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Interleukin 1 pretreatment decreases ischemia/reperfusion injury

(O_2 radicals/neutrophils/antioxidants/vascular injury/heart)

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ABSTRACT **Hearts isolated from rats treated 36 hr before with interleukin 1 (IL-1) had increased glucose-6-phosphate dehydrogenase (G6PD) activity and decreased hydrogen peroxide levels and injury after global ischemia (I, 20 min)/reperfusion (R, 40 min) compared with hearts from untreated rats. Hearts isolated from rats treated 6 hr earlier with IL-1 also had increased polymorphonuclear leukocytes (PMN), H_2O_2 levels, and oxidized glutathione (GSSG) contents compared with hearts from untreated rats. Depletion of circulating blood PMN by prior treatment with vinblastine prevented both early (from treatment 6 hr before study) IL-1-induced increases in myocardial PMN accumulation, H_2O_2 levels, and GSSG contents and late (from treatment 36 hr before study) increases in myocardial G6PD activity and protection against I/R. Our results indicate that IL-1 pretreatment causes an early (6 hr after IL-1 treatment) myocardial PMN accumulation and most likely an H_2O_2-dependent oxidative stress, which contributes to late (36 hr after IL-1 treatment) increases in myocardial G6PD activity and decreases in I/R injury.**

After ischemia, tissues are subjected to a reperfusion injury that appears to involve generation of toxic O_2 metabolites (1–3). The O_2 metabolite premise implies that increases in endogenous antioxidant enzyme activity should decrease reperfusion damage; indeed, hearts from rats pretreated with endotoxin had increased catalase activity and resisted ischemia/reperfusion (I/R) injury (4). Another link between endogenous antioxidant enzyme activity and susceptibility to oxidant injury is manifest in erythrocytes that selectively lack glucose-6-phosphate dehydrogenase (G6PD) activity and, as a result, are extremely sensitive to lysis by oxidants (5). G6PD activity is needed for effective detoxification of H_2O_2, most likely because optimal G6PD activity is necessary for optimal regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG). The mechanism responsible for increases in myocardial antioxidant enzyme activity is unknown but oxidants might increase antioxidant enzyme activity. For example, increases in lung antioxidants and tolerance to lung injury from hyperoxia occur after pretreatment with endotoxin (6), hypoxia (7), nitrogen dioxide (8), sublethal hyperoxia (9), or tumor necrosis factor and interleukin 1 (IL-1) (10)—all factors that may increase O_2 metabolite generation *in vivo* (11). Moreover, IL-1 treatment has induced mRNA for superoxide dismutase in cell systems *in vitro* (12). Accordingly, we hypothesized that IL-1 pretreatment would increase myocardial antioxidant enzyme and decrease I/R injury.

MATERIALS AND METHODS

Preparation and Pretreatment of Rats with IL-1. Sprague–Dawley rats (350 ± 25 g; Harlen; Sprague–Dawley) were fed diets containing 0.7 g of sodium tungstate (ICN) per kg of body weight or standard diets (2). In some experiments, sodium molybdate (60 mg/kg in 5.0 mM potassium phosphate buffer, pH 7.4) was given i.p. 6 hr before heart isolation. Rats were injected i.p. with 30 μg of human recombinant IL-1α per kg of body weight (specific activity, 3×10^8 units/mg by D10 assay with <0.8 units of endotoxin per mg of protein by limulus assay) in sterile 5.0 mM potassium phosphate-buffered saline (pH 7.4) containing 0.5 mg of endotoxin-free bovine serum albumin per ml or with albumin/potassium phosphate-buffered saline alone 36, 6, or 1 hr before heart isolation (13). Rats were also injected with 6-aminonicotinamide (AMN; 30 mg/kg i.p.)—a competitive inhibitor of NADPH-dependent reactions—4 hr before heart isolation (7), with vinblastine sulfate (VBL; 0.75 mg/kg i.v.) 1 hr or 4 days before IL-1 injection, and/or with aminotriazole (300 mg/kg in potassium phosphate-buffered saline, pH = 7.4, i.p.) 90 min before heart isolation (2).

