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Induction of Stress Proteins in Rat Cardiac Myocytes by Antimony

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The effects of nonlethal concentrations of potassium antimonyl tartrate (PAT) were examined in cultured neonatal rat cardiac myocytes. PAT (5, 10 μM) significantly increased cellular reduced glutathione (GSH) and heme oxygenase activity after 18 h. GSH levels and heme oxygenase activity were increased 2.5- and 5.4-fold, respectively, by 10 μM PAT after 18 h. In addition, total cytochrome P450 levels were decreased by PAT after an 18-h exposure. PAT exposures were associated with the induction of specific stress proteins. Nonlethal concentrations of PAT produced a dose-dependent increase in HO-1, HSP70, and HSP25/27 protein levels but did not increase HSP60 levels. Pretreatment of cardiac myocytes with low concentrations of PAT (0.5–10 μM) protected against a subsequent lethal concentration of PAT (200 μM). This protection was blocked if cells were treated with the protein synthesis inhibitor cycloheximide. Results demonstrate that low concentrations of PAT increase GSH levels and stress protein synthesis, which may be responsible for the protection that low-level PAT exposure offers against the subsequent toxicity of higher concentrations of PAT.

Key Words: **antimony; heme oxygenase; heat shock proteins; glutathione; stress proteins; cardiac myocytes; heart; potassium antimonyl tartrate.**

Antimony is a hard, brittle metal that has extensive uses in industry. Compounds containing antimony are used in the manufacture of paints, ceramics, pyrotechnics, fire retardants, and glass (Carson *et al.*, 1986; Stokinger, 1981). Antimony is also used in the semiconductor industry and has limited but important use as an antiparasitic drug (Winship, 1987). Antimony-containing compounds produce cardiac functional alterations and toxicity in both experimental animals and humans. Breiger *et al.* (1954) observed degeneration of the myocardium in rats and rabbits chronically exposed to dusts containing antimony trisulfide. Injection of antimony (10 mg/kg) into the coronary circulation of an isolated perfused dog heart reduced contractile force by 50% within 1 h and a comparable treatment in the intact dog was fatal (Bromberger-Barnea and Stephens, 1965). Altered electrocardiograms (EKG) and death from cardiotoxicity have been documented following administration of antimony-containing drugs (NIOSH, 1978; Sundar *et al.*, 1998; Thakur *et al.*, 1998; Winship, 1987). High incidence of cardiotoxicity following the administration of sodium antimony gluconate has been reported in recent years in the treatment of visceral leishmaniasis (Sundar *et al.*, 1998). In a study reported by Thakur *et al.* (1998), an estimated 40% of a total of 80 patients treated with sodium antimony gluconate exhibited electrocardiographic changes and 5% died of cardiotoxicity. EKG changes associated with antimony treatment include increased amplitude of the P wave, fusion of the S-T segment and T wave, presence of U wave, lengthening of Q-T interval, and ventricular dysrhythmia (Winship, 1987). At a factory manufacturing resinoid grinding wheels, six sudden deaths were reported in workers following exposure to antimony trisulfide for 8 to 24 months. Heart disease was suspected in all but one of the cases. A subsequent study conducted at this factory reported that 37 of 75 workers exhibited electrocardiogram changes that involved T wave modifications (Brieger *et al.*, 1954).

Despite the numerous case reports of antimony-induced cardiotoxicity, few studies have examined underlying mechanisms responsible for adverse cardiac reaction to antimony. To address this, our laboratory has conducted a series of studies investigating the effects of antimony on cultured cardiac myocytes. These studies demonstrated that potassium antimonyl tartrate (PAT) induces a lethal oxidative stress in cardiac myocytes (Tirmenstein *et al.*, 1995) that may arise from impairment of antioxidant defenses rather than via direct reduction of molecular oxygen (Tirmenstein *et al.*, 1997). This distinguishes antimony from other metals such as iron and copper that can reduce oxygen to produce reactive oxygen species. Elevation of intracellular calcium was also found to be an early event in PAT-induced cardiac myocyte death (Wey *et al.*, 1997). In contrast, nonlethal doses of PAT reduced the capacity of cardiac myocytes to mobilize calcium during excitation–contraction (Toraason *et al.*, 1997). Although the specific mechanism responsible for reduced calcium mobilization was not defined, one mechanism proposed was increased intracellular buffering of calcium by calcium binding proteins as part of a stress response. Such a response includes expression

