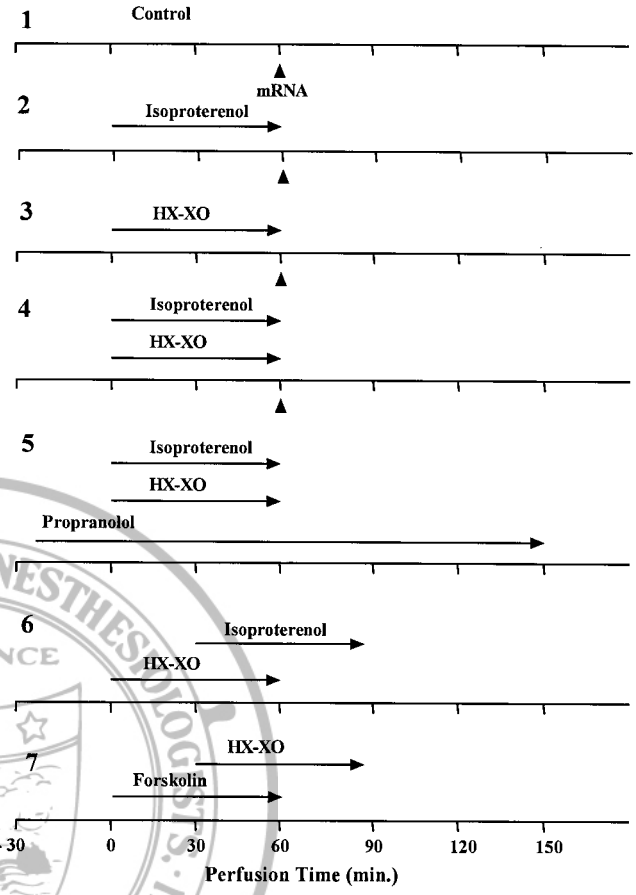

Fig. 1. Protocols used in isolated heart perfusions. HX = hypoxanthine; XO = xanthine oxidase.

times milliliter per minute). To determine if the cytotoxicity in the L929 assay was caused by TNF-α, a guinea pig antimouse TNF-α antiserum in a final dilution of 1:500 was added to selected samples of coronary effluent before L929 assay. All test agents, hypoxanthine, XO, isoproterenol, propranolol, and forskolin, were added directly into the L929 cell assay. All were without effect on the assay.

Slot Blot Analysis for Interleukin-6

Coronary effluent (800 μl) collected at 90 min was concentrated using a Microcon centrifugal filter tube with a nominal molecular weight cutoff of 3,000 (Millipore, Bedford, MA). Equal amounts of samples were slot blotted to supported nitrocellulose membranes by vacuum transfer. The membranes were also reversibly stained with Ponceau S to reconfirm equivalence of sample loading. After blocking with 5% nonfat milk, the membranes were incubated with a monoclonal antirat interleukin-6 antibody (R&D Systems Inc., Minneapolis, MN) and followed by horse radish peroxidase (HRP) conjugated secondary antibody (Amersham, Piscataway, NJ). The binding of antibody was detected by enhanced chemiluminescence.

Measurement of Cyclic Adenosine Monophosphate

For the measurement of cyclic AMP released from the heart, 1.8-ml aliquots of coronary effluent were collected into 200 μl 1N HCl at 0, 1, 2, 3, 4, 5, 10, 20, 30, 60, 90, 120, and 150 min. Cyclic AMP was determined by radioimmunoassay as described previously.[30] Data are expressed as release in picomoles per minute, determined as the concentration of cyclic AMP in the coronary effluent multiplied by the flow (picomoles per minute times milliliters per minute).

RNase Protection Analysis

Five hearts in each of the groups (control, HX–XO, HX–XO plus isoproterenol, and isoproterenol alone) were perfused for 60 min after a 30-min equilibration period. The hearts were removed from the perfusion apparatus, and the atria were trimmed away. Ventricular tissue was cut into approximately 2-mm cubes and stored in 5 ml RNA/later® fluid (Ambion, Austin, TX) before extraction of RNA. Antisense RNA probes labeled with [α-^{32}P] uridine triphosphate (UTP) were synthesized under the direction of T7 polymerase using DNA templates of rat cytokines, rCK1 multiprobe template set (Pharmingen, San Diego, CA), and purified by ammonium acetate–ethanol precipitation. The templates contained in the set included interleukin-1α and -1β, TNF-β, interleukin-3, -4, -5, -6, and -10, TNF-α, interleukin-2, INFγ, and the housekeeping genes, *L32* and *GAPDH*. Total cellular RNA was prepared from ventricular tissue homogenates by acid guanidium isothyocynate-phenol-chloroform extraction as previously described.[31] Determination of target mRNA was conducted using the Direct Protect® kit (Ambion) according to the manufacturer's instructions. Protected fragments of mRNA were separated by 5% polyacrylamide–8 M urea gel electrophoresis and exposed to x-ray film. Film was digitized, and integrated band density was analyzed using the NIH IMAGE program (written by Wayne Rasband, M.S., Computer Science, Research Service Branch, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/nih-image/index.html). Values for band density are expressed as the ratios of interleukin-1β, -4, and -6 band densities to that of the housekeeping gene, *L32*.

