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Antimony-induced alterations in thiol homeostasis and adenine nucleotide status in cultured cardiac myocytes[1]

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Abstract

Cultured cardiac myocytes were exposed for up to 4 h to 50 and 100 μM potassium antimonyl tartrate (PAT). After 4 h, 50 and 100 μM PAT killed 14 and 33% respectively of the cardiac myocytes. PAT-induced alterations in both protein and nonprotein thiol homeostasis. Transient increases in oxidized glutathione disulfide (GSSG) levels were detected after cells were treated with 100 μM PAT for 2 h. After 4 h, both concentrations of PAT significantly depleted reduced glutathione (GSH) levels. Protein thiols levels were also decreased after a 2-h exposure to 50 and 100 μM PAT. Cells treated with 50 μM and 100 μM PAT had a 15% and 40% reduction respectively in protein thiols after 4 h. PAT also significantly inhibited glutathione peroxidase and pyruvate dehydrogenase activity in cardiac myocytes. Pyruvate dehydrogenase activity levels were inhibited as early as 1 h after cells were treated with both concentrations of PAT. Cardiac myocyte ATP levels were also decreased by PAT, but only after a 4-h exposure to 50 μM and 100 μM PAT. Decreases in cellular ATP levels paralleled PAT toxicity put appeared to be secondary to other cellular changes initiated by PAT exposure. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

Antimony has extensive uses in industry both as a constituent of nonmetal products and metal alloys. Antimony-containing compounds are used in the manufacture of paints, pigments, ceramics, pyrotechnics, fire retardants and glass (Carson et al., 1986; Stokinger, 1981). Antimony is also used

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in the semiconductor industry and has limited but important usage as an antiparasitic drug (Winship, 1987). The National Institute for Occupational Safety and Health (NIOSH) estimates that more than 250 000 individuals in the United States are exposed to antimony-containing compounds at work (NIOSH, 1988, 1989, 1990).

Antimony-containing compounds produce cardiac functional alterations and toxicity in both experimental animals and humans. Bradley and Fredrick (1941) demonstrated that the administration of antimony-containing compounds to rats induced cardiac toxicity. The toxicity to the heart was said to be specific and was observed at the lowest doses of the compounds tested. Administration of potassium antimonyl tartrate (PAT) to experimental animals has been associated with degeneration of the myocardium and an increase in the fibrous and connective tissue of the heart (Bradley and Fredrick, 1941; Brieger et al., 1954).

The cardiac effects of antimony in humans have been observed as a toxic side effect of antimonial drug administration. Altered electrocardiograms and autopsies that revealed cardiac toxicity as the cause of death have been reported in individuals treated with antimonial drugs (NIOSH, 1978; Hepburn et al., 1994; Honey, 1960; Winship, 1987). Cardiac toxicity has also been associated with occupational exposures to antimony. Six sudden deaths were reported in workers following exposure to antimony trisulfide for a period of 8–24 months at a factory manufacturing resinoid grinding wheels. Heart disease was suspected in all but one of these 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).

Although antimony administration has been associated with cardiac toxicity, little is known concerning the biochemical effects of antimony on heart cells. Our laboratory has recently conducted a series of experiments using cultured cardiac myocytes isolated from neonatal rats to investigate the cellular effects of antimony. We previously reported that PAT induced oxidative stress in cardiac myocytes. Both lipid peroxidation and cell death were induced in a concentration-dependent manner after a 4-h exposure to PAT (Tirmenstein et al., 1995). Antioxidants protected against both lipid peroxidation and cell death following exposure to 100–200 μM PAT for 4 h. However, our studies also demonstrated that antioxidants protected against lipid peroxidation but not cell death following an 18-h exposure to 100 μM PAT. Our results suggest that other mechanisms besides lipid peroxidation may contribute to cell death following long-term exposures to PAT. Thiol-containing compounds were very effective in preventing PAT toxicity following both 4- and 18-h exposures to PAT (Tirmenstein et al., 1995). This protection most likely relates to the ability of thiol compounds to interact and form complexes with trivalent antimony compounds. The present study was conducted to further characterize the cellular effects of PAT on cardiac myocytes from neonatal rats. In the workplace, cardiovascular disease has been associated with exposure to antimony oxides or sulfides which are relatively insoluble in water. However, PAT, antimony trisulfide, antimony pentasulfide, antimony trioxide or antimony pentaoxide all have been demonstrated to produce comparable cardiac toxicity in rats that can culminate in myocardial failure and death (Bradley and Fredrick, 1941). Since PAT is a soluble form of antimony, it was used to expose cardiac myocytes to antimony in culture medium.