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of a multitude of stress proteins (Ribeiro *et al.*, 1995), including the metal binding protein metallothionein (Camhi *et al.*, 1995).

Numerous studies have reported the induction of heat shock or stress proteins in a wide variety of cellular systems following exposure to metals (Bauman *et al.*, 1993; Goering *et al.*, 1993). Stress proteins, such as HSP25/27, HSP60, and HSP70, are a ubiquitous group of proteins that are synthesized in response to heat and other environmental stress and are thought to afford protection against cardiac cell toxicity (Das *et al.*, 1993). Recently, evidence has suggested that induction of stress proteins may protect the heart against ischemic injury (Hutter *et al.*, 1994). The stress protein heme oxygenase (HO-1) is especially susceptible to induction by metals (Maines and Kappas, 1977). Drummond and Kappas (1981) demonstrated that PAT as well as other antimony-containing compounds were potent inducers of HO-1 in the liver and kidney of rats, but the effects of PAT on the heart were not examined.

Our observations that low-levels of antimony alter calcium homeostasis in cultured cardiac myocytes (Toraason *et al.*, 1997) lead to the hypothesis that this same exposure could induce a stress response that would protect cardiac myocytes against subsequent exposures to antimony. This could have important implications for therapeutic administration of antimony compounds where cardiac toxicity is a concern (Thakur *et al.*, 1998). Therefore, the objectives of the present investigation were to (1) determine if PAT induces a stress response in cardiac myocytes, (2) characterize the stress response, and (3) assess the protection the response provides against lethal doses of PAT.

METHODS

Chemicals. Newborn calf serum was purchased from Hyclone (Logan, UT). PAT was obtained from Aldrich (Milwaukee, WI). All other chemicals and reagents, unless otherwise stated, were purchased from Sigma (St. Louis, MO).

Preparation of myocytes. Cardiac ventricular cells were isolated from 2- to 4-day-old Sprague–Dawley rats by a method previously described (Toraason *et al.*, 1990). Rat pups were obtained from a breeding colony maintained in the animal quarters of NIOSH, which is accredited by the American Association for Accreditation of Laboratory Animal Care. In order to reduce the percentage of fibroblasts in the cultures, ventricular heart cells were preplated at a density of 10^6 cells/ml in incubation flasks in M199 medium containing 10% newborn calf serum and 100 U penicillin–streptomycin/ml. After 1 h, flasks were gently swirled, which suspended unattached myocytes and left the majority of fibroblasts attached. The cell suspension was then either plated at 5×10^6 cells/100-mm dish (for HO-1 and glutathione determinations) or 5×10^4 cells/well/microplate (for HSP determination) for 48 h prior to exposure to PAT. This plating density assured nearly confluent cultures of myocytes within 24 h. Myocytes were easily identified because of their distinctive morphology and spontaneous beating. Nonmyocytes constituted less than 10% of the culture as determined by microscopic inspection.

Glutathione determinations. Reduced gluthatione (GSH) levels were determined by high-performance liquid chromatography based on the procedures of Reed *et al.* (1980). Cells were washed twice in Hanks' balanced salt solution (HBSS) prior to the addition of 2 ml of 10% perchloric acid containing 1 mM EDTA. Plates were scraped to remove cells, and the cell suspension was centrifuged at 14,000*g* for 10 min. The supernatant was analyzed for GSH and the pellet was used to assay total protein according to the methods of Lowry *et al.* (1951) as modified by Peterson (1977) with bovine serum albumin as a standard.