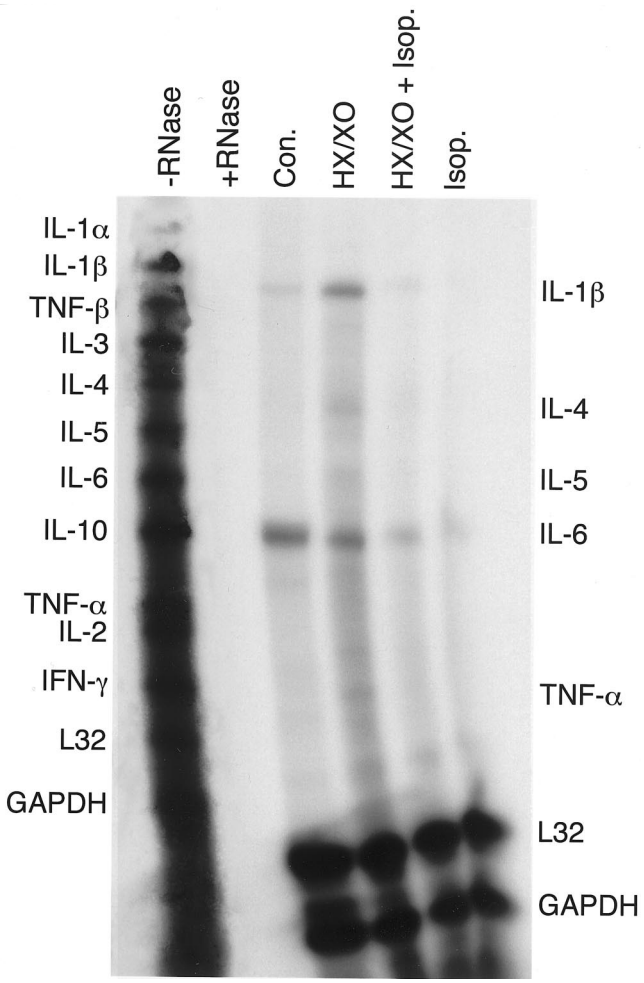

Fig. 2. Isoproterenol suppression of hypoxanthine–xanthine oxidase (HX–XO)-stimulated myocardial cytokine gene transcription. RNase protection assay typical of five experiments. In this experiment, only mRNA encoding interleukin (IL)-1β and IL-6 was detected in homogenates of ventricular tissue from control hearts (Con). HX–XO increased mRNA encoding for IL-1β, IL-4, IL-5, IL-6, and tumor necrosis factor α (TNF-α) (HX–XO). When isoproterenol was simultaneously infused with HX–XO, mRNA encoding all expressed cytokines became undetectable or was reduced (HX–XO + Isop). Isoproterenol alone had no detectable effect. The first two lanes on left are unprotected probes for the screened cytokines and the housekeeping genes, *L32* and *GAPDH*, in the absence (−) and presence (+) of RNase.

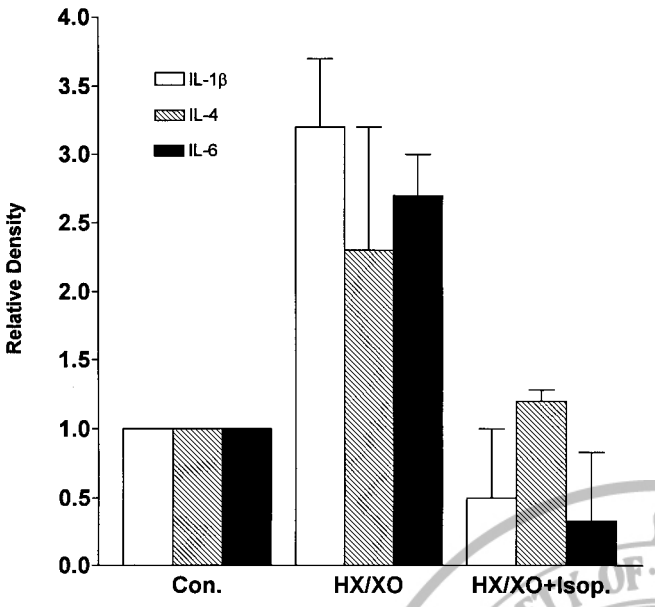

Fig. 3. Densitometric quantification of myocardial mRNA encoding cytokines detected in control hearts, interleukin (IL)-1β, IL-4, and IL-6. Perfusion with hypoxanthine–xanthine oxidase (HX–XO) significantly increased density for all three cytokines by approximately threefold ($P < 0.05$ *vs.* control [Con]). Simultaneous administration of isoproterenol with HX–XO was associated with band densities not significantly different form control. Density values are expressed as fold change from control and relative to the *L32* housekeeping gene to correct for loading. Values are mean ± SE for IL-6 in five hearts and IL-1β and IL-4 in three hearts.

Statistical Analysis

Data were analyzed by two-way analysis of variance with repeated measures. Values are expressed as mean ± SE.