The present results provide additional information characterizing the cellular changes induced by PAT in cardiac myocytes. We demonstrate that a 4-h exposure to 50–100 μM PAT leads to depletion of glutathione (GSH) and protein thiols, inhibition of certain key enzymes involved in energy production and oxidant defense, decreases in adenine nucleotides and ultimately to cell death. These cellular alterations may represent important sites of action of antimony in the heart. Additional in vivo studies are needed to confirm that these changes also occur in the heart after the administration of antimony to experimental animals.

2. Materials and methods

2.1. Chemicals

Newborn calf serum was purchased from Hyclone, (Logan, UT). PAT hydrate (+99%, CAS No. 28300-74-5) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). All other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific Co. (Cincinnati, OH).

2.2. Preparation and incubation 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 the 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 plated at 6×10^6 cells per 100-mm dish for biochemical and cytotoxicity assays 48 h after plating. 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. Cardiac myocytes plated for 48 h in 100-mm wells were washed with Hanks' balanced salt solution (HBSS) and exposed to PAT in serum-free M199. M199 solutions containing test concentrations of PAT were added as 10-ml aliquots to culture dishes. At the end of the exposure period, buffer was removed from culture dishes and cells were washed twice with HBSS prior to subsequent biochemical assays. In our experiments viable cultured myocytes adhere to the surface of the culture dish while dead myocytes tend to detach from the dish. All cultures were extensively washed prior to biochemical determinations in order to minimize the number of dead cells present in our assays.

2.3. Thiol Determinations

Reduced (GSH) and glutathione disulfide (GSSG) levels were determined as previously described (Tirmenstein et al., 1995). Protein thiols were assayed by reacting cells with the thiol specific reagent monobromobimane (mBBr). These procedures were based on the methods of Cotgreave et al. (1988). After washing cardiac myocytes with HBBS, cells were incubated with 4 mM mBBr in 2 ml of reaction buffer (250 mM sucrose, 1 mM EDTA, 5 mM HEPES, 25 mM *N*-ethylmorpholine, pH 7.4) at room temperature for 5 min. Following this reaction, cells were washed twice with HBBS. Cells were scraped off dishes after the addition of 2 ml cold 66% (v/v) ethanol, sonicated and transferred to microcentrifuge tubes. Proteins were pelleted by centrifuging for 5 min at $14\,000 \times g$. Proteins were washed twice more by sonication in 66% (v/v) ethanol. Final protein pellets were resuspended in 100 μl 10% sodium dodecyl sulfate and then diluted with 4.9 ml water prior to measuring fluorescence at excitation/emission wavelengths of 394/480 nm. Aliquots were withdrawn for total protein determinations. Protein thiol standards were prepared from bovine serum albumin (BSA), and a value of 0.7 –SH/mol was used for quantitation of protein thiols (Andersson, 1966).

2.4. Enzyme assays

Lactate dehydrogenase (LDH) was measured with a Sigma kit (Procedure No. 228-UV) in cell treatment buffer and in cells following a 10-min incubation in 1% Triton X-100 in HBSS solution at 37°C. LDH activity is expressed as percentage of LDH in treatment buffer relative to total LDH in the cell culture dish. LDH leakage from the cell into the treatment buffer indicates irreversibly increased membrane permeability with loss of cytosolic constituents and was used as an index of cell killing as previously described (Shier and DuBourdieu, 1992).

