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Antimony-Induced Oxidative Stress and Toxicity in Cultured Cardiac Myocytes[1]

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Cardiac myocytes were exposed to concentrations of potassium antimonyl tartrate (PAT) ranging from 1 to 1000 μM for 1 to 24 hr. Toxicity was assessed by measuring lactate dehydrogenase (LDH) release and by monitoring chronotropic depression. Lipid peroxidation was assessed by measuring the release of thiobarbituric acid reactive substances (TBARS). PAT produced a concentration- and time-dependent depression in chronotropy and an increase in the release of LDH and TBARS. A 4-hr exposure to 100 μM PAT stopped beating and induced significant increases in TBARS and LDH release in the myocyte cultures. The lipid peroxidation and LDH release induced by 100–200 μM PAT at 4 hr could be prevented by pretreatment of the cardiac myocytes with vitamin E or by the simultaneous addition of other antioxidants. Vitamin E continued to protect against lipid peroxidation up to 18 hr after the addition of 100 μM PAT, but failed to provide significant protection against LDH release at this timepoint. Both 50 and 100 μM PAT decreased cardiac myocyte glutathione (GSH) levels after a 4-hr exposure. A series of thiol-containing compounds was evaluated for their effects on PAT toxicity. The addition of dithiothreitol, GSH, and 2-mercaptoethanol afforded some degree of protection against lipid peroxidation and LDH release up to 18 hr after the addition of 100 μM PAT. These results suggest that PAT induces lipid peroxidation in cultured cardiac myocytes but that other mechanisms may contribute to cell death with long-term exposures to PAT. Our results also suggest that PAT interacts with thiol-containing compounds.

Antimony is a hard, brittle, bluish–white metal that has numerous applications in industry. Antimony alloys are used in ammunition, type-setting metals, battery grids, and bearings. In addition to its use as an metal alloy, antimony-containing compounds are used in the manufacture of paints, pigments, ceramics, pyrotechnics, fire retardants, and glass (Carson *et al.*, 1986; Stokinger, 1981). The metal hydride of antimony, stibine, is a highly toxic gas that can be generated when antimony is exposed to reducing acids or when storage batteries are overcharged (Stokinger, 1981). High-purity stibine is also used as an n-type gas-phase dopant in the production of semiconductors. Antimony-containing compounds also have limited but important usage as antiparasitic drugs and have been used in the treatment of schistosomiasis and leishmaniasis (Winship, 1987). The National Institute for Occupational Safety and Health estimates that over 250,000 individuals are exposed to antimony compounds at work, and approximately 25% of these workers are women (NIOSH, 1988, 1989, 1990).

Antimony-containing compounds produce cardiac functional alterations and toxicity in humans. This conclusion is based on clinical studies on the effects of antimonial drugs on patient electrocardiograms and several cases in which autopsies revealed that cardiac toxicity was the cause of death in patients treated with antimonial drugs (NIOSH, 1978; Honey, 1960; Winship, 1987). Animal studies have also reported cardiac toxicity following administration of antimony-containing compounds. Rats injected ip with median lethal doses of antimony, potassium antimonyl tartrate (PAT), antimony trisulfide, antimony pentasulfide, antimony trioxide, or antimony pentaoxide exhibited cardiac toxicity at autopsy (Bradley and Fredrick, 1941). The toxicity to the heart was said to be specific and was observed at the lowest doses of the compounds tested. Rats receiving an ip dose of 11 mg PAT/kg body wt exhibited an increase in the fibrous and connective tissue of the heart (Bradley and Fredrick, 1941). Breiger *et al.* (1954) observed degeneration of the myocardium in rats and rabbits chronically exposed to dusts containing antimony trisulfide. In experiments conducted by Bromberger-Barnea and Stephens (1965), PAT administration to dogs irreversibly decreased myocardial contractile force and led to ventricular fibrillation.

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Six sudden deaths were reported in workers following exposure to antimony trisulfide for a period of 8 to 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).