Treatment of cells for heme oxygenase and total P450 assays. Cardiac myocytes plated for 48 h in 100-mm plates were washed with HBSS and exposed to PAT in serum-free M199. At the end of the exposure period (18 h), medium was removed from culture dishes, and plates were washed three times with HBBS. One milliliter of 0.1 M Tris buffer (pH 7.4) was added to each plate and myocytes were removed by gentle scraping. Myocytes were pelleted by centrifugation at 1000*g* for 10 min, rinsed with HBSS, and resuspended in microsomal preparation buffer consisting of 0.015 KCl/0.05 M Tris (pH 7.4) and disrupted by pulsed-sonication (3×20-s pulses, 40% duty cycle; Heat Systems–Ultrasonics, Inc. Farmingdale, NY). Lysates were kept on ice throughout these procedures. Lysates were centrifuged at 10,000*g* for 10 min at 4°C; supernatants were collected and centrifuged at 100,000*g* for 1 h (60 Ti rotor, Beckman Model L5–50 Ultracentrifuge). The microsomal pellets were resuspended and assayed for heme oxygenase activity. Total protein was determined according to Lowry *et al.* (1951).

Assay of heme oxygenase activity and total P450. Heme oxygenase activity was determined in myocyte microsomes by the method of Saunders *et al.* (1991) with slight modifications. Rat liver cytosol was used as a source of biliverdin reductase, and 1 ml incubations were stopped after 30 min with the addition of 5 ml ethyl acetate to precipitate protein and extract bilirubin. Sample tubes were vortexed for 30 s and centrifuged at 3000*g* for 10 min at 4°C. Samples were extracted twice with 5 ml of ethyl acetate, and the combined ethyl acetate extracts were evaporated to dryness under nitrogen. Residues were resuspended in 1 ml methanol, and the difference in absorption between 453 and 530 nm was determined. Bilurubin formed was estimated using an extinction coefficient of 60.7 $mM^{-1}cm^{-1}$. Total microsomal P450 was measured by the method of Omura and Sato (1964).

Treatment of cells for induction of heat shock proteins and immunocytochemical detection. Cardiac myocytes plated for 48 h in 96-well microplates were washed with HBSS and exposed to PAT in serum-free M199. To serve as a positive control, one plate of cells was incubated for 1 h at 42°C and returned to 37°C for 18 h. At the end of the 18-h exposure period, medium was removed from the wells, and plates were washed three times with HBBS. After washing, 200 μl of cold cell fixative (70% EtOH with HCl, pH 5) was added to each well and plates were stored at −20°C for 1 h prior to removal of fixative by aspiration. One hundred microliters of 50% fetal bovine serum in phosphate-buffered saline was added as a blocking agent, and plates were incubated for 1 h at 37°C. The blocking agent was removed, and plates were washed three times and incubated at 37°C for 2 h with primary antibody (anti-HSP70, -HSP60 Sigma, and anti-HSP25/27, Stressgen, Victoria, British Columbia, Canada). The unbound primary antibody was removed, and the plates were washed three times. The plates were then incubated for 1 h with 200 μl/well of antimouse-HRP conjugate. The unbound secondary antibody was removed, plates were washed at least three times, and 200 μl of GIBCO (Grand Island, NY) premixed ELISA peroxidase substrate was added to each well. After 30 min, the plate was read at 405 nm. The absorbance of cell-containing wells was compared to a standard curve of known amounts of HSP70 (HSP25/27 are reported as absorbance units/well). Protein (mg/well) was estimated by the method of Lowry *et al.* (1951) after removing substrate and adding NaOH to solubilize the adherent cells.