Cell homogenates were prepared from washed cells by scraping myocytes from plates in phosphate buffered saline. Cell suspensions were sonicated for 20 s and then centrifuged for 10 min at 14 000 × *g*. Supernatants were used to assay glutathione peroxidase (Lawrence and Burk, 1976), glutathione reductase (Worthington and Rosemeyer, 1974) superoxide dismutase (Spitz and Oberley, 1989) and catalase (Wall et al., 1993) according to established spectrophotometric methods. Pellets were resuspended in 50 mM Tris–HCl, 0.5 mM EDTA and 0.2% Triton pH 7.8 and were used for determination of pyruvate dehydrogenase activity according to the procedures of Elnageh and Gaitonde (1988). Lipoamide dehydrogenase was used as an electron carrier, although similar results were obtained if phenazine methosulfate was substituted as the electron carrier. Aliquots were used to determine total proteins in both the supernatant and pellet.

2.5. Adenine nucleotide determinations

Cells were washed with HBSS, and then 1 ml of 0.6 M trichloroacetic acid was added to culture dishes. Dishes were scraped and the resulting acid-cell suspension was sonicated and allowed to remain on ice for 10 min. The acid-cell suspension was centrifuged at 14 000 × *g* for 2 min and the acid supernatant was withdrawn for adenine nucleotide determinations by HPLC as described by Pogolotti and Santi (1982). Pellets were assayed for total protein.

2.6. Protein assays

Total protein was measured according to Lowry et al. (1951) as modified by Peterson (1977) with BSA used as a standard.

2.7. Statistical analysis

One-way analysis of variance was performed using the Statgraphics, version 5 (STSC Inc., Rockville, MD) statistical package. Differences between controls and specific treatments were determined using Scheffe's test for multiple comparisons.

3. Results

3.1. Effects of PAT on cell viability

The time course for 50 and 100 μM PAT-induced LDH release from cardiac myocytes was examined. In these studies, LDH release was used as a measure of toxicity. However, similar results were also obtained when trypan blue exclusion was used as an index of PAT-induced myocyte toxicity (data not shown). The pooled results from three separate experiments indicate that 100 μM PAT killed 9.4 ± 6.2% (mean ± SD) of the cells after 3 h and 33.4 ± 11.5% (mean ± SD) of the cells after 4 h. Exposures to 50 μM PAT killed 13.8 ± 11.0% of the cells after 4 h. Only a 4-h exposure to 100 μM PAT produced a statistically significant increase in LDH release as compared to control cultures ($P < 0.05$). Less than 4% of the cells were killed in control cultures during the 4-h incubation period.

3.2. Effects of PAT on cellular thiol levels

Cellular GSH and GSSG levels were measured in untreated controls and PAT-treated cardiac myocytes following 2 and 4 h exposures (Table 1). Control levels of GSH were about 4.5 nmol per mg protein, and GSSG levels were below the limits of detection. There was no effect on GSH levels following a 2-h exposure to 50 μM PAT. However, GSH levels were reduced to 30% of control values after a 4-h exposure to 50 μM PAT. There was a significant decrease in GSH levels following a 2-h exposure to 100 μM PAT and also a detectable increase in GSSG levels at this time point. Following a 4-h exposure to 100 μM PAT, GSH levels were reduced to less than 10% of controls.

The effects of PAT on protein thiols were also measured (Fig. 1). Both 50 and 100 μM PAT reduced protein thiol levels to some extent at 2 h, although this decrease was only significantly different from controls for cells treated with 100 μM PAT. Protein thiols were also significantly decreased following 4-h exposures to 50 μM PAT and 100 μM PAT. Protein thiols were reduced by 15% with 50 μM PAT and 40% with 100 μM PAT relative to 4-h controls.

Table 1
Effects of PAT on glutathione and adenine nucleotide levels (nmol/mg protein) in cardiac myocytes

Treatment	GSH	GSSG	AMP	ADP	ATP
Untreated					
2 h	4.62 ± 0.45[a]	ND[b]	ND[c]	4.97 ± 0.94	34.98 ± 5.69
4 h	4.45 ± 0.78	ND	ND	4.42 ± 0.93	34.97 ± 4.82
PAT (50 μM)					
2 h	4.67 ± 0.40	ND	ND	4.96 ± 1.34	36.87 ± 5.36
4 h	1.39 ± 0.35[d]	ND	0.99 ± 0.97	4.64 ± 1.25	28.78 ± 1.96
PAT (100 μM)					
2 h	2.94 ± 0.58[d]	0.41 ± 0.13	ND	4.95 ± 1.17	41.94 ± 4.09
4 h	0.44 ± 0.11[d]	ND	2.54 ± 2.38	2.46 ± 0.36[d]	10.24 ± 3.63[d]

[a]All values expressed as nmol/mg protein and represent the mean ± SD, $n \geq 6$ from three separate experiments.
[b]Not detectable (GSSG values less than 0.15 nmol/mg protein).
[c]Not detectable (AMP values less than 0.5 nmol/mg protein).
[d]Values significantly different from controls ($P < 0.05$).