Isolation and Treatment of Perfused Hearts. After anesthesia (pentobarbital; 60 mg/kg i.p.), thoracotomy, and heparinization (500 units via inferior vena cava), hearts were rapidly excised and perfused in retrograde fashion at the aortic root with Kreb's Henseleit solution (2). For some experiments, IL-1 was infused into isolated hearts at 0.5 μg/min for 10 min before ischemia and throughout reperfusion. For other experiments, aminotriazole (7.5 mg/min) in 5 mM potassium phosphate–buffered saline (pH 7.4) was infused for 15 min before and 15 min after ischemia or for 30 min in hearts not subjected to ischemia.

Assay of Myocardial Antioxidant Enzyme Activity. Hearts were isolated and perfused for 10 min, which eliminated perfusate hemoglobin and catalase activity. Next, hearts were weighed, placed in 10 ml of 50 mM potassium phosphate, pH 7.8/0.1 mM EDTA, and homogenized for 30 s at maximum speed at 4°C (Tissue Miser, 60S, maximum speed, Cole–Parmer). Aliquots of homogenates were then centrifuged at 20,000 × *g* for 10 min, and supernatants were analyzed for protein, G6PD, catalase superoxide dismutase, GSH peroxidase, and GSH reductase activity (10, 13–16). Values for antioxidant enzyme activity were adjusted by using standard measurements and were expressed as units of activity divided by mean values for supernatant protein.

Assessment of Myocardial H_2O_2 Levels. After I/R with aminotriazole, isolated hearts were placed in liquid N_2 and homogenized in 5 mM potassium phosphate buffer (pH 7.8). After centrifugation at 20,000 × *g* for 10 min, supernatants were assayed for catalase activity (2). Absorbance change per min was recorded, and catalase activity was calculated as units/g (wet weight) of myocardium (2). H_2O_2 index was (1 −

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Abbreviations: PMN, polymorphonuclear leukocytes; I/R, ischemia/reperfusion; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; GSSG, oxidized GSH; DP, ventricular developed pressure; AMN, 6-aminonicotinamide; IL-1, interleukin 1.

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remaining myocardial catalase activity)/initial myocardial catalase activity.

Assessment of Myocardial Function. Hearts were isolated, and a water-filled latex balloon was placed through the left atrium into the left ventricle and adjusted to a left-ventricular-end diastolic pressure of 6 mmHg (2). Pacing wires (rate, 350 beats per min; 2.5× threshold, turned off during ischemia) were attached, and after equilibration for 10 min, ventricular developed pressure (DP) and positive ($+dP/dt$) and negative ($-dP/dt$) first derivatives were recorded using direct pressure and differentiator amplifiers. Patterns of values for $+dP/dt$ and $-dP/dt$ were always similar to ventricular DP and are not shown. Subsequently, hearts were subjected to global (aortic root stopcock) normothermic (37°C) ischemia for 20 min and reperfusion for 40 min.

Assessment of Myocardial Polymorphonuclear Leukocyte (PMN) Accumulation. A cross section was made through the midportion of each excised heart, which included right and left ventricles. Immediately tissue samples were fixed by immersion in 10% buffered formalin. Each section was then embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. PMN in five random high-power (×40) fields in the left ventricle wall, right ventricle wall, and septum were counted without knowledge of the identity of the samples.

Assessment of Myocardial GSH Contents. Excised hearts were perfused blood-free and freeze-clamped (7, 13). Samples for GSSG were ground under liquid N_2 and homogenized in twice the volume of buffer (100 mM potassium phosphate, pH = 6.5) containing 5 mM EDTA and 0.05 M *N*-ethylmaleimide. Samples for GSH were ground under liquid N_2 and homogenized in 100 mM potassium phosphate, pH 7.5/5 mM EDTA. Proteins were precipitated with 20% metaphosphoric acid (1:4, vol/vol; Alpha–Ventron, Danvers, MA), and samples were centrifuged at 15,000 × *g* for 10 min and assayed for GSH and GSSG content. GSSG/GSH ratios were GSSG/(total GSH + GSSG) (17).