Results

Figure 2 shows results of an RNase protection assay that is typical of results obtained from assays performed on five hearts in each group. Control hearts were perfused for 60 min before RNA was purified from ventricles. Message encoding for interleukin-6 was detected in five of five control hearts, and for interleukin-1β and -4 in three of five control hearts. TNF-α and interleukin-5 message was not detected in any control heart (fig. 2). In hearts perfused for 60 min with HX–XO, mRNA encoding interleukin-1β, -4, -5, -6, and TNF-α was increased in all five hearts as represented by the increased intensity of each of the specific bands in figure 2. In hearts infused with isoproterenol and HX–XO simultaneously, the intensity of bands representing interleukin-1β, -4, and -6 message was reduced to less than control, and message for TNF-α and interleukin-5 was no longer detected. The administration of isoproterenol alone had no discernable effects on gene expression. Figure 3 shows band densities of mRNA relative to control for interleukin-1β, -4, and -6 normalized to the *L32* housekeeping gene. (Band densities for TNF-α and interleukin-5 mRNA were not included because this message could not be detected in the control state or after administration of HX–XO plus isoproterenol.) Messages for interleukin-1β, -4, and -6 were increased approximately threefold above control by HX–XO infusion. Simultaneous administration of isoproterenol with HX–XO prevented the increase of mRNA caused by HX–XO.

Cyclic AMP was not detected in the coronary effluent of control hearts or hearts perfused with HX–XO alone. However, as seen in figure 4, infusion of isoproterenol was associated with release of cyclic AMP. Concentrations of cyclic AMP peaked rapidly at 1 min (240 ± 65 pmol/min, n = 4 hearts), then declined but remained elevated for the duration of the experiment (33 ± 2 pmol/min at 150 min). The addition of 0.1 μM propranolol to the perfusion buffer significantly attenuated the isoproterenol-stimulated release of cyclic AMP at all time points (fig. 4).

In rat hearts perfused with buffer alone, no TNF-α was detectable in the coronary effluent for the entire duration of the experiment (150 min). It is possible that TNF-α was produced but was below the detection limit (1 pg) of the L929 cell cytotoxicity assay. Figure 5 shows that in hearts treated with HX–XO, TNF was reliably detected in the coronary effluent at 60 min after the initiation of XO (728 ± 330 pg/min, n = 7 hearts). Even though the infusion of XO was stopped at 60 min,

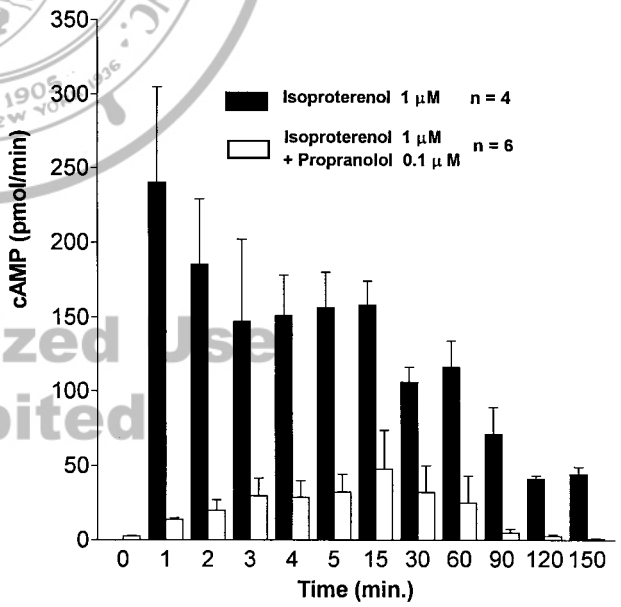

Fig. 4. Cyclic adenosine monophosphate (cAMP) release into the coronary effluent after administration of isoproterenol (1 μM) for 60 min and the inhibition of release by propranolol (0.1 μM). cAMP was undetectable in the coronary effluent before administration of isoproterenol, was at a maximum at 2 min, and remained elevated for the duration of the experiment. Inclusion of propranolol in the perfusion buffer significantly inhibited isoproterenol-stimulated cAMP release at all time points ($P < 0.05$). Time is perfusion time in minutes. Values are mean ± SE.