3.3. Effects of PAT on antioxidant enzymes

We examined the effects of PAT on antioxidant enzymes to determine if inhibition of these enzymes by PAT could in part account for the oxidative stress associated with PAT exposures. PAT decreased glutathione peroxidase activity levels in a time- and concentration-dependent manner in cardiac myocytes (Fig. 2). Enzyme activity levels were unchanged after 3 h with 50 μM PAT but were decreased by 27% after a 3-h exposure to 100 μM PAT. A PAT-induced decrease in enzyme activity of 40% was seen after a 4-h exposure to 50 μM PAT, and a 60% decrease was seen with 100 μM PAT. The addition of 50 or 100 μM PAT to control cell homogenates did not directly inhibit glutathione peroxidase activity (data not shown).

Studies also examined the effects of PAT on glutathione reductase, superoxide dismutase and catalase activity levels. No significant inhibition of these enzymes was detected after exposing cardiac myocytes for up to 4 h to 100 μM PAT (data not shown).

3.4. Effects of PAT on pyruvate dehydrogenase

PAT has a high affinity for vicinal dithiols (Basinger and Jones, 1981). Therefore, enzymes containing vicinal dithiols which are required for activity may be especially susceptible to inhibition by PAT. Pyruvate dehydrogenase is an example of such an enzyme. The effects of PAT on pyruvate dehydrogenase activity are shown in Fig. 3. Pyruvate dehydrogenase was inhibited in a time- and concentration-dependent manner by PAT. Both 50 and 100 μM PAT produced inhibition of pyruvate dehydrogenase as early as 1 h after the addition of PAT. Following 4-h exposures to 100 μM PAT, activity was reduced to about 7% of controls. When 100 μM PAT was added to control cell homogenates and incubated at room temperature for 30 min, there was a dramatic reduction in pyruvate dehydrogenase activity (data not shown).

3.5. Effects of PAT on adenine nucleotide levels

The effect of PAT on the adenine nucleotide status of the cell is reported in Table 1. In control cells, ATP levels were measured at 35 nmol per mg protein (seven times greater than ADP levels). AMP could not be measured in control cells (less than 0.5 nmol per mg protein). The addition of 50 or 100 μM PAT to myocyte cultures did not significantly decrease ATP levels after 2 h. In fact, there was a slight nonsignificant increase in ATP levels in myocytes exposed for 2 h to 100 μM PAT. ATP levels decreased after 4-h exposures to 50 or 100 μM PAT. ATP levels declined by approximately 18% with 50 μM PAT and 70% with 100 μM PAT at 4 h. Significantly decreased

ADP levels were evident only after 4-h exposures to 100 μM PAT. AMP levels increased after cells were treated with 50 or 100 μM PAT for 4 h, but total cellular adenine nucleotide (AMP + ADP + ATP) levels were decreased after a 4-h exposure to 50 or 100 μM PAT.

4. Discussion

The present study provides new information on the biochemical effects of antimony on cardiac myocytes and further supports the association between antimony exposures and the generation of oxidative stress in cultured cardiac myocytes (Tirmenstein et al., 1995). Using LDH release as an index of cell death, our results indicate that no