Western immunoblotting. HO-1 protein levels were estimated in myocytes by Western immunoblotting as described by Saunders *et al.* (1991). Briefly, PAT-exposed cells were washed three times with HBBS. One milliliter of 0.1 M Tris buffer (pH 7.4) was added to each plate, and myocytes were removed by gentle scraping. Myocytes were pelleted by centrifugation at 1000*g* for 10 min, rinsed with HBSS, and resuspended in preparation buffer consisting of 0.015 KCl/0.05 M Tris (pH 7.4) and disrupted by pulsed-sonication (3×20-s pulses, 40% duty cycle) Ten micrograms of cell lysate

was separated by electrophoresis on 12% SDS–polyacrylamide gels as described byLaemmli (1970) and electroblotted onto nitrocellulose membranes. The immunodetection of protein bands (HO-1) on the nitrocellulose membranes was carried out as described previously using the appropriate secondary antibodies conjugated to alkaline phosphatase (Towbin *et al.* 1979). The relative band intensities on the immunoblots were scanned and quantified using a Scanmaster 3 reflecting densitometer and Master Scan version 3.1 analysis software (Scananalytics, Billerica, MA).

Protective effect of low concentrations of PAT on subsequent PAT exposures. Cardiac myocytes were treated with M199 containing 0.5–10 μM PAT 48 h after initial isolation and plating. In experiments in which cylcoheximide was used to inhibit protein synthesis, cells were treated with 2 μg/ml cyclohexamide alone or in combination with the low doses of PAT described above. Twenty-four or 48 h later the medium was removed and replaced by fresh M199 containing 0–200 μM PAT. After an additional 4 h, cell viability was measured. Cell viability was assessed by measuring the release of lactate dehydrogenase (LDH) as previously described (Tirmenstein *et al.*, 1995; Toraason *et al.*, 1990). Beat rates were evaluated as previously described (Tirmenstein *et al.*, 1995; Toraason *et al.*, 1990).

Statistical analysis. Analysis of variance was performed using the Statgraphics, version 5 (STSC, Inc., Rockville, MD) statistical package. Differences between groups were determined using Scheffe's test for multiple comparisons.

RESULTS

Myocyte Toxicity

Exposure of rat neonatal cardiac myocytes to low concentrations of PAT (0–25 μM) did not produce significant cytotoxicity after 18 h as assessed by both cell attachment and LDH release. However, spontaneous beating rate was reduced more than 50% by the 10–25 μM PAT. Data are not shown as results are similar to those previously reported (Tirmenstein, 1995; Toraason *et al.*, 1997).

GSH, Heme Oxygenase, and P450

Control cells were treated with potassium tartrate, which had no significant effect on cellular GSH, heme oxygenase activity, or cytochrome P450 levels. Low concentrations of PAT (5, 10 μM) significantly increased cellular GSH levels in a dose-dependent manner after 18 h (Table 1). PAT at 25 μM did not increase GSH above that induced by 10 μM PAT (data not shown). Low concentrations of PAT also significantly increased heme oxygenase activity and decreased total cytochrome P450 after 18 h. Heme oxygenase activity was induced about ninefold by 5 μM PAT and fivefold by 10 μM PAT.

TABLE 1

Effects of PAT on Glutathione Levels, Heme Oxygenase Activity, and Total P450 in Rat Neonatal Cardiac Myocytes Treated for 18 h.

Treatment	Glutathione (nmol/mg protein)	Heme oxygenase (nmol/mg protein)	Total P450 (nmol/mg protein)
Controls[a]	15.56 ± 2.35[b]	0.55 ± 0.24	0.295 ± 0.55
5 μM PAT	22.05 ± 5.33[c]	4.74 ± 0.63[c]	0.155 ± 0.014[c]
10 μM PAT	38.65 ± 2.34[c]	2.95 ± 0.55[c]	0.135 ± 0.020[c]

[a] Controls cells were treated with 10 μM potassium tartrate (heme oxygenase and total P450) or 25 μM potassium tartrate (glutathione). Cell treatments, reduced glutathione, heme oxygenase activity, and total P450 determinations were performed as described in Methods.

[b] All values are means ± SD ($n = 3$).

[c] Statistically different from controls ($p < 0.05$).