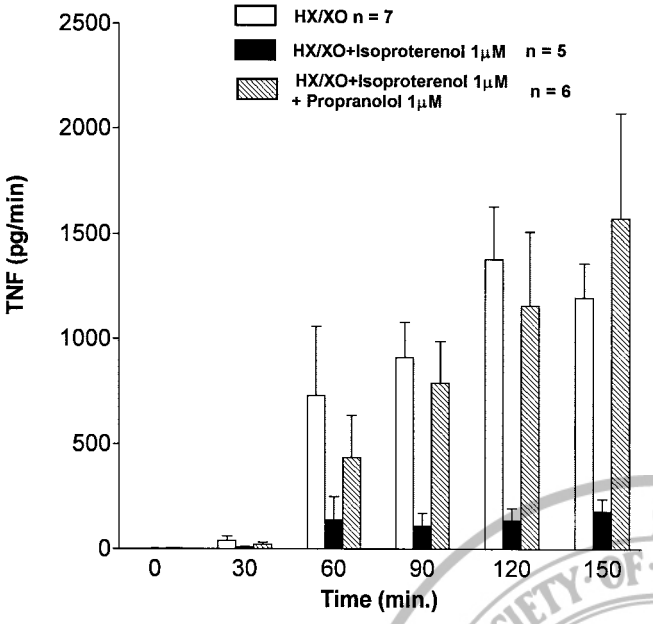

Fig. 5. Isoproterenol significantly reduced hypoxanthine–xanthine oxidase (HX–XO)-stimulated tumor necrosis factor α (TNF-α) release (picograms per minute) from the isolated rat heart ($P < 0.05$ *vs.* HX–XO alone), and this inhibition was blocked by propranolol. A 60-min infusion of HX–XO alone was begun at zero time (open bars). Closed bars show a simultaneous 60-min infusion of HX–XO and isoproterenol (1 μM). Hatched bars represent the effect of propranolol (0.1 μM) on the inhibition of HX–XO-stimulated TNF-α release induced by isoproterenol. Time is perfusion time in minutes. Values are mean ± SE.

cardiac release of TNF-α continued to increase, with maximum release measured at 120 min (1,378 ± 251 pg/min). A simultaneous 60-min infusion of isoproterenol (1 μM) with XO significantly inhibited HX–XO-stimulated release of TNF-α at all time points (figure 5). For instance, at 120 min, isoproterenol infusion reduced release of TNF-α by 90% (135 ± 56 pg/min, n = 5 hearts) compared with HX–XO alone ($P < 0.01$). The cytotoxicity of the coronary effluent obtained from three hearts perfused with HX–XO on the L929 cells was completely blocked with an antibody to TNF-α, confirming cardiac release of biologically active cytokine.

The addition of propranolol to the perfusion buffer blocked the inhibition of HX–XO-stimulated release induced by isoproterenol (fig. 5). For instance, at 120 min with propranolol in the perfusion buffer and isoproterenol infusion, HX–XO-stimulated release of TNF-α was 1,156 ± 355 pg/min (n = 6 hearts), which was not significantly different from HX–XO alone (1,378 ± 251 pg/min) but significantly greater than HX–XO-stimulated release with infusion of isoproterenol in the absence of propranolol (135 ± 56 pg/min). In other experiments, forskolin induced release of cyclic AMP from hearts stimulated with HX–XO (fig. 6). In these experiments (n = 3 hearts) no TNF-α was detected in the coronary effluent.

In figure 7, slot blots of interleukin-6 released into the coronary effluent are shown and are typical of three sets of experiments each set conducted on the same day. In each of the three control hearts, interleukin-6 was detected and perhaps reduced when isoproterenol alone was given. Infusion of HX–XO was associated with interleukin-6 release in all three hearts, and release was reduced by simultaneous infusion of isoproterenol. Although band intensities varied among the individual hearts, the relation always held, *i.e.*, HX–XO always increased interleukin-6, and simultaneous infusion of isoproterenol always decreased it in three separate experiments.

Figure 8 shows the effect of isoproterenol infusion begun 30 min after the initiation of XO perfusion on TNF-α released into the coronary effluent. In hearts perfused for 60 min with HX–XO alone, TNF-α was again reliably detected in the coronary effluent at 60 min (360 ± 79 pg/min, n = 8 hearts) and increased to a maximum of 1,501 ± 250 pg/min at 120 min. In hearts where isoproterenol (1 μM) was begun 30 min after the initiation of XO infusion and continued for 1 h, TNF-α in the coronary effluent at 60 min (421 ± 188 pg/min, n = 6 hearts) was no different from HX–XO alone. However, at 90, 120, and 150 min, delayed isoproterenol infusion significantly reduced the release of TNF-α. For instance, maximal release at 120 min was 638 ± 181 pg/min ($P < 0.05$ *vs.* HX–XO alone) or 45% of that seen with HX–XO alone.

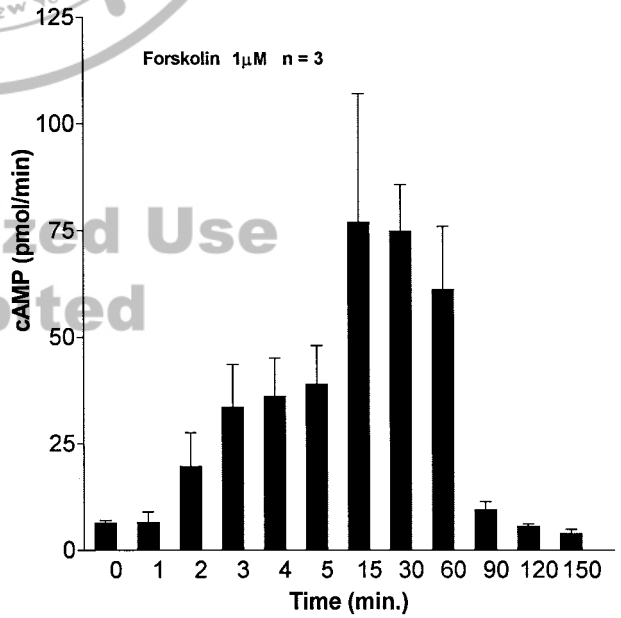

Fig. 6. Time course of release of cyclic adenosine monophosphate (cAMP) from hearts perfused with hypoxanthine–xanthine oxidase. Induced by 10 μM forskolin. Tumor necrosis factor α release could not be detected in these experiments. Time is perfusion time in minutes. Values are mean ± SE.