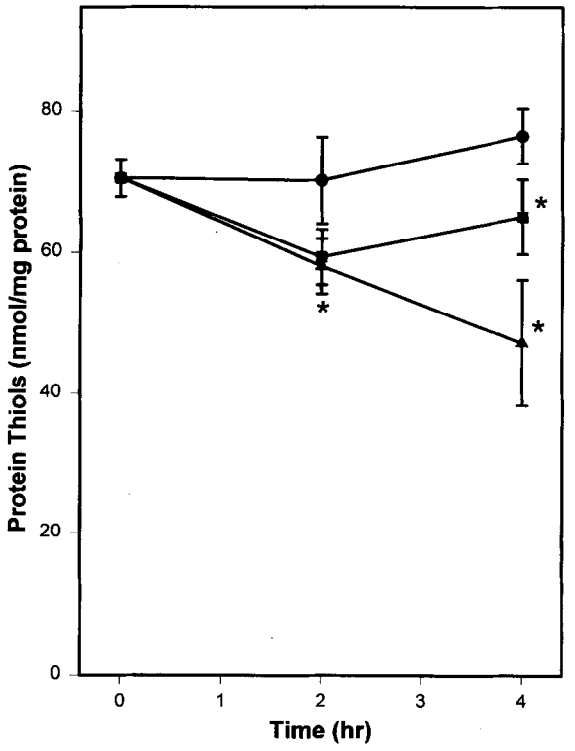

Fig. 1. Effects of PAT on protein thiol levels in neonatal cardiac myocytes. Protein thiol levels were measured for untreated controls (●) and cultures which had been treated with 50 μM (■) or 100 μM (▲) PAT. *Significantly different from untreated controls at corresponding time point ($P < 0.05$). All values are expressed as the mean ± SD, $n = 6$ from three separate experiments.

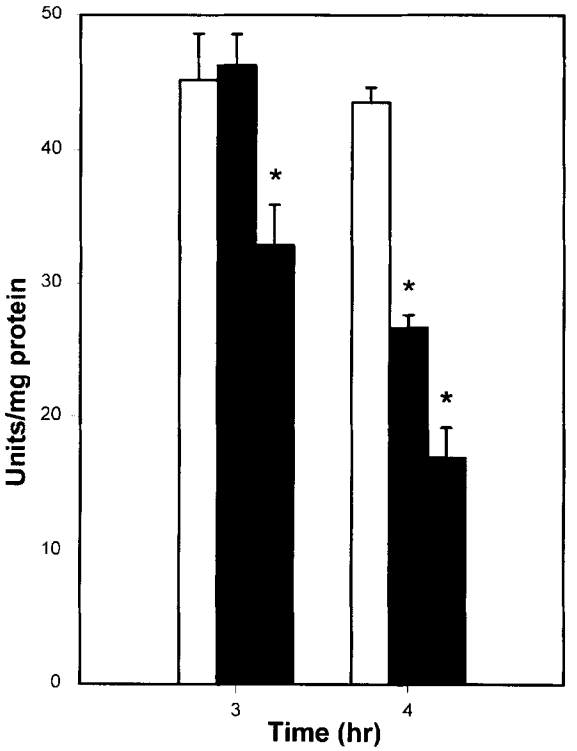

Fig. 2. Effects of PAT on glutathione peroxidase activity in neonatal cardiac myocytes. Glutathione peroxidase activity levels were measured for untreated controls (□) and cultures which had been treated with 50 μM (▩) and 100 μM (■) PAT. *Significantly different from control values ($P < 0.05$). All values are expressed as the mean ± SD, $n \geq 3$ from three separate experiments.

significant increase in cell death relative to controls occurred following exposures to 50 and 100 μM PAT after 2 and 3 h. Exposures to 50 μM and 100 μM PAT for 4 h increased LDH release by about 14 and 33% respectively. These concentrations were used in all subsequent experiments to minimize toxicity so that cellular changes which precede cell death could be distinguished from those which follow cell death.

Our results indicate that PAT produces significant alterations in cellular thiol homeostasis in cardiac myocytes. These events are detectable as early as 2 h after the addition of PAT. Changes were detected in both protein thiols and cellular GSH and GSSG levels. Following exposure to 100 μM PAT, there was a transient increase in GSSG levels detected in cardiac myocytes. In fact,

about 20% of the total glutathione measured in cells was present in the disulfide form at this time point. GSSG levels in rat heart are normally present at 2.5% of the total glutathione (Ishikawa and Sies, 1984). This increase in GSSG levels provides additional support for the concept of PAT-induced oxidative stress in cultured cardiac myocytes. By 4 h, both concentrations of PAT had significantly reduced GSH levels although no GSSG was detected at this time point.