Despite considerable evidence indicating antimony is a cardiac toxicant, virtually nothing is known regarding its mechanism of action. The following study was conducted to assess the effects of a soluble form of antimony, PAT, on cultured cardiac myocytes from neonatal rats and to confirm *in vitro* that PAT is indeed cardiotoxic. Isolated cardiac myocytes from neonatal rat are a commonly used model that has proven to be useful for assessing the effects of xenobiotics at the cellular and subcellular level (Shier and DuBourdieu, 1992). This model was selected for assessing the cardiac toxicity of antimony because whole animal studies indicated a direct and rapid action of antimony on the heart. The use of isolated cells allowed for complete control of the extracellular environment in the absence of confounding factors such as hemodynamics that must be confronted when doing studies in intact animals. Furthermore, the use of isolated cardiac myocytes provided the opportunity for rapid assessment of injury induced by antimony in the presence and absence of potential inhibitors of toxicity. Our results indicate that exposure to PAT induces oxidative stress, depression of spontaneous beating, and cell death in cultured cardiac myocytes.

MATERIALS AND METHODS

Chemicals. Newborn calf serum was purchased from Hyclone (Logan, UT). PAT was obtained from Aldrich (Milwaukee, WI). All other chemicals and reagents 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 the National Institute for Occupational Safety and Health 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 hr, flasks were gently swirled, which suspended unattached myocytes and left the majority of fibroblasts attached. The cell suspension was then plated at 10^6 cells per 35-mm well for lipid peroxidation and cytotoxicity assessment 48 hr after plating. This plating density assured nearly confluent cultures of myocytes within 24 hr. 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.

Treatment of cells for assessment of lipid peroxidation and cytotoxicity. Cardiac myocytes plated for 48 hr in 35-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 2-ml aliquots to culture dishes. Antioxidants and thiol-containing compounds were added at the same time that PAT was added. Vitamin E and deferoxamine were exceptions to this procedure. Vitamin E was added to serum-supplemented M199 24 hr before washing cells with HBSS in preparation for PAT exposure. Deferoxamine was added to serum-supplemented M199 1 hr prior to washing the cells and was also present along with PAT during the 4-hr exposure period. At the end of the exposure period, buffer was removed from culture dishes and assayed for thiobarbituric acid reactive substances (TBARS) release and lactate dehydrogenase (LDH) leakage from myocytes.

Lactate dehydrogenase determination. 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 the treatment buffer relative to the 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). In the present studies, LDH values greater than 90% of total LDH were associated with complete lysis of virtually all the cells in the culture dish as determined by microscopic examination.

Thiobarbituric acid reactive substances assay. Lipid peroxidation was assessed by measuring TBARS released in culture dishes by the method of Yagi (1976) as modified by Casini *et al.* (1982). Test solutions obtained from cultured cells were cooled to 4°C and centrifuged for 10 min at 1000*g* to remove cells. Aliquots (750 μl) of the supernatant were combined with an equal volume of cold 12% trichloroacetic acid and centrifuged for 10 min at 1000*g* at 4°C to remove precipitated protein. One milliliter of supernatant was added to 1 ml thiobarbituric acid reagent (0.6% thiobarbituric acid, 0.01% BHT, 1.0 mM EDTA), and the mixture was heated at 100°C for 20 min. The mixture was allowed to cool, and TBARS were extracted with 3 ml of 1-butanol. Appropriate blanks were included to assess the reactivity of medium and inhibitors toward the thiobarbituric acid reagent. Only M199 showed significant chromophore production and these blanks values were subtracted from sample values. A 1.0 mM stock solution of tetraethoxypropane in water was diluted in various amounts of 0.01 N HCl to produce malondialdehyde; these solutions were used as TBARS standards. The 1-butanol fractions and malondialdehyde standards were spectrofluorometrically analyzed with an excitation wavelength of 520 nm and an emission wavelength of 553 nm. TBARS, in nanomoles, was calculated using the linear regression obtained from the standards.

Glutathione determinations. Reduced (GSH) and oxidized (GSSG) gluthatione levels were determined by high-performance liquid chromatography utilizing fluorometric detection based on the procedures of Reed *et al.* (1980) as modified by Martin and White (1991). Cells were washed twice in HBSS prior to the addition of 2 ml of cold 5% perchloric acid containing 2 mM diethylenetriaminepentaacetic acid, 0.2 M boric acid, and 5 mg/liter cresol red indicator. Plates were scraped to remove cells, and the acid buffer–cell suspension was centrifuged at 14,000*g* for 10 min. The supernatant was analyzed for GSH and GSSG and the pellet was used to assay total protein. Total protein was measured according to Lowry *et al.* (1951) as modified by Peterson (1977) with bovine serum albumin used as a standard.