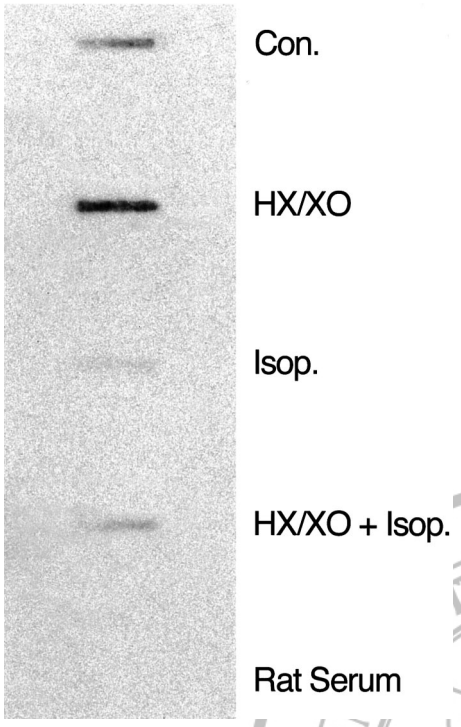

Fig. 7. Slot blot analysis of interleukin-6 in coronary effluent collected at 90 min of perfusion. Interleukin-6 was detected in the coronary effluent from the control heart (Con) and was also detected in the heart infused with isoproterenol (Isop) alone. Release of interleukin-6 increased in the heart perfused with hypoxanthine–xanthine oxidase (HX–XO) alone. Simultaneous infusion of isoproterenol inhibited HX–XO-stimulated release of interleukin-6 into the coronary effluent. The figure represents measurements from four hearts and is typical of results from three experiments. "Rat Serum" represents blotting with nonimmune serum as a control for specificity.

Discussion

In these experiments, we have shown that an enzyme–substrate system (HX–XO) that generates reactive oxygen intermediates and that is activated in episodes of myocardial ischemia–reperfusion, triggers myocardial gene expression for multiple cytokines, interleukin-1β, -4, -5, -6, and TNF-α. Infusion of the β-adrenergic agonist isoproterenol suppressed gene expression for each of these cytokines. Furthermore, we determined that TNF-α and interleukin-6 were both released into the coronary effluent by HX–XO and were inhibited by simultaneous infusion of isoproterenol and the consequent increase in cyclic AMP. Only interleukin-6 was detected in the coronary effluent of control hearts. These release data are consistent with the mRNA findings of expression of interleukin-6 in control hearts but not TNF-α, whereas both were expressed after administration of HX–XO, and both were inhibited by isoproterenol. Including propranolol in the perfusion buffer inhibited isoproterenol-stimulated release of cyclic AMP from the heart and blocked the isoproterenol-induced inhibition of HX–XO-stimulated TNF-α release. In addition, forskolin induced release of cyclic AMP and inhibited HX–XO-stimulated TNF-α release. These experiments with propranolol and forskolin indicate that the isoproterenol effect on cytokine gene expression was caused by activation of the cardiac β-adrenergic receptor and the consequent increase in myocardial cyclic AMP. Finally, when isoproterenol infusion was delayed 30 min after the start of HX–XO administration, TNF-α that was increasing in the coronary effluent was attenuated, suggesting a dynamic relation between elements regulating cytokine gene expression and β-adrenergic stimulation.

The cell type within the myocardium that is the source of cytokine expression was not identified in these experiments. In previous studies, the inflammatory cytokines interleukin-1 and -6 and TNF-α or their mRNAs have been identified in myocytes, endothelial cells, or vascular smooth muscle cells.[32–41] To our knowledge, gene expression for interleukin-5 or the antiinflammatory cytokine, interleukin-4, has not been previously demonstrated in the myocardium. For the cytokines identified in this study for which NF-κB is a common regulator, namely, interleukin-1β, interleukin-6, and TNF-α, activation of this nuclear factor by reactive oxygen could initiate expression. On the other hand, expression of interleukin-4 and -5 by mast cells has been shown to be independent of NF-κB activation.[42] The exact mechanism by which β-adrenergic stimulation inhibits myocardial cytokine gene transcription was not determined in the current study. Inhibition of activation of