The manner in which GSH levels are depleted by PAT in cardiac myocytes is unknown. Trivalent antimony is known to react with thiol groups to form thioantimonites (Stemmer, 1976). The formation of a GSH–antimony complex and the export of this complex from the cell may lead to the depletion of cellular GSH levels. Trivalent arsenic, which shares similar chemical properties with trivalent antimony, forms a reversible complex with three GSH molecules (Delnomdedieu et al., 1994). Dissociation of this complex was found to be catalyzed by extremes in pH. Delnomdedieu et al. (1994) reported that the GSH–arsenic complex was stable in the pH range from 1.5 to 7. The procedures we used for GSH and GSSG analysis exposed cell extracts first to acidic and then basic conditions, both of which fell outside this pH stability range. These extremes in pH may preclude the detection of the GSH–antimony complex. Therefore, it is likely that the actual level of free GSH present in the cell is overestimated by our procedures. The presence of the GSH–antimony complex may also prevent the oxidation of GSH to GSSG in myocytes and this may in part explain why GSSG was only detected after a 2 h exposure to 100 μM PAT. Further experiments are required to fully delineate the mechanism of antimony-induced GSH depletion.

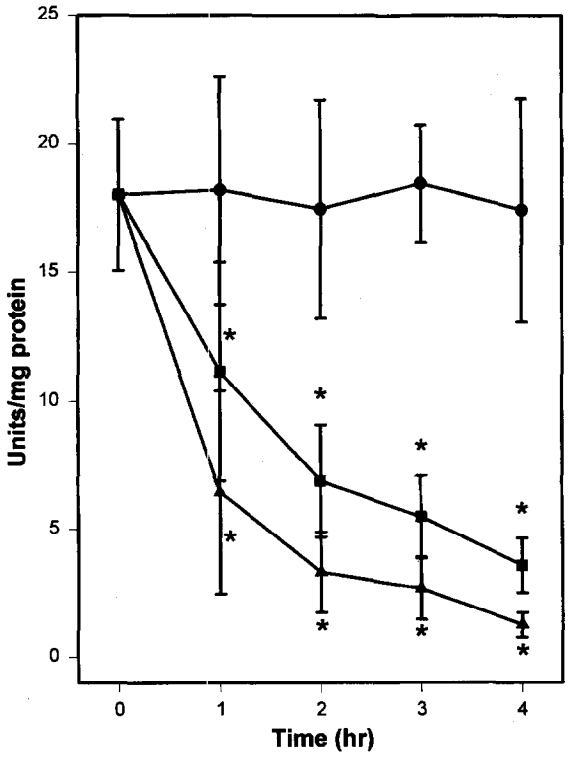

Fig. 3. Effects of PAT on pyruvate dehydrogenase activity in neonatal cardiac myocytes. Pyruvate dehydrogenase activity levels were measured for untreated controls (●) and cultures which had been treated with 50 μM (■) or 100 μM (▲) PAT. *Significantly different from control values ($P < 0.05$). All values are expressed as the mean ± SD, $n \geq 3$ from three separate experiments.

Thiol-antimony complexes may also form between antimony and protein thiols. Our experiments indicate that by 2 h both 50 and 100 μM PAT depleted protein thiols by about 15%. After a 4 h exposure to 100 μM PAT, protein thiols were reduced to about 40% of controls. Procedures for the measurement of protein thiols did not involve treating cellular homogenates with extremes in pH. This may explain why there is a 15% depletion of protein thiols following a 2 h exposure to 50 μM PAT without an apparent loss of GSH at this time point.