PAT decreased total cytochrome P450 activity in a dose-dependent manner after 18 h. Exposure of cardiac myocytes to 10 μM PAT led to a 54% decrease in total P450 levels.

Induction of Heat Shock Proteins

Myocytes exposed to low concentrations of PAT for 18 h had increased levels of HO-1 as determined by Western blot (Fig. 1) and HSP70 as determined by immunocytochemistry (Fig. 2). Nonlethal heat shock (42°C for 1 h) did not increase HO-1 protein levels, but did increase HSP70 protein levels after 18 h. HSP25/27 was significantly increased above controls at PAT concentrations from 0.5 to 5 μM (Fig. 3). Treatment with 5 μM PAT induced about a 13-fold induction in HSP25/27 protein levels after 18 h, and a comparable increase occurred following treatment with 42°C for 1 h. Heat treatment also increased HSP60 levels, but PAT at 5 or 10 μM did not affect HSP60 levels (data not shown).

Protective Effect of Low Concentrations of PAT on Subsequent PAT Exposures

Exposure to 200 μM PAT resulted in release of 73% of the total myocyte LDH into the culture medium after 4 h (Fig. 4). Prior treatment with low concentrations of PAT (0.5–10 μM) for 48 h protected against a subsequent 200 μM PAT challenge. The extent of this protection was dose dependent. Pre-

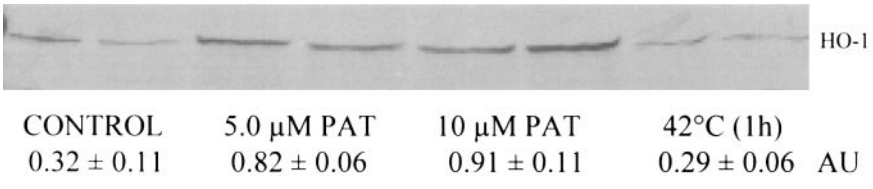

FIG. 1. Effects of PAT treatment (0–10 μM) or heat shock (1 h at 42°C) on cellular HO-1 protein levels in rat neonatal cardiac myocytes. Control cells were treated with 10 μM potassium tartrate. Protein levels were measured following an 18-h exposure to the indicated PAT concentration or 18 h after heat shock treatment. Absorbance units (AU) are the means ± SD of duplicate measurements.

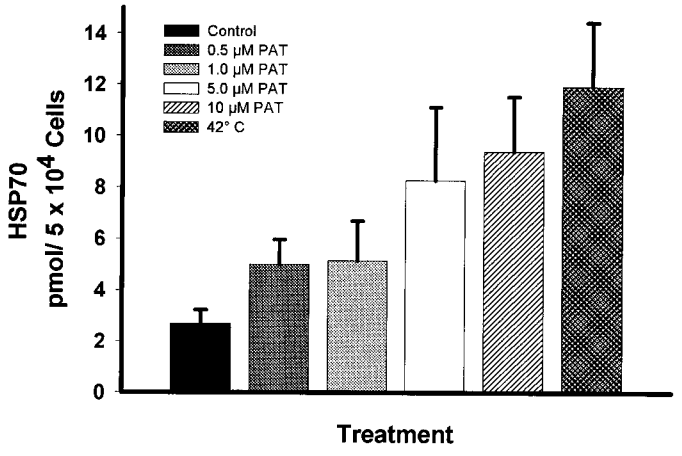

FIG. 2. Effects of PAT treatment (0–10 μM) or heat shock (1 h at 42°C) on HSP 70 levels in rat neonatal cardiac myocytes. Control cells were treated with 10 μM potassium tartrate. Protein levels were measured following an 18-h exposure to the indicated PAT concentration or 18 h after heat shock treatment. Bars, means + SD; $n = 6$. All treatment responses are significantly greater than the control ($p < 0.05$).