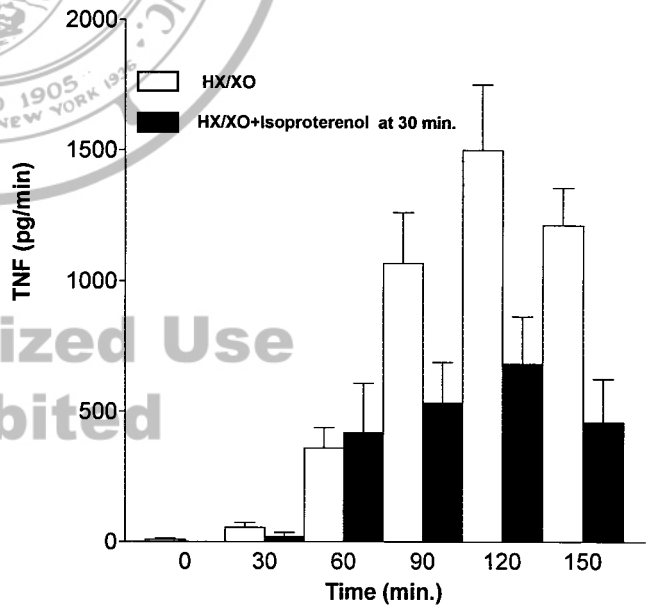

Fig. 8. The effect of isoproterenol administered 30 min after the initiation of hypoxanthine–xanthine oxidase (HX–XO) infusion on TNF-α release from the isolated rat heart. Open bars represent a 60-min infusion of HX–XO initiated at zero time. Closed bars show the effect of a 60-min infusion of isoproterenol (1 μM) begun at 30 min after the initiation of HX–XO. Values are mean ± SE for eight hearts treated with HX–XO alone and six hearts receiving HX–XO and isoproterenol. Isoproterenol significantly reduced HX–XO-stimulated tumor necrosis factor (TNF) release ($P < 0.05$ *vs.* HX–XO alone).

NF-κB by cyclic AMP can possibly be eliminated as a potential mechanism because activation was not affected by elevation of cyclic AMP in coronary smooth muscle cells, although TNF-α release by the cells was suppressed.[43] Similar findings have been reported in endothelial cells where elevated cyclic AMP failed to block activation and translocation of NF-κB to the nucleus.[44] A potential mechanism for the regulation of NF-κB–dependent genes by cyclic AMP was determined in human monocytes and endothelial cells.[45] It is known that transcriptional activation of genes by NF-κB requires multiple cofactors.[46] Activation of cyclic AMP–dependent protein kinase A induced phosphorylation of cyclic AMP response element binding protein (CREB). CREB then competed with NF-κB for a limited amount of an essential coactivator, cyclic AMP response element binding protein binding protein (CBP), to inhibit NF-κB–dependent gene transactivation.[45] Perhaps such a mechanism is active in cells within the myocardium that produce these inflammatory cytokines.

In the current study we found that the acute administration of isoproterenol suppressed cytokine production by the heart. Just the opposite has been recently reported for chronic administration of isoproterenol to rats for 7 days.[47] Chronic administration of catecholamines is known to be associated with free radical generation, myocardial ischemia, focal areas of myocardial necrosis, and failure.[48] In these 7-day experiments, results similar to ours were found in normal myocardium in that mRNA encoding TNF-α was not detected while interleukin-6 was present in low levels. After chronic administration of isoproterenol, both mRNA and protein for TNF-α, interleukin-1β, and increased interleukin-6 message were detected in the myocardium. Chronic administration of isoproterenol at the doses used resulted in inflammatory cell infiltrates, but the cytokines were only detected in myocardial cells and blood vessels and were not found in serum. As stated by Murray *et al.*,[47] their experiments could not distinguish between elevated cyclic AMP or increased heart rate, myocardial ischemia, free radical generation, and calcium overload as the cause of myocardial cytokine production. Our results suggest that free radicals will stimulate and elevated cyclic AMP will inhibit myocardial cytokine production, and this finding is supported by results in isolated myocytes where adenosine inhibited TNF-α expression through a cyclic AMP–dependent mechanism.[26] It is possible that chronic administration of isoproterenol was associated with receptor desensitization and reduced myocardial cyclic AMP concentrations, thereby removing an inhibitory regulation as well as inducing reactive oxygen intermediates, which stimulated cytokine production. Collectively, these results indicate a role for the sympathetic nervous system in regulating myocardial inflammatory cytokine production. Moreover, they emphasize the complexity of this regulation in chronic disease states such as heart failure, which is accompanied by high circulating levels of catecholamines, reduced cardiac tissue levels, and reduced cardiac response to these adrenergic agonists.