Measurement of Myocardial Xanthine Dehydrogenase/Oxidase Activity. Excised hearts were freeze-clamped, weighed, and homogenized in potassium phosphate buffer containing 0.2 M Na_2CO_3/HCO_3 (pH = 10.0), 1 mM phenylmethylsulfonyl fluoride, 1 mM dithioerythritol, and 0.2 mM EDTA. After centrifugation for 30 min at 45,000 × *g*, supernatants were passed over a potassium phosphate buffer-equilibrated Sephadex G-25 column at 4°C. Xanthine oxidase activity was determined by measuring uric acid made in the presence of xanthine and ambient O_2 (2).

Statistical Analysis. All values were compared by analysis of variance and corrected by post hoc Student *t* tests for differences between groups. Each figure value is the mean ± SEM of the number of determinations shown within the parentheses.

RESULTS

Antioxidant Enzyme Activity, H_2O_2 Levels, and Ventricular Function in Hearts from Rats Treated 36 hr Before Heart Isolation with IL-1. *Myocardial antioxidant enzyme activity before I/R.* Hearts harvested from rats treated 36 hr before with IL-1 had the same ($P > 0.05$) weights (1.41 ± 0.05 g vs. 1.37 ± 0.03 g), supernatant protein values (0.61 ± 0.33 mg/g of tissue vs. 0.67 ± 0.33 mg/g of tissue), and wet-to-dry ratios (4.9 ± 0.3 vs. 4.8 ± 0.4) as untreated rats. However, hearts harvested from rats treated 36 hr earlier with IL-1 had increased ($P < 0.05$) G6PD activity (0.50 ± 0.02 unit/mg of protein, $n = 5$) compared with hearts from untreated control rats (0.37 ± 0.01 unit/mg of protein, $n = 5$). By comparison, catalase, superoxide dismutase, GSH peroxidase, and GSH reductase activities were the same ($P > 0.05$) in hearts from IL-1-pretreated and untreated control rats (data not shown). Moreover, when expressed as activity per heart (data not shown), G6PD (but not catalase, superoxide dismutate, GSH peroxidase, or GSH reductase) activity was also increased ($P < 0.05$) in hearts from IL-1-pretreated (36 hr before isolation) but not ($P > 0.05$) untreated rats.

Myocardial H_2O_2 levels after I/R. Measurement of catalase activity in aminotriazole-treated hearts revealed a baseline H_2O_2 index of 0.21 ± 0.04 ($n = 3$) in hearts perfused for 60 min without ischemia. By comparison, hearts subjected to I/R had an increased ($P < 0.05$) H_2O_2 index (0.49 ± 0.02, $n = 3$) compared with hearts perfused for 60 min without ischemia. In contrast, after I/R, hearts from IL-1-pretreated (36 hr before isolation) rats had a decreased ($P < 0.05$) H_2O_2 index (0.30 ± 0.05, $n = 3$) compared with hearts from untreated rats (0.49 ± 0.02, $n = 3$).

Ventricular function after I/R. DP was the same ($P > 0.05$) in all hearts studied before I/R or after perfusion for 60 min without ischemia (Fig. 1). However, after I/R, hearts had decreased DP compared with hearts studied before I/R or hearts perfused for 60 min without ischemia. In contrast, after I/R, hearts from rats treated with IL-1 36 hr before I/R had increased ($P < 0.05$) DP compared with hearts from untreated rats. By comparison, after I/R, hearts from rats treated with IL-1 at 1 hr before study or infused with IL-1 during perfusion had the same ($P > 0.05$) DP as hearts from untreated rats.