Measurement of spontaneous beating rate. Neonatal rat cardiac myocytes start beating spontaneously in culture within 24 hr of plating. At 24 hr, beating is often irregular and the entire dish does not beat in synchrony. This is primarily because all cells are not physically linked via gap junctions which is essential for the entire dish to beat as a syncytium (Toraason *et al.*, 1992). When plated at 10^6 per 35-mm dish, a confluent monolayer that beats spontaneously as a syncytium is clearly established by 48 hr. At this time, cells were exposed to PAT by replacing culture medium in wells with medium containing from 0 to 100 μM PAT. Beating activity was monitored at various intervals over the next 24 hr. Contractions were

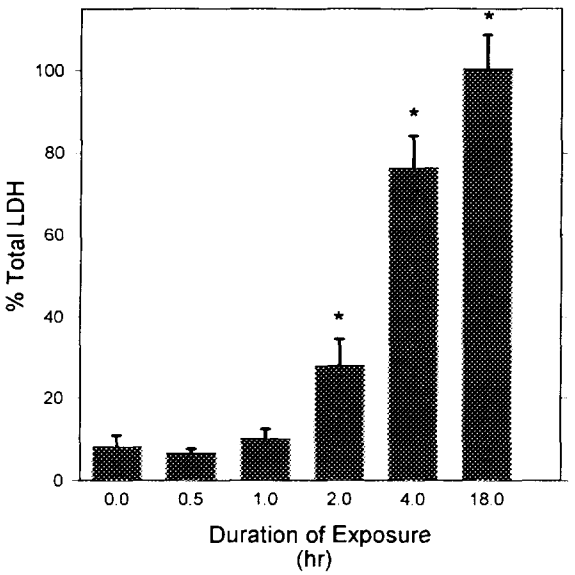

FIG. 1. Effects of the duration of exposure to 100 μM PAT on LDH release from neonatal cardiac myocytes. Myocytes were exposed to 100 μM PAT for the length of time indicated and then washed and incubated in medium without PAT. The amount of LDH released into the medium was determined 18 hr after the initial addition of PAT. *Significantly different from control values ($p < 0.05$). All values are expressed as the means ± SD, $n \geq 4$ for all time points.

counted by eye for 15 sec in three separate areas in each well. Experimental groups were randomized, and the data recorder was blind to the treatments.

Statistical analysis. One-way 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

Effects of PAT on LDH release and lipid peroxidation. The effects of duration of exposure to PAT on LDH release from rat cardiac myocytes were examined (Fig. 1). LDH release was used as a measure of toxicity. Myocytes were exposed to 100 μM PAT for varying lengths of time and then washed and transferred to medium without PAT. Eighteen hours after PAT was first introduced, the amount of LDH released into the incubation medium was determined. Results suggest that myocytes must be exposed to 100 μM PAT for periods in excess of 1 hr in order for toxicity to occur. PAT exposure periods of 2 and 4 hr produced significant decreases in cell viability when compared to controls (Fig. 1).

The effects of increasing concentrations of PAT on the release of LDH and TBARS from rat cardiac myocytes are shown in Figs. 2 and 3. LDH and TBARS were assayed after a 4-hr (Fig. 2) and 18-hr (Fig. 3) exposure to PAT. TBARS formation was used to assess lipid peroxidation. As Figs. 2 and 3 indicate, increases in TBARS production paralleled increases in LDH release at both the 4- and 18-hr time points. The absolute TBARS values with an 18-hr exposure were approximately 60% of those at 4 hr despite a higher LDH release after the 18-hr exposure. This may be due to loss of TBARS due to metabolism or cell binding. The extent of toxicity was related to the duration of exposure to PAT. Exposure to 500 μM PAT released 70% of the total LDH after 4 hr, while about 90% of the total LDH was released in incubations containing only 50 μM PAT after 18 hr.

Experiments were conducted on the effects of vitamin E pretreatment on the concentration dependence of PAT on LDH release and lipid peroxidation after 4- and 18-hr exposures (Figs. 2 and 3). Cultures were pretreated with 10 μM vitamin E for 24 hr prior to the addition of PAT. Vitamin E pretreatment effectively inhibited TBARS production at both the 4- and 18-hr time points. Vitamin E pretreatment