References

1. Basaran Y, Basaran MM, Babacan KF, Ener B, Okay T, Ozdemir M: Serum tumor necrosis factor levels in acute myocardial infarction and unstable angina pectoris. Angiology 1993; 44:332–7
2. Latini R, Bianchi M, Correale E, Dinarello, CA, Fantuzzi G, Fresco C, Maggioni AP, Mengozzi M, Romano S, Shapiro L, Sironi M, Tognoni G, Turato R, Ghezzi P: Cytokines in acute myocardial infarction: Selective increases in circulating tumor necrosis factor, its soluble receptor, and interleukin-1 receptor antagonist. J Cardiovasc Pharmacol 1994; 23:1–6
3. Maury CPJ, Teppo A-M: Circulating tumour necrosis factor-α (cachectin) in myocardial infarction. J Internal Med 1989; 225:333–6
4. Hirschl MM, Gwechenberger M, Binder T, Binder M, Graf S, Stefenlli T, Rauschat F, Laggner AN, Sochor H: Assessment of myocardial injury by serum tumour necrosis factor alpha measurements in acute myocardial infarction. Eur Heart J 1996; 17:1852–9
5. Lissoni P, Pelizzoni F, Mauri O, Perego M, Pittalis S, Barni S: Enhanced secretion of tumour necrosis factor in patients with myocardial infarction. Eur J Med 1992; 1:277–80
6. Biasucci LM, Vitelli A, Liuzzo G, Altamura S, Caligiuri G, Monaco C, Rebuzzi AG, Ciliberto G, Maseri A: Elevated levels of Interleukin-6 in unstable angina. Circulation 1996; 94:874–7
7. Neumann F-J, Ott I, Gawaz M, Richardt G, Holzapfel H, Jochum M, Schömig A: Cardiac release of cytokines and inflammatory responses in acute myocardial infarction. Circulation 1995; 93:748–55
8. Tashiro H, Shimokawa H, Yamamoto K, Nagano M, Momohara M, Muramatu K, Takeshita A: Monocyte-related cytokines in acute myocardial infarction. Am Heart J 1995; 130:446–52
9. Wan S, DeSmet J-M, Barvais L, Goldstein M, Vincent J-L, LeClerc J-L: Myocardium is a major source of proinflammatory cytokines in patients undergoing cardioplumonary bypass. J Thorac Cardiovasc Surg 1996; 112:806–11
10. Hennein HA, Ebba H, Rodriguez JL, Merrick SH, Keith FM, Bronstein MH, Leung JM, Mangano DT, Greenfield LJ, Rankin JS: Relationship of the proinflammatory cytokines to myocardial ischemia and dysfunction after uncomplicated coronary revascularization. J Thorac Cardiovasc Surg 1994; 108:626–35
11. Meldrum DR, Meng X, Dinerallo CA, Ayala A, Cain BS, Shames BD, Ao L, Banerjee A, Harken AH: Human myocardial tissue TNF-α expression following acute global ischemia *in vivo*. J Mol Cell Cardiol 1998; 30:1683–9
12. Wan S, Yim APC, Vincent J-L: Inflammatory response to cardiopulmonary bypass. New Horizons 1999; 7:462–71
13. Ashraf M, Zhai X: Pathophysiology of myocardial reperfusion injury: Role of oxygen free radicals. Transplant Proc 1995; 27:2800–1
14. Ferrari R, Agnoletti L, Comini L, Gaia G, Bachetti T, Cargnoni A, Ceconi C, Curello S, Visioli O: Oxidative stress during myocardial ischaemia and heart failure. Eur Heart J 1998; 19(Suppl B):B2–11
15. Hansen PR: Myocardial reperfusion injury: Experimental evidence and clinical relevance. Eur Heart J 1995; 16:734–40
16. McCord JM: Oxygen-derived free radicals in postischemic tissue injury. N Eng J Med 1985; 312:159–63
17. Meldrum DR, Dinerallo CA, Cleveland JC, Cain BS, Shames BD, Meng X, Harken AH: Hydrogen peroxide induces tumor necrosis factor α-mediated cardiac injury by a P38 mitogen-activated protein kinase-dependent mechanism. Surgery 1998; 124:291–6
18. Chandrasekar B, Freeman GL: Induction of nuclear factor κB and activation protein 1 in postischemic myocardium. FEBS Lett 1997; 401:30–4
19. Meldrum DR, Shenkar R, Sheridan BC, Cain BS, Abraham E, Harken AH: Hemorrhage activates myocardial NF-κB and increases TNF-α in the heart. J Mol Cell Cardiol 1997; 29:2849–54
20. Newman WH, Zhang L-M, Lee DH, Dalton ML, Warejcka DJ, Castresana MR, Leeper-Woodford SK: Release of tumor necrosis factor-α from coronary smooth muscle: Activation of NF-κB and inhibition by elevated cyclic AMP. J Surg Res 1998; 80:129–35
21. Taffet SM, Singhel KJ, Overholtzer JF, Shurtleff SA: Regulation of tumor necrosis factor expression in a macrophage-like cell line by lipopolysaccharide and cyclic AMP. Cell Immunol 1989; 120:291–300
22. Haskó G, Németh ZH, Szabó C, Zsilla G, Salzman AL, Vizi ES: Isoproterenol inhibits IL-10, TNF-α, and nitric oxide production in Raw 264.7 macrophages. Brain Res Bull 1998; 45:183–7
23. Meldrum DR, Cain BS, Cleveland JC Jr, Meng X, Ayala A, Banerjee A, Harken AH: Adenosine decreases post-ischemic cardiac TNF-alpha production: Anti-inflammatory implications for preconditioning and transplantation. Immunology 1997; 92:472–7
24. Bergman MR, Holycross BJ: Pharmacological modulation of myocardial tumor necrosis factor-α production by phosphodiesterase inhibitors. J Pharmacol Exp Ther 1996; 279:247–54

25. Bergman MR, Kao RH, McCune SA, Holycross BJ: Myocardial tumor necrosis factor-α secretion in hypertensive and failure-prone rats. Am J Physiol 1999; 277(Heart Circ Physiol 46):H543–50