Effect of AMN Treatment on Increases in G6PD Activity and Ventricular Function in Hearts Isolated from Rats Treated 36 hr Before with IL-1. *Myocardial G6PD activity before I/R.* Hearts from rats pretreated with both AMN (4 hr before isolation) and IL-1 (36 hr before isolation) had decreased (*P*

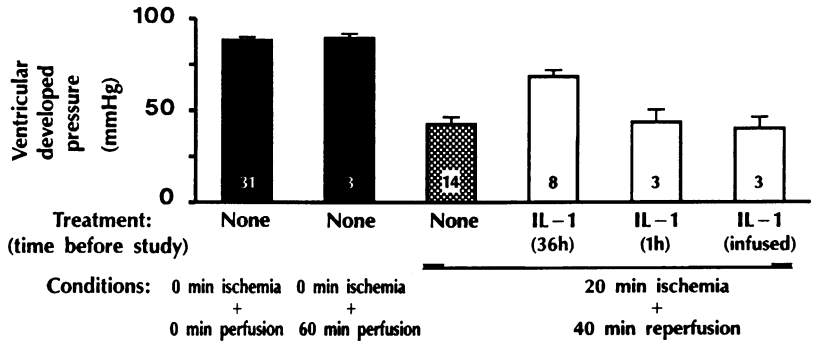

FIG. 1. Ventricular function in isolated buffer-perfused hearts from rats treated 36 hr earlier with IL-1. After I/R, control hearts had decreased ($P < 0.05$) DP compared with hearts studied before ischemia or after perfusion without ischemia. In contrast, after I/R, hearts from rats treated with IL-1 (30 μg/kg i.p.) 36 hr prior to heart isolation had increased ($P < 0.05$) DP compared with hearts from untreated rats. In contrast, after I/R, hearts from rats treated with IL-1 1 hr before isolation or infused with IL-1 at 0.5 μg/min for 10 min before ischemia and throughout reperfusion had the same ($P > 0.05$) DP as hearts from untreated rats.

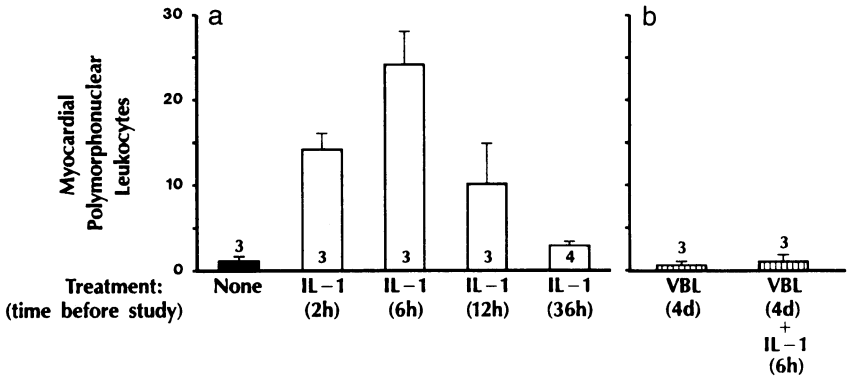

FIG. 2. PMN in hearts isolated from rats treated 6 hr earlier with IL-1. Hearts isolated from rats treated 2, 6, or 12 hr earlier with IL-1 had increased ($P < 0.05$) numbers of PMN following IL-1 treatment compared with hearts from control rats. Hearts from rats treated with vinblastine 4 days before IL-1 treatment [VBL (4d)] or with VBL (4d) + IL-1 36 hr before isolation [VBL (4d) + IL-1 (36 h)] had the same ($P > 0.05$) numbers of PMN as hearts from untreated control rats and had decreased ($P < 0.05$) PMN compared with hearts from IL-1-pretreated rats.

< 0.05) G6PD activity (0.38 ± 0.01 unit/mg of protein, $n = 5$) compared with hearts from rats pretreated only with IL-1 (0.50 ± 0.02 unit/mg of protein, $n = 5$).