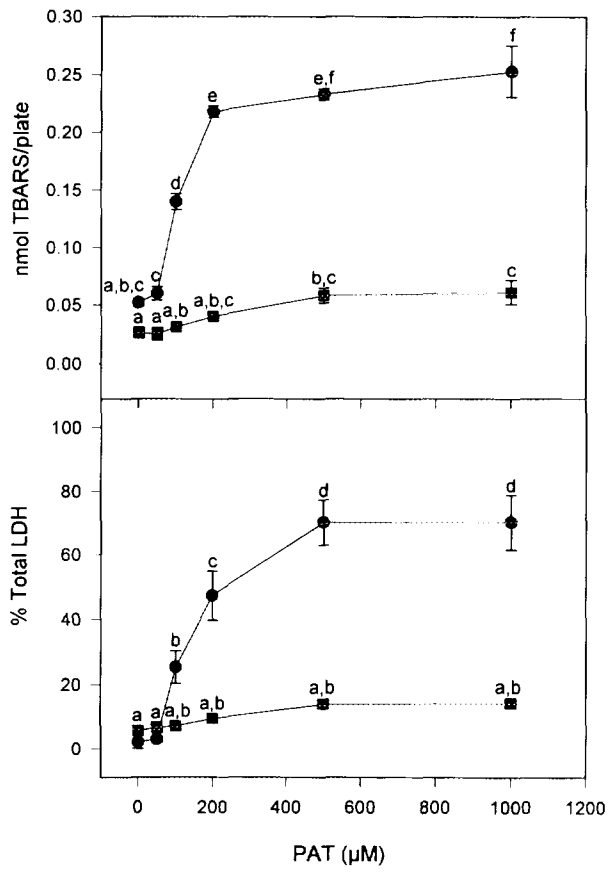

FIG. 2. Effects of varying concentrations of PAT on LDH and TBARS release from neonatal cardiac myocytes. PAT was added to untreated myocyte cultures (●) or to cultures which had been pretreated with 10 μM vitamin E (■) for 24 hr prior to the addition of PAT. Exposure periods were for 4 hr. Data points not having letters in common are significantly different ($p < 0.05$). All values are expressed as the means ± SD, $n = 4$.

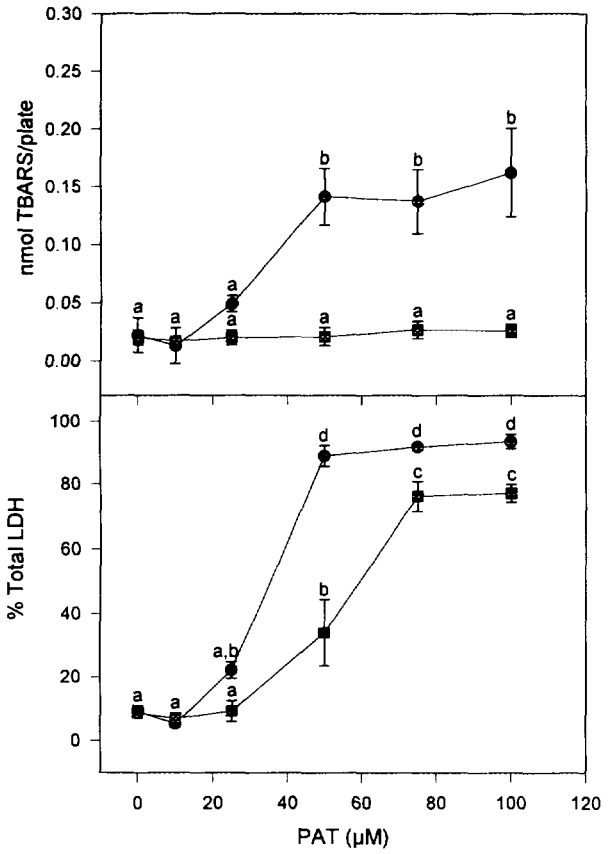

FIG. 3. Effects of varying concentrations of PAT on LDH and TBARS release from neonatal cardiac myocytes. PAT was added to untreated myocyte cultures (●) or to cultures which had been pretreated with 10 μM vitamin E (■) for 24 hr prior to the addition of PAT. Exposure periods were for 18 hr. Data points not having letters in common are significantly different ($p < 0.05$). All values are expressed as the means ± SD, $n = 4$.

also was very effective in inhibiting LDH release following a 4-hr exposure to various concentrations of PAT. However, vitamin E, despite reducing TBARS release, provided only slight protection against toxicity resulting from an 18-hr exposure to 75 and 100 μM PAT. The vitamin E pretreatment did afford more effective protection against toxicity induced by 50 μM PAT after 18 hr.

