Chronic exposure to interleukin 1β induces a delayed and reversible alteration in excitation–contraction coupling of cultured cardiomyocytes

Abstract While proinflammatory cytokines can depress cardiac contractility, the mechanism by which this occurs remains unclear. To clarify the cellular effects of interleukin (IL)-1β, we assessed contractility, calcium homeostasis, and gene expression in cardiomyocytes exposed to this proinflammatory cytokine. Neonatal rat cardiomyocytes were exposed to IL-1β in the presence or absence of an inhibitor of nitric oxide (NO) synthase. Videomicroscopy was used to follow calcium transients (Fura-2 fluorescence) and amplitude of contraction, both unstimulated and after isoproterenol challenge. Gene expression was assessed by Northern and Western blot analyses. Both basal contractility (amplitude of contraction, maximum speed of contraction and relaxation) and amplitude of calcium transients were decreased, respectively, ca. 60% (*P*≤0.05) and ca. 40% (*P*≤0.05) after 3 days of IL-1β exposure. Contractile function and amplitude of calcium transients returned to control values when cells where cultured an additional 3 days in the absence of IL-1β. IL-1β-treated cells had reduced responses to isoproterenol as evidenced by a lack of enhanced amplitude of contraction and a reduction in cAMP production. IL-1β decreased the expression of genes important to the regulation of calcium homeostasis (phospholamban, sarcoplasmic reticulum calcium ATPase) at both the transcript and protein level. Alterations in contractile function did not occur through NO-mediated pathways. These results support the hypothesis that IL-1β may play an important role in contractile dysfunction through alterations in calcium homeostasis.

Keywords Calcium transients · Cardiomyocyte · Contractile function · Gene expression · Proinflammatory cytokine

Introduction Proinflammatory cytokines (including IL-1β and TNF-α), classically characterized by their mediation of inflammatory processes after synthesis by macrophages and other immunoregulatory cells, are elevated in the serum and cardiac tissue of patients with endstage heart failure [13, 27, 50, 52]. Furthermore, they are believed to depress cardiac function in heart failure, transplant rejection, myocarditis, sepsis, and burn shock [1, 12, 15, 25, 27, 36, 50]. In experimental models, IL-1β and TNF-α have been shown to depress contractile function in intact animals, isolated hearts, and papillary muscles [11, 35, 43]. A more explicit demonstration of the potential role of proinflammatory cytokines in the development of heart failure arises from murine models in which cardiac-specific overexpression of TNF-α leads to cardiac hypertrophy, fibrosis, reduced ejection fraction, loss of adrenergic responsiveness, and induction of a fetal gene program [24].

In order to understand the cellular biology of proinflammatory cytokine exposure, investigators have studied the effects of a variety of cytokines in neonatal and adult myocytes in culture. Although myocytes challenged with TNF-α or IL-1β uniformly show depressed contractility, the various studies have also yielded conflicting results [17, 57]. For example, contractile responses and alterations in adrenergic responsiveness are alternatively reported to be either dependent or independent of inducible nitric oxide synthase (iNOS) induction and NO production [34, 49, 57].
Additionally, proinflammatory cytokines have been reported to (1) enhance or depress the spontaneous contraction rates of neonatal cardiomyocytes [37, 42]; (2) enhance, depress, or leave unchanged the basal contractile activity of neonatal and adult cardiomyocytes [3, 4, 25, 49]; (3) increase or decrease the peak systolic intracellular calcium level [4, 57]; (4) increase or leave unchanged the level of transcripts for sarcomeric proteins [39, 40, 51]; and (5) induce or not induce cardiomyocyte protein synthesis [30, 39, 51]. These disparities may be partly attributable to differences in culture techniques and inherent differences between adult and neonatal cardiomyocytes. However, the existing inconsistencies might also be attributable to various doses and durations of cytokine treatment, a reliance on transcript levels without assessing the effects of cytokines on protein expression, and a failure to correlate changes in gene expression with functional responses. Indeed, most studies evaluating the effects of IL-1β on myocyte contractility and/or calcium homeostasis have focused on short-term (min to a few h) effects of the cytokine on heart cells and IL-1 concentrations used in these studies were extremely high, ranging from 10 ng ml⁻¹ to 500 ng ml⁻¹ [4, 8, 25, 49, 54]. However, lower IL-1 plasma concentration have been measured in pathological conditions such as heart failure (up to 250 pg ml⁻¹ [50]) or severe septic shock (up to 500 pg ml⁻¹ [7]) even if the presence in the plasma of IL-1RII, the soluble form of type II IL-1 receptor, reduces the detection of IL-1β in clinical samples by at least 50% [7].

Therefore, to clarify the effects of prolonged (3 days) cytokine exposure on myocyte cell biology, we assessed the effects of physiological concentrations of IL-1β on contractile function, calcium homeostasis, and transcript and protein expression in neonatal cardiac myocytes.