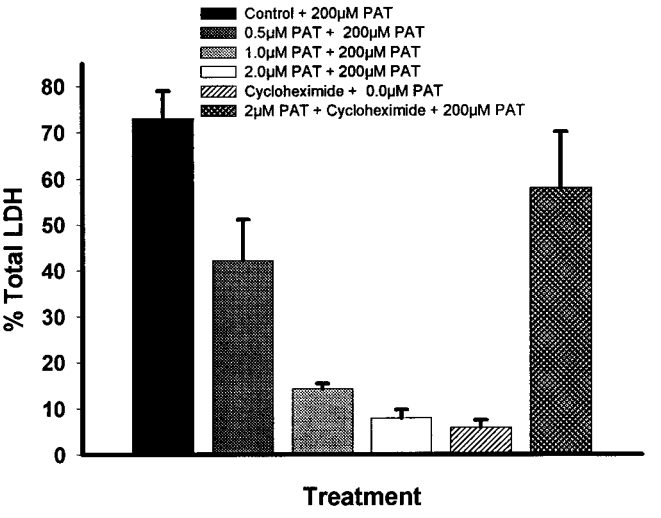

FIG. 4. Effect of low-level PAT pretreatment and protein synthesis inhibition on PAT toxicity in rat neonatal cardiac myocytes. Control cells were treated with 2 μM potassium tartrate. Cells were pretreated with PAT (0–2 μM), cycloheximide (2 μg/ml), or 2 μM PAT + cycloheximide (2 μg/ml) for 48 h. After the 48-h period, PAT-exposed and PAT + cycloheximide-treated cells were then exposed to a 200 μM PAT challenge. After an additional 4 h, the percentage of total LDH in the culture dish that was released from myocytes into the culture medium was determined. Bars, means + SD; $n = 6$. All treatment responses are significantly less than the control + 200 μM PAT ($p < 0.05$) with the exception of 2 μM PAT + cycloheximide + 200 μM PAT.

treatment with 2 μM PAT reduced the LDH release induced by 200 μM PAT from 73% to less than 10%. The extent of this protection by low concentrations of PAT was also time dependent. If cells were pretreated with 1 μM PAT for 24 h, 200 μM PAT resulted in release of 30% of total LDH (data not shown). In contrast, if cells were pretreated with 1 μM PAT for 48 h, 200 μM PAT resulted in release of only 14% of total LDH (Fig. 3). Cotreatment of cardiac myocytes with the protein synthesis inhibitor, cycloheximide (2 μg/ml), eliminated the protection afforded to cells by pretreatment with low concentrations of PAT. In cells treated only with cycloheximide, LDH in the culture medium was only 6% of total LDH in the culture dish.

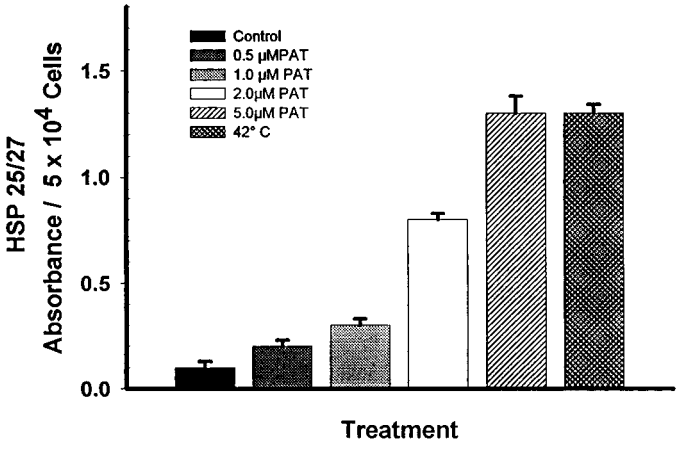

FIG. 3. Effects of PAT treatment (0–10 μM) or heat shock (1 h at 42°C) on HSP 25/27 levels in rat neonatal cardiac myocytes. Control cells were treated with 10 μM potassium tartrate. Protein levels were measured following an 18-h exposure to the indicated PAT concentration or 18 h after heat shock treatment. Bars, means + SD; $n = 6$. All treatment responses are significantly greater that the control ($p < 0.05$).