Ventricular function after I/R. Hearts from AMN-pretreated (4 hr before isolation) rats perfused for 60 min without ischemia had the same ($P > 0.05$) DP (90.3 ± 4 mmHg, $n = 5$) as hearts studied before ischemia or after perfusion for 60 min without ischemia. However, after I/R, hearts from untreated rats had decreased ($P < 0.05$) DP (46.2 ± 0.2 mmHg, $n = 5$) compared with hearts isolated from rats treated with IL-1 36 hr earlier or hearts studied before ischemia or perfused for 60 min without ischemia. By comparison, after I/R, hearts from rats pretreated with AMN 4 hr before isolation and IL-1 36 hr before isolation had decreased ($P < 0.05$) DP (54.0 ± 5.6 mmHg, $n = 5$) compared with hearts from rats pretreated with IL-1 alone (67.6 ± 3.7 mmHg, $n = 5$).

PMN Accumulation, H_2O_2 Levels, and GSSG Contents in Hearts Isolated from Rats Treated 6 hr Before with IL-1: Effects of Vinblastine or Tungsten Treatment. The next series of investigations was conducted to determine early (after treatment 6 hr before heart isolation) consequences of IL-1 treatment. Since PMN and xanthine oxidase might generate H_2O_2 *in vivo*, we sought to assess their contribution by standard depletion techniques prior to IL-1 treatment. PMN were depleted by using vinblastine treatment (0.75 mg/kg i.v.; one dose) 4 days prior to IL-1 treatment. Xanthine oxidase activity was decreased by tungsten feeding for 4 weeks prior to heart isolation (4) and then rapidly restored before perfusion by treatment with molybdenum (2).

Vinblastine treatment. Vinblastine treatment 4 days prior to IL-1 treatment decreased ($P < 0.05$) blood PMN counts 95% from 1160 ± 250 to 63 ± 20. By comparison, the vinblastine treatment alone decreased total leukocyte counts only 40% from 7700 ± 1800 to 4100 ± 500, lymphocyte counts 40% from 6440 ± 1400 to 3860 ± 450, monocyte counts 50% from 187 ± 70 to 95 ± 30, and platelet counts 30% from 993,000 ± 94,000 to 633,700 ± 47,000.

Tungsten treatment. Hearts isolated from rats fed tungsten for 4 weeks had decreased ($P < 0.05$) xanthine oxidase and xanthine oxidase/dehydrogenase activity (<5% of control hearts) compared with hearts from untreated rats. However, 6 hr after molybdenum treatment, hearts from the tungsten-fed rats had increased ($P < 0.05$) xanthine oxidase and xanthine oxidase/dehydrogenase activity compared with hearts from rats treated with tungsten alone (2).

Myocardial PMN accumulation. Hearts from rats pretreated with IL-1 had an 18-fold increase ($P < 0.05$) in PMN after 6 hr compared with hearts from untreated rats (Fig. 2*a*). Hearts from rats treated with vinblastine 4 days before IL-1 treatment and then with IL-1 6 hr before study had decreased ($P < 0.05$) PMN compared with hearts from rats treated with