We examined the effects of PAT on antioxidant enzymes to determine if inhibition of these enzymes could explain in part the association between PAT exposures and the generation of oxidative stress in cardiac myocytes. PAT exposures had no effect on glutathione reductase, superoxide dismutase and catalase activity. However, glutathione peroxidase activity was inhibited in cells exposed to PAT. This inhibition appears to be by an indirect mechanism since no inhibition of the enzyme was detected when PAT was added to untreated homogenates. Previous studies have shown that glutathione peroxidase is inhibited by reactive oxygen species such as superoxide radical (Blum and Fridovich, 1985). Inhibi-

tion of glutathione peroxidase was also seen in experiments in which isolated cardiac rat myocytes were subjected to oxidative stress (Kirshenbaum et al., 1995). PAT-induced oxidative stress may therefore be responsible for the inhibition of glutathione peroxidase activity in cardiac myocytes. Between 3 and 4 h, there is significant inhibition of glutathione peroxidase activity in myocytes treated with 50 and 100 μM PAT as well as a depletion of glutathione. This impairment of the GSH–GSH peroxidase system is likely to increase the susceptibility of cardiac myocytes to oxidative stress.

Pyruvate dehydrogenase is a multienzyme complex found in the mitochondria matrix. Since pyruvate dehydrogenase contains the coenzyme dihydrolipoic acid, it is inhibited by agents which have an affinity for vicinal dithiols. Arsenite is a well known inhibitor of pyruvate dehydrogenase, and is thought to inhibit the enzyme by binding to the dihydrolipoic acid moiety of pyruvate dehydrogenase (Aposhian, 1989). Antimony also appears to be a direct inhibitor of pyruvate dehydrogenase. In our experiments, the addition of PAT to homogenates of cardiac myocytes led to a dramatic decrease in pyruvate dehydrogenase activity. Pyruvate dehydrogenase is a key enzyme in cellular metabolism and regulates whether glucose is oxidized by anaerobic or by aerobic metabolism. Since the aerobic metabolism of glucose generates 18 times more ATP than glycolysis, inhibition of this enzyme would be expected to restrict the aerobic metabolism of glucose and eventually lead to decreases in cellular ATP levels. Analysis of the effects of PAT on adenine nucleotides indicates that as expected PAT decreases ATP levels. After a 4-h exposure to 100 μM PAT, ATP levels were reduced to less than 30% of controls. However, the depletion of ATP appears to be a late event in PAT-induced killing of cardiac myocytes. ATP levels were actually increased relative to controls after a 2-h exposure to 100 μM PAT. This increase may be related to the PAT-induced cessation of spontaneous beating activity in cardiac myocytes at this time point (Tirmenstein et al., 1995), and the resulting reduced metabolic demand. Our results suggest that there is an apparent lag between inhibition of pyruvate dehydrogenase activity and decreases in ATP levels. The depletion of ATP is likely to contribute to the death of cardiac myocytes although this depletion appears to be secondary to other events initiated by PAT exposures.

In summary, this study demonstrates that PAT induces several important biochemical alterations in cardiac myocytes. The disruption of cellular thiol homeostasis including the depletion of GSH and protein thiols are early events which may contribute to cellular toxicity. Enzymes which contain vicinal dithiols such as pyruvate dehydrogenase also appear to be primary targets for the actions of PAT. In this regard, antimony is similar to arsenic in its mode of action. Additional studies in our laboratory indicate that PAT also disrupts cellular calcium homeostasis and that this disruption is also an early event in cell toxicity (Wey et al., 1995). GSSG levels are elevated as soon as 2 h after the addition of PAT suggesting that oxidative stress may be induced prior to cell death. However, the source of this PAT-induced oxidative stress is unknown. In experiments in our laboratory, we have been unable to demonstrate that antimony can directly reduce molecular oxygen (using oxidation of 2-deoxyguanosine as an indictor of hydroxyl radical formation, data not shown). Thus, antimony is distinct from metals such as iron and copper which have been shown to produce reactive oxygen species through the direct reduction of molecular oxygen. The inhibition of glutathione peroxidase and the depletion of GSH are likely to increase the toxic effects of any background levels of oxidative stress or any increased reactive oxygen species generated in response to PAT exposures.

Further studies are required to investigate whether the biochemical alterations which we have observed following antimony exposures to cardiac myocytes also occur in the hearts of rats exposed to antimony. The present study identifies several biochemical alterations which may be critical targets of antimony exposure in vivo. These targets include the disruption of thiol homeostasis, the inhibition of dithiol containing enzymes such as pyruvate dehydrogenase and the loss of cellular ATP. These events may lead to the loss of beating activity or the death of certain popula-

tions of cardiac myocytes thereby contributing to antimony-induced cardiac toxicity.

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