DISCUSSION

Previously, we have shown that concentrations of PAT greater than 50 μM induce significant increases in the release of thiobarbituric acid reactive substances and LDH in neonatal cardiac myocytes after a 4-h exposure (Tirmenstein *et al.*, 1995). In these studies, pretreatment with various antioxidants prevented or attenuated this PA-induced lipid peroxidation and cytotoxicity. In addition, PAT significantly decreased protein thiols, cellular glutathione, and ATP levels in cardiac myocytes (Tirmenstein *et al.*, 1997). In the present study, low, nonlethal concentrations of PAT produced a dose-dependent increase in cellular GSH levels after 18 h. While this is the first report of increased cellular GSH content in response to a low-level antimony exposure, a similar response has been reported in rats exposed to other metals. In a study conducted by Sasame and Boyd (1978), cobaltous chloride, cadmium chloride, manganese chloride, and lead nitrate all increased hepatic GSH levels in rats 24 h after administration. Increases in GSH levels *in vivo* and *in vitro* have been suggested to occur in response to oxidative stress. Woods and Ellis (1995) demonstrated that prolonged exposures to methyl mercury produced oxidative stress in rat kidneys and a two- to threefold elevation in renal cortical GSH levels. This increase in GSH was associated with

an up regulation of γ-glutamylcysteine synthetase expression, the rate-limiting enzyme involved in the synthesis of GSH (Woods and Ellis, 1995). A similar induction of γ-glutamylcysteine synthetase may explain the increases in GSH levels observed in cardiac myocytes following PAT exposures.

Treatment of neonatal rat cardiac myocytes with 1–10 μM PAT resulted in dose-dependent increases of heme oxygenase activity and microsomal HO-1 protein levels as well as a concurrent loss of cytochrome P450. HO-1 is the inducible form of heme oxygenase, the rate-limiting enzyme in heme degradation. HO-1 is induced by a wide variety of conditions, including oxidative stress, sulfhydryl reactive compounds, metals, and heme compounds (Maines, 1988). *In vivo* and *in vitro* studies have reported that oxidative stress and exposure to metals increase heme oxygenase activity and HO-1 protein levels in cardiac tissue. Maines and Kappas (1977) demonstrated a loss of the heme-containing enzyme cytochrome P450 and induction of heme oxygenase activity in the livers of rats treated with metals. In this study, heme oxygenase activity was also induced in the heart by iron, cobalt, nickel, copper, platinum, and mercury. The mechanism for the loss of cytochrome P450 following heme oxygenase induction is not known. Heme oxygenase induction may lead to the loss of cytochrome P450 by limiting the availability of heme for synthesis of cytochrome P450 or by directly catalyzing the degradation of the enzyme. Other *in vivo* studies have demonstrated that metals (Neil *et al.*, 1995), ischemia–reperfusion (Katayose *et al.*, 1993), and hemodynamic stress (Maulik *et al.*, 1996) induce heme oxygenase activity and HO-1 protein levels in the heart. Hoshida *et al.* (1996) using rat neonatal cardiac myocytes examined the effect of tissue culture and oxidative stress on HO-1 induction. They found that HO-1 levels increased 12–48 h after isolation in cardiac myocytes and oxidative stress caused further increases.