Antioxidant protection against PAT toxicity. A series of compounds was tested for their effects on PAT-induced toxicity and lipid peroxidation after exposure to 200 μM PAT for 4 hr (Table 1). In all cases, treatment protocols without PAT did not significantly elevate either LDH or TBARS values above those seen with untreated controls after 4 hr (data not shown). Potassium tartrate alone was also without effect. All of the compounds tested offered at least some degree of protection against PAT-induced toxicity. In general, those compounds which protected against loss in cell viability at the 4-hr time point also protected against the induction of lipid peroxiation. Vitamin E, the iron chelator deferoxamine, dithiothreitol (DTT), *N,N'*-diphenyl-*p*-phenylenediamine (DPPD), and chlorpromazine reduced both TBARS and LDH release in this experiment. Ascorbate, however, did not follow this trend. The addition of 1 mM ascorbate to PAT-exposed cells doubled the lipid peroxidation but also produced a slight but significant reduction in LDH release.

The addition of 50 μM DPPD to myocyte cultures completely blocked increases in lipid peroxidation but did not protect against toxicity following an 18-hr exposure to 100 μM PAT (data not shown). In this regard, the effects of DPPD are similar to those seen with vitamin E-pretreated cells at this time point (Fig. 3).

Thiol-containing compounds protect against PAT toxicity. As Table 1 indicates, 1.5 mM DTT protected against toxicity and lipid peroxidation following a 4-hr exposure to 200 μM PAT. Studies were conducted to assess the effectiveness of DTT and other thiol-containing compounds in protecting against long-term (18 hr) exposures to PAT. The addition of 0.5 mM DTT to neonatal cardiac myocytes incubations prevented 100 μM PAT-induced toxicity and lipid peroxidation after an 18-hr exposure period (Table 2). Lower concentrations of DTT (0.1 mM) prevented lipid peroxidation and provided about a 56% reduction in LDH release at the 18-hr time point. GSH and 2-mercaptoethanol at concentrations of 1 mM reduced PAT-induced TBARS production by about 45% and provided some de-

TABLE 1
Protective Effects of Antioxidants on Toxicity and Lipid Peroxidation Induced by a 4-hr PAT Exposure[a]

Treatment[b]	LDH (% of total)	TBARS (pmol/plate)
Untreated controls	4.2 ± 1.0[c,d]	69 ± 6[d]
Potassium tartrate (1 mM)	4.0 ± 0.5[d]	58 ± 3[d]
PAT (200 μM)	45.8 ± 4.0[e]	192 ± 35[e]
+ Deferoxamine (1 mM)	18.6 ± 5.6[d,e]	98 ± 21[d]
+ Ascorbate (1 mM)	33.1 ± 2.8[d,e]	404 ± 61[d,e]
+ Chlorpromazine (10 μM)	24.3 ± 1.8[d,e]	84 ± 7[d]
+ Vitamin E (10 μM)	9.5 ± 0.7[d]	40 ± 2[d]
+ Dithiothreitol (1.5 mM)	2.8 ± 1.1[d]	81 ± 2[d]
+ DPPD (50 μM)	2.6 ± 0.6[d]	72 ± 1[d]

[a] LDH and TBARS were assayed 4 hr after the addition of potassium tartrate or 200 μM PAT.

[b] Cultures were supplemented with vitamin E for 24 hr and with deferoxamine for 1 hr prior to the addition of PAT. Cells were washed before initiation of the PAT exposure period. Vitamin E was not present during the 4-hr exposure period. All other compounds, including additional deferoxamine, were added just prior to the addition of PAT and were present during the entire 4-hr exposure period.

[c] All values expressed as means ± SD, $n = 4$.

[d] Values significantly different from 200 μM PAT treatment group ($p < 0.05$).

[e] Values significantly different from controls ($p < 0.05$).

TABLE 2

Protective Effects of Thiol-Containing Compounds on Toxicity and Lipid Peroxidation Induced by an 18-hr PAT Exposure[a]

Treatment[b]	LDH (% of total)	TBARS (pmol/plate)
Untreated controls	5.4 ± 1.1[c,d]	32 ± 26[d]
Potassium tartrate (1 mM)	8.9 ± 1.2[d]	8 ± 5[d]
PAT (100 μM)	97.4 ± 6.8[e]	185 ± 46[e]
+ Dithiothreitol (0.1 mM)	41.4 ± 4.8[d,e]	29 ± 4[d]
+ Dithiothreitol (0.5 mM)	9.9 ± 1.1[d]	27 ± 3[d]
+ Glutathione (1 mM)	39.8 ± 12.1[d,e]	103 ± 3[d,e]
+ 2-mercaptoethanol (1 mM)	62.4 ± 6.2[d,e]	104 ± 9[d,e]

[a] LDH and TBARS were assayed 18 hr after the addition of potassium tartrate or 100 μM PAT.