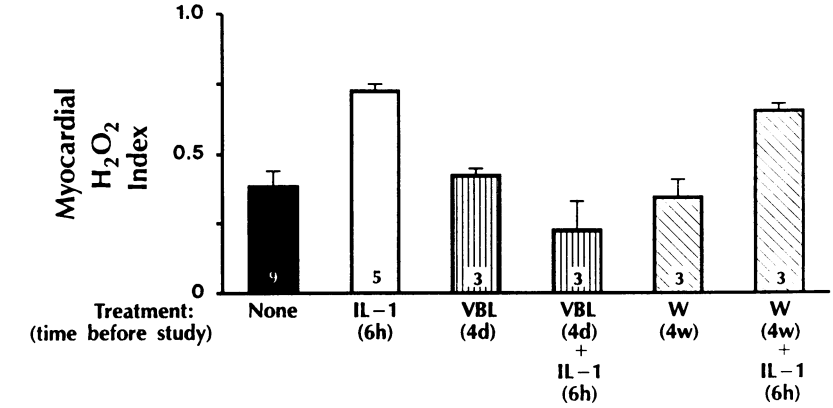

FIG. 3. H_2O_2 levels in hearts isolated from rats treated 6 hr earlier with IL-1. Hearts from IL-1-pretreated (6 hr before isolation) rats had an increased ($P < 0.05$) H_2O_2 index compared with hearts from untreated control rats. Hearts isolated from rats treated with vinblastine 4 days before IL-1 treatment [VBL (4d)], with VBL (4d) + IL-1 6 hr before isolation [VBL (4d) + IL-1 (6 h)], or with tungsten [fed for 4 weeks before isolation; W (4w)] had the same ($P > 0.05$) H_2O_2 index as hearts from untreated control rats. In contrast, hearts from rats fed tungsten for 4 weeks and then pretreated with IL-1 (6 hr before isolation) had an increased ($P < 0.05$) H_2O_2 index compared with hearts from rats treated only with tungsten and had the same ($P > 0.05$) H_2O_2 index as hearts from rats treated only with IL-1. Each value is the mean ± SEM of the number of determinations shown within the parentheses.

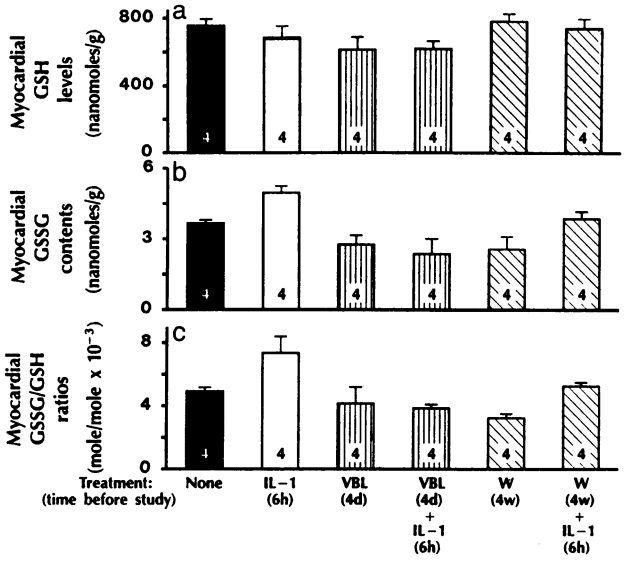

FIG. 4. GSSG activity in hearts isolated from rats treated 6 hr earlier with IL-1. GSH contents were the same ($P > 0.05$) in all hearts from IL-1-pretreated rats compared with hearts from respective control rats (*a*). However, GSSG (*b*) and GSSG/GSH ratios (*c*) were increased ($P < 0.05$) in hearts isolated from rats pretreated 6 hr before with IL-1 compared with hearts from untreated control rats. In contrast, GSSG and GSSG/GSH values were the same ($P > 0.05$) in hearts isolated from rats treated with vinblastine 4 days before IL-1 treatment [VBL (4d)] compared with VBL (4d) + IL-1 treatment 36 hr before isolation [VBL (4d) + IL-1 (6 h)]. Furthermore, GSSG and GSSG/GSH were increased ($P < 0.05$) in hearts isolated from rats fed tungsten for 4 weeks and treated with IL-1 at 6 hr before isolation compared with hearts from rats pretreated only with tungsten.

only IL-1 and the same ($P > 0.05$) PMN accumulation as hearts from vinblastine-pretreated or untreated rats (Fig. 2*b*).

Myocardial H_2O_2 levels. Hearts from rats treated 6 hr before isolation with IL-1 had an increased ($P < 0.05$) H_2O_2 index compared with hearts from untreated rats (Fig. 3). Hearts from rats treated with vinblastine 4 days before the IL-1 treatment had a decreased ($P < 0.05$) H_2O_2 index compared with hearts from rats pretreated with IL-1 alone. Hearts from rats fed tungsten for 4 weeks and treated with IL-1 6 hr before isolation had the same increased ($P > 0.05$) H_2O_2 index as hearts from rats pretreated with IL-1 alone.