Several studies have concluded that the induction of heat shock or stress proteins can protect the heart from toxicity. HSP70 is induced in cardiac tissue by heat stress (Hutter *et al.*, 1994), hypoxia/reoxygenation (Iwaki *et al.*, 1993), and chemical treatments (Hoshida *et al.*, 1997). Researchers have hypothesized that elevated levels of the heat shock proteins HSP70 (Hutter *et al.*, 1994; Iwaki *et al.*, 1993; Hoshida *et al.*, 1997), HSP25/27 (Sharma *et al.*, 1996), and HSP60 (Lau *et al.*, 1997) can contribute to protection of cardiac tissue from subsequent insults. Marber *et al.* (1995) using transgenic mice that over-express HSP70 reported that the expression of high levels of HSP70 protected isolated hearts against ischemic injury. Similarly, Martin *et al.* (1997) using recombinant methods constructed HSP25/27 over-expressing cardiac myocytes. Cells that over-expressed HSP25/27 displayed significant protection against ischemia. In the present study, PAT treatments were observed to induce HSP70 and HSP25/27 protein expression in cardiac myoctes in a dose-dependent manner. HSP60, a stress protein associated with mitochondria, was elevated in cardiac myocytes exposed to heat shock (1 h at 42°C) but not in PAT-exposed cells.

Pretreatment of rat neonatal cardiac myocytes with nonlethal concentrations of PAT (0.5–10 μM) for 24–48 h prevented or diminished rates of cell death when cells were later treated with PAT concentrations that are normally lethal to cardiac myocytes. This protection was blocked when the protein synthesis inhibitor cycloheximide was added to the PAT pretreatment. These data suggest that the PAT pretreatment induces the synthesis of proteins that subsequently protect against PAT-induced cell death. The identity of these proteins are not known, but stress proteins are likely candidates. The ability of low doses of toxicants to protect against subsequent lethal doses of the same toxic agent has been previously demonstrated. In studies conducted by Kampinga *et al.* (1995), pretreatment of HeLa S3 cells with arsenite, ethanol, diamide, and heat protected against subsequent lethal doses of the same agent. Protection was associated with the induction of stress proteins. We previously demonstrated that addition of glutathione to cultured cardiac myocytes provided a degree of protection from the lethality of PAT (Tirmenstein *et al.*, 1995). The protection provided by exogenous GSH is most likely due to the binding of antimony by sulfhydryl groups present in GSH (Tirmenstein *et al.*, 1997). Taken together with present results, it is reasonable to conclude that increased GSH levels produced by low-level PAT exposure is a factor in the protection against subsequent lethal concentrations of PAT.

This study reports that treatment of rat neonatal cardiac myocytes with nonlethal concentrations of PAT increases cellular GSH and heme oxygenase activity and decreases total cytochrome P450 levels. Low doses of PAT also increased protein levels of the stress proteins HO-1, HSP70, and HSP25/27. Pretreatment of cardiac myocytes with 0.5–10 μM PAT for 24–48 h protected cells against a later 200 μM PAT exposure. This protection was abrogated by inhibiting protein synthesis. Increased GSH levels and heme oxygenase activity and the induction of stress protein have been associated with protection against oxidative stress. Since antimony has been demonstrated to induce oxidative stress in neonatal cardiac myocytes (Tirmenstein *et al.*, 1995), these alterations may protect against antimony-induced cell death.

The concentrations of PAT used in the present study and previous cardiac myocyte toxicity studies (Tirmenstein *et al.*, 1995, 1997; Toraason *et al.*, 1997; Wey *et al.*, 1997) ranged from 0.5–200 μM PAT. These solutions contain 0.06–24 μg/ml antimony and are within the range of therapeutic antimony concentrations. For example, leishmaniasis patients receiving daily intramuscular injections of sodium stibogluconate or meglumine antimoniate maintained blood antimony concentrations of 1–12 μg/ml (Chulay *et al.*, 1988). In a study of the efficacy of pentavalent antimony in combination with other drugs, serum concentrations of antimony in dogs ranged from 0.125 to 37.1 μg/ml during the 24 h following administration of therapeutic doses (Belloli *et al.*, 1995). In asymp-

tomatic workers exposed to antimony trioxide or stibine compounds in a battery factory, whole blood concentrations of antimony reached 0.01 μg/ml (Kenter *et al.*, 1995). Therefore, it is reasonable to anticipate that PAT effects observed in cultured cardiac myocytes may occur to some degree during therapeutic treatment with or occupational exposure to antimony compounds.

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