[b] All compounds were added just before the addition of PAT and were present during the entire 18-hr exposure period.

[c] All values expressed as means ± SD, $n = 4$.

[d] Values significantly different from 100 μM PAT treatment group ($p < 0.05$).

[e] Values significantly different from controls ($p < 0.05$).

gree of protection against toxicity in this study. Incubations containing the thiol-containing compound but without PAT released less than 8% of the total LDH after 18 hr (data not shown).

Effects of PAT on cellular glutathione levels. Exposure of cultured myocytes to 50 μM PAT reduced cellular GSH levels to about 30% of control values after 4 hr (Fig. 4). LDH assays indicate that 15.6 ± 5.4% (mean ± SD, $n = 6$) of the total LDH was released following this exposure. A 4-hr exposure to 100 μM PAT reduced GSH levels to less than 10% of controls after 4 hr. LDH values corresponded to 34.4 ± 1.4% (mean ± SD, $n = 6$) of the total for this time point. In both cases, LDH data suggest that the extent of GSH depletion observed could not be explained solely by loss of cell viability. No GSSG was detected with either concentration of PAT in these experiments.

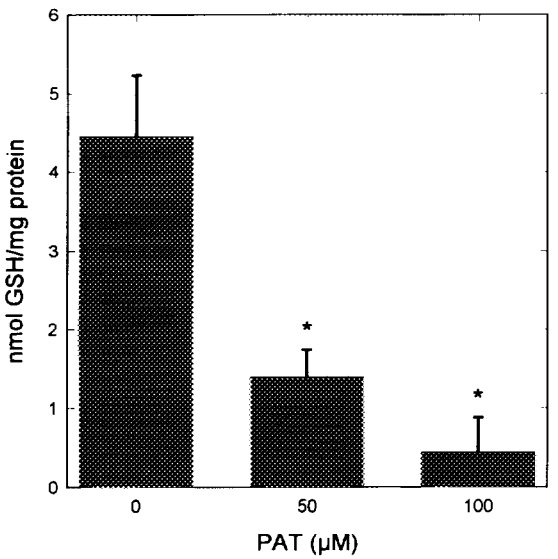

FIG. 4. Effects of PAT on glutathione levels in neonatal cardiac myocytes. Myocytes were exposed to 50 and 100 μM for 4 hr. *Significantly different from control values ($p < 0.05$). All values are expressed as the means ± SD, $n \geq 6$.

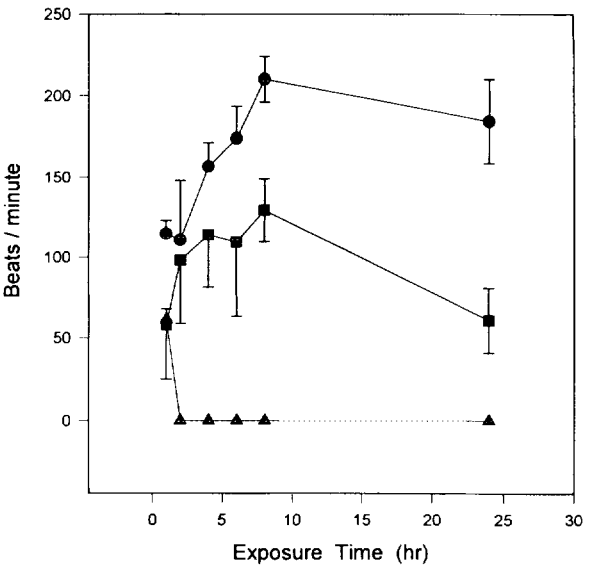

FIG. 5. Effects of PAT on spontaneous beating activity of cardiac myocytes. Myocytes were exposed to 0 μM (●), 10 μM (■), or 100 μM (▲) PAT and beating activity was recorded after 1, 2, 4, 6, 8, and 24 hr of exposure. Values shown are the means ± SD, $n = 3$.