Myocardial GSSG contents. Hearts from rats treated 6 hr before isolation with IL-1 had the same ($P > 0.05$) GSH and total GSH + GSSG contents as control hearts (Fig. 4). However, GSSG contents and GSSG/GSH ratios were increased ($P < 0.05$) in hearts from the IL-1-pretreated rats compared with untreated rats. Hearts from rats treated with vinblastine 4 days before IL-1 treatment and IL-1 6 hr before study or with vinblastine alone had the same ($P > 0.05$) GSSG and GSSG/GSH contents compared with each other and decreased ($P < 0.05$) GSSG levels compared with hearts from rats pretreated with IL-1 alone. By comparison, hearts from rats fed tungsten for 4 weeks and treated with IL-1 6 hr before isolation had increased ($P > 0.05$) GSSG content and GSSG/GSH ratios compared with hearts from rats pretreated with tungsten alone.

Ventricular function before and after I/R. Hearts from rats treated with IL-1 6 hr before isolation did not have significantly different ($P > 0.05$) DP before (92.6 ± 2.3 mmHg vs. 89.1 ± 1.7 mmHg, $n = 5$) or after (59.2 ± 5.7 mmHg vs. 67.6 ± 3.7 mmHg, $n = 5$) I/R compared with hearts from untreated rats.

G6PD Activity and Ventricular Function in Hearts Isolated from Rats Pretreated 36 hr Before with IL-1: Effect of Vinblastine or Tungsten Treatment. Since vinblastine but not tungsten treatment decreased the early effects (after 6 hr) of IL-1 pretreatment on myocardial PMN accumulation, H_2O_2 indices, and GSSG contents, we next assessed the effect of vinblastine or tungsten treatment on the late increases in G6PD activity and ventricular function after I/R in rats treated 36 hr before I/R with IL-1.

Myocardial G6PD activity before I/R. Hearts from rats pretreated with vinblastine (4 days before IL-1 treatment) and then IL-1 (36 hr before isolation) had decreased ($P < 0.05$) G6PD activity (0.38 ± 0.01 unit/mg of protein, $n = 5$) compared with hearts from rats pretreated (36 hr) with only IL-1 (0.50 ± 0.02 unit/mg of protein, $n = 5$). Hearts from rats pretreated with tungsten (for 4 weeks before isolation) and IL-1 (36 hr before isolation) had the same ($P > 0.05$) G6PD activity (0.47 ± 0.03 unit/mg of protein, $n = 5$) as hearts from rats pretreated with IL-1 alone (0.50 ± 0.02 unit/mg of protein, $n = 5$).

Ventricular function after I/R. After I/R, hearts from rats pretreated with vinblastine 4 days before IL-1 treatment and then with IL-1 36 hr before isolation had decreased ($P < 0.05$) DP compared with hearts from rats pretreated with IL-1 alone (Fig. 5). By comparison, hearts from rats pretreated with vinblastine for only 1 hr before the IL-1 pretreatment at 36 hr had the same ($P > 0.05$) DP (75.5 ± 3.2 mmHg, $n = 5$) as hearts from rats pretreated with IL-1 alone and increased ($P < 0.05$) DP compared with hearts from rats pretreated with vinblastine 4 days prior to IL-1. After I/R, hearts from rats