### Materials and methods

**Preparation and treatment of neonatal rat cardiomyocytes**

Cardiomyocytes were prepared from 1- to 2-day-old Sprague-Dawley rats and cultured in DF-5% on Pronectin (Biosource)-coated tissue culture plates or glass coverslips as previously described [30]. Cells were cultured in DF-5% for 24–48 h before the initiation of experiments. In preliminary studies, we evaluated the dose response of amplitude of contraction and maximal amplitude of calcium transients to 3 days of treatment with various concentrations of IL-1β. As shown in Fig. 1, the effects of IL-1β treatment on myocyte contractility (Fig. 1B) and amplitude of calcium transients (Fig. 1D) showed a concentration dependence, with a significant decrease observed at 0.1 ng ml⁻¹ and a maximal effect with concentrations ≥2 ng ml⁻¹. Therefore, in all the experiments described we used 2 ng ml⁻¹ of IL-1β, a concentration which is in the range of that described in human pathological conditions (0.5–1 ng ml⁻¹) [7]. Cells were treated with murine IL-1β (2 ng ml⁻¹) from Biosource, or vehicle (phosphate buffered saline; PBS). Cells were evaluated at three time points: after 24 h or 3 days of cytokine exposure, and 3 days after cessation of cytokine challenge. Fresh media containing the same cytokines was added every 24–36 h, similarly fresh vehicle was added to the control cells every 24–36 h and cytokine free media was added every other day during the recovery period. Dexamethasone (3 µmol l⁻¹, Sigma), and IL-1β neutralizing antibody (Biosource) were used as reported [30]. For studies on the role of NO production, cells were cultured in DF-5% for 24 h followed by arginine-free media (Select Amine, Life Technologies) for 4 h prior to the addition of either 1 mmol l⁻¹ L-NAME (N⁶-monomethyl-L-arginine, see below) or 1 mmol l⁻¹ arginine as previously described [26]. Spontaneous rates of contraction were determined from cells grown on plastic dishes in DF-5% in the presence or absence of IL-1β. Cells were observed with an inverted phase microscope at either 100× or 200× magnification. Rates of contraction were determined over a 20-s interval and contractions per minute calculated.

**Analysis of cytosolic calcium and contraction–relaxation**

To provide high-contrast spots for tracking contractile activity, glass beads (2.1±0.5 mm; Duke Scientific, Palo Alto, Calif., USA) were added to the neonatal myocytes. The preparation was illuminated with red light through a dichroic mirror, and a video edge-detection system (VAD 104; Crestview Electronics, Sandy, Utah, USA) was used to record the motion of glass beads attached to the surface of contracting myocytes. Calcium transients were followed in cells loaded with the acetoxy methylester form of Fura-2 (Molecular Probes). Cardiomyocytes were placed in Tyrode’s solution containing (in mmol l⁻¹): NaCl 137, KCl 5, glucose 15, HEPES 5, CaCl₂ 1.3, NaH₂PO₄ 1.2, MgCl₂ 0.6, DMSO 0.1, 20 µM D-Pluronic (Molecular Probes); 3 µl of 25% wt/wt in dimethyl sulfoxide) for 20 min. Myocytes were then rinsed with Tyrode’s solution and maintained for 15 min at room temperature to allow for de-esterification of the dye. Coverslips were transferred to a temperature regulated chamber (33°C) mounted on a Nikon Diaphot 300 inverted microscope stage and cells perfused with prewarmed Tyrode’s solution. While the cultures contained a spontaneously contracting monolayer of cells, they were paced by electrical field stimulation at 1 Hz (15 V/4 ms pulse duration. Grass S11 Stimulator, Grass Instruments) using platinum electrodes embedded in the wall of the perfusion chamber [30]. Fluorescence of intracellular Fura-2 was determined by illuminating the cells at 360 nm (the isosbestic wavelength for Fura-2) at both the beginning and end of the time interval in which cells were continuously exposed to 380-nm light, which stimulates fluorescence emission from Fura-2 that is complexed with Ca²⁺. Light emission at 520 nm was continuously recorded (Ionoptix), and the fluorescent light due to calcium transients calculated from the ratio between that stimulating by 380-nm light, and that calculated as a linearly interpolated numerator from the two 360-nm excitations (Ionoptix). The sampling rate for collection of ratio values was 100 Hz. Because the Fura-2 fluorescence ratio is an extremely sensitive indicator of changes in free calcium levels, and significant uncertainties remain in calibrating the Fura-2 fluorescence ratio to absolute intracellular free calcium levels, the fluorescence ratio (generated from the light intensity emitted by 360-nm and 380-nm excitations) was used to monitor changes in intracellular free calcium levels. A data analysis program (IonWizard 4.3, Ionoptix) was used to measure Fura-2 parameters (diastolic and peak systolic calcium) and calculate maximum speed of contraction, maximum speed of relaxation, and peak amplitude of contraction. Amplitude of calcium transients were reported both as the fluorescence ratio at the peak calcium transient as well as the difference between the diastolic baseline ratio and that observed at the calcium transient peak. Calibration of contractile distance was determined by using Cell-VU grid coverslips (Erie Scientific), Assessment of cellular contractile responsiveness to 0.1 mmol l⁻¹ isoproterenol challenge was performed as previously described with one cell recorded per coverslip [30].

**Media nitrite measurements**

Cells were cultured in arginine-free media as described above supplemented with 1 mmol l⁻¹ L-arginine (control), 1 mmol l⁻¹ arginine and 2 ng ml⁻¹ IL-1β, or 1 mmol l⁻¹ L-NAME and 2 ng ml⁻¹ IL-1β.