Effect of PAT on spontaneous beating rate. After 48 hr in culture, control cardiac myocytes contracted at the rate of 117 ± 21 beats per min (BPM). Over the next 8 hr, beating activity increased to 202 ± 17 BPM and maintained that rate for the next 16 hr. After 1 hr exposure, 100 μM PAT significantly reduced beating activity. At 2 hr, 100 μM PAT caused a cessation of beating. During the initial 8 hr of exposure to 10 μM PAT, beating rates increased to only 135 ± 28 BPM, which was significantly less than the rate for controls. After 24 hr of exposure to 10 μM PAT, cells were beating at 61 ± 20 BPM. A 24-hr pretreatment with 10 μM vitamin E was without effect on cells exposed to 10 or 100 μM PAT (data not shown). A summary of these results are illustrated in Fig. 5, which is representative of replicate experiments. In a separate experiment (data not shown), a 24-hr exposure to 1 μM PAT had a small but nonsignificant inhibitory effect on myocyte beat rates. Also, potassium tartrate was without significant effect on beating rate.

DISCUSSION

The present findings are the first demonstration that PAT is toxic to cultured cardiac myocytes. As such, these

results are consistent with *in vivo* studies. Exposure to PAT increased LDH release and decreased spontaneous beat rates. The toxic response of cardiac myocytes to PAT was dependent on both the concentration and duration of exposure to PAT. Spontaneous beating was particularly sensitive to long-term exposures to PAT, as a concentration of 10 μM PAT reduced beat rates to about 30% of controls after 24 hr. However, 10 μM PAT did not significantly increase LDH release when assayed after 18 hr.

Our results also suggest that PAT is capable of inducing oxidative stress in cardiac myocytes. PAT depleted cellular GSH and induced lipid peroxidation. Vitamin E and other antioxidants afforded short-term protection against lipid peroxidation and cell death as measured by LDH release. However, vitamin E was much less effective in protecting against cell death in long-term exposures to PAT. Vitamin E provided protection against LDH release after an 18-hr exposure to 50 μM PAT but only slight protection against an 18-hr exposure to 75 and 100 μM PAT. Vitamin E completely prevented PAT-induced lipid peroxidation at all the concentrations of PAT tested in the 18-hr exposure experiments. This apparent dissociation between lipid peroxidation and LDH release suggests that additional causes, in addition to lipid peroxidation, are important in contributing to PAT-induced loss of cardiac myocyte viability in long-term exposures to PAT. Protection against lipid peroxidation appears to delay but not prevent the onset of PAT-induced death of cardiac myocytes. When lipid peroxidation is prevented, myocytes eventually succumb to other PAT-induced alterations in cellular function.

Lipid peroxidation is known to be induced by numerous other metals. Cadmium, cobalt, copper, lead, mercury, nickel, tin, and vanadium compounds have been shown to induce lipid peroxidation in various experimental systems (Sunderman, 1986). However, *in vitro* studies have suggested that lipid peroxidation is not a direct cause of cell death in hepatocytes incubated with cadmium (Stacey *et al.*, 1980) or mercury (Stacey and Kappus, 1982).

In addition to antioxidants, thiol-containing compounds were effective in preventing PAT-induced cell death. Cysteine, GSH, 2-mercaptoethanol, and DTT afforded at least some degree of protection against PAT-induced toxicity after an 18-hr exposure. The reagent DTT, which is a vicinal dithiol, was particularly effective in this regard. The protection provided by these compounds is most likely due to their ability to chelate trivalent antimony compounds. Trivalent antimony is known to readily react with thiol groups and form thioantimonites (Stemmer, 1976). Vicinal dithiols seem to be especially reactive toward trivalent antimony compounds. Basinger and Jones (1981) tested a number of chelating agents for their ability to protect against PAT-induced toxicity in mice and found water-soluble vicinal dithiols to be the most effective antidotes for PAT-induced intoxication.

Antimony compounds have been demonstrated to differ in their relative toxicities. Bradley and Fredrick (1941) ranked PAT and antimony metal as the most toxic followed by the sulfides and then the oxides. Accumulated evidence indicates that the trivalent forms of the sulfides and oxides are more toxic than the pentavalent forms. This difference in toxicity may relate to the increased reactivity of the trivalent form of antimony with thiol compounds.

PAT exposure in the present study caused lipid peroxidation and cell death in a concentration-dependent manner. If similar events occurred *in vivo*, severe myocardial toxicity would be the result. This is consistent with clinical observations and animal studies which indicate that acute