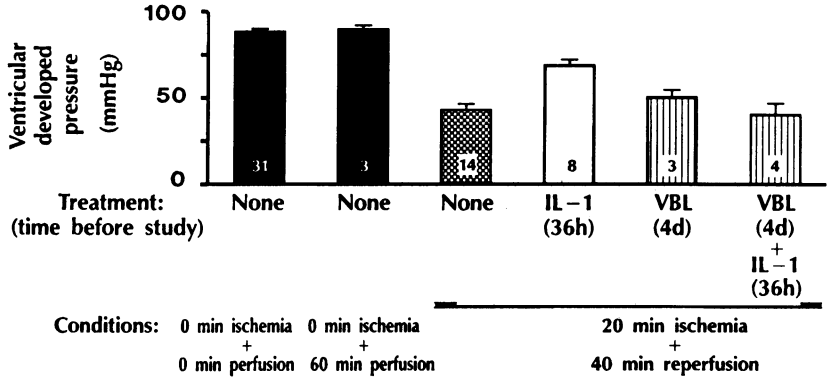

FIG. 5. Effect of VBL on ventricular function after I/R in isolated buffer-perfused hearts isolated from rats treated 36 hr earlier with IL-1. Hearts subjected to I/R had decreased ($P < 0.05$) DP compared with hearts studied before ischemia or after perfusion without ischemia. In contrast, after I/R, hearts from IL-1-pretreated (36 hr before isolation) rats had increased ($P < 0.05$) DP compared with control hearts. After I/R, hearts from rats treated with vinblastine for 4 days before IL-1 pretreatment [VBL (4d)] or with VBL (4d) + IL-1 at 36 hr before isolation [VBL (4d) + IL-1 (36h)] had the same ($P > 0.05$) DP as hearts from untreated control rats and decreased ($P < 0.05$) DP compared with hearts from rats pretreated only with IL-1.

pretreated with tungsten (for 4 weeks before isolation) and with molybdenum 6 hr before isolation (one dose) had decreased ($P < 0.05$) DP compared with hearts from rats pretreated with tungsten (for 4 weeks) or IL-1 at 36 hr alone and the same ($P > 0.05$) DP as hearts from untreated control rats. Furthermore, after I/R, hearts from rats pretreated with tungsten for 4 weeks, IL-1 at 36 hr, and then molybdenum at 6 hr had increased ($P < 0.05$) DP compared with hearts from tungsten/molybdenum-pretreated rats and with hearts from untreated rats and had the same ($P > 0.05$) DP as hearts from rats pretreated with IL-1 at 36 hr.

DISCUSSION

Our results indicate that a prior oxidant stress can induce protection against a subsequent oxidant insult. Pretreating rats with IL-1 36 hr before heart isolation increased myocardial G6PD activity and decreased I/R damage. Increases in G6PD activity and resistance to I/R injury appeared to depend on earlier IL-1-induced increases in PMN accumulation and H_2O_2 generation in myocardium.

IL-1-mediated protection against I/R depended on delayed rather than immediate effects, as hearts from rats pretreated 1 hr before isolation or perfused with IL-1 did not show decreased injury following I/R. The mechanism responsible for IL-1-mediated protection against I/R damage appeared to involve alterations in myocardial oxidant–antioxidant balance. Hearts from IL-1-pretreated rats had increased G6PD activity and decreased tissue H_2O_2 levels after I/R compared with hearts from untreated rats. Moreover, pretreatment with the hexose monophosphate shunt inhibitor AMN partially decreased G6PD activity and resistance to I/R injury in hearts from IL-1-pretreated rats.

Hearts from rats pretreated 6 hr before with IL-1 also had an 18-fold increase in PMN. These PMN could be adhering, activated, and perhaps releasing increased O_2 metabolites as suggested by the increased H_2O_2 levels and GSSG contents in hearts from PMN-replete but not PMN-depleted rats pretreated 6 hr before with IL-1. The findings suggested that H_2O_2 generated by IL-1-recruited and -stimulated PMN may have been involved in subsequent increases in G6PD activity and resistance to I/R damage. The latter hypothesis was supported when vinblastine-induced PMN depletion also decreased myocardial G6PD activity and related resistance to reperfusion injury in hearts isolated from rats 36 hr after IL-1 treatment. By comparison, inactivating xanthine oxidase—another source of H_2O_2—had no effect on the development of early and late myocardial changes seen in rats treated with IL-1. Taken *en toto*, these findings indicate that an early oxidant stress may decrease a subsequent oxidant insult by increasing endogenous antioxidant and perhaps other defense mechanisms.

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