26. Wagner DR, Combes A, McTiernan C, Sanders VJ, Lemster B, Feldman AM: Adenosine inhibits lipopolysaccharide-induced cardiac expression of tumor necrosis factor-α. Circ Res 1998; 82:47–56

27. Smart KR, Warejcka DJ, Castresana MR, Dalton ML, Webb JG, Newman WH: Isoproterenol inhibits bacterial lipopolysaccharide-stimulated release of tumor necrosis factor-α from human heart tissue. Am Surg 2000; 66:947–51

28. Newman WH, Castresana MR, Webb JG, Wang Z, Warejcka DJ: Stimulation of β-adrenergic receptors inhibits release of tumor necrosis factor-α from the isolated rat heart. Crit Care Med 2000; 28:3593–8

29. Flick DA, Gifford GE: Comparison of *in vitro* cell cytotoxic assays for tumor necrosis factor. J Immunol Meth 1984; 68:167–75

30. Brooker GJ, Harper JF, Terasaki WL, Moylan RD: Radioimmunoassay of cyclic AMP and cyclic GMP. Adv Cyclic Nucleotide Res 1979; 10:1–33

31. Chomczynski P, Sacci N: Single-step method of RNA isolation by acid guanidinium isothiocynate-phenol-chloroform extraction. Analytical Biochem 1987; 162:156–9

32. Newman WH, Zhang LM, Leeper-Woodfrod SK, Castresana MR: Human blood vessels release tumor necrosis factor alpha from a smooth muscle cell source. Crit Care Med 1996; 24:294–7

33. Newman WH, Zhang LM, Leeper-Woodford SK, Shaker IJ, Erceg SK, Castresana MR: Inhibition of release of tumor necrosis factor-α from human vascular tissue and smooth muscle cells by glucocorticoids. Crit Care Med 1997; 25:519–22

34. Warner SJC, Libby P: Human vascular smooth muscle cells: Target for and source of tumor necrosis factor. J Immunol 1989; 142:100–9

35. Ito T, Ikeda U, Shimpo M, Yamamoto K, Shimada K: Serotonin increases Interleukin-6 synthesis in human vascular smooth muscle cells. Circulation 2000; 102:2522–7

36. Browatzki M, Schmidt J, Kubler W, Kranzhofer R: Endothelin-1 induces Interleukin-6 release via activation of the transcription factor NF-kappaB in human vascular smooth muscle cells. Basic Res Cardiol 2000; 95:98–105

37. Loppnow H, Libby P: Functional significance of human vascular smooth muscle cell-derived interleukin-1 in paracrine and autocrine regulation pathways. Exp Cell Res 1992; 198:283–90

38. Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S: Cytokine gene expression after myocardial infarction in rat hearts: Possible implication in left ventricle remodeling. Circulation 1998; 98:149–56

39. Sterpetti AV, Cucina A, Morena AR, Di Donna S, D'Angelo LS, Cavalarro A, Stipa S: Shear stress increases the release of interleukin-1 and interleukin-6 by aortic endothelial cells. Surgery 1999; 114:911–4

40. Gwechenberger M, Mendoza LH, Youker KA, Frangogiannis NG, Smith CW, Michael LH, Entman ML: Cardiac myocytes produce Interleukin-6 in culture and in viable boarder zone of reperfused infarctions. Circulation 1999; 99: 546–51

41. Francis SE, Holden H, Holt CM, Duff GW: Interleukin-1 in myocardium and coronary arteries of patients with dilated cardiomyopathy. J Mol Cell Cardiol 1998; 30:215–23

42. Marquardt DL, Walker LL: Dependence of mast cell IgE-mediated cytokine production of nuclear factor-kappaB activity. J Allergy Clin Immunol 2000; 105:500–5

43. Newman WH, Zunzunegui RG, Warejcka DJ, Dalton ML, Castresana MR: A Reactive oxygen-generating system activates nuclear factor-κB and release tumor necrosis factor-α in coronary smooth muscle cells. J Surg Res 1999; 85:142–7

44. Ollivier V, Parry GCN, Cobb RR, de Prost D, Mackman N: Elevated cAMP inhibits NF-kB-mediated transcription in human monocytic cells and endothelial cells. J Biol Chem 1996; 271:20828–35

45. Parry GCN, Mackman N: Role of cyclic AMP response element-binding protein in cyclic AMP inhibition of NF-κB-mediated transcription. J Immunol 1997; 159:5450–6

46. Sheppard K-A, Rose DW, Haque ZK, Kurokawa R, McInerney E, Westin S, Thanos D, Rosenfeld MG, Glass CK, Collins T: Transcriptional activation by NF-κB requires mutiple cofactors. Mol Cell Biol 1999; 19:6367–78

47. Murray DR, Prabhu SD, Chandrasekar B: Chronic β-adrenergic stimulation induces myocardial proinflammatory cytokine expression. Circulation 2000; 101: 2338–41

48. Mann DL: Basic mechanisms of disease progression in the failing heart: The role of excessive adrenergic drive. Prog Cardiovasc Disease 1998; 41(Suppl 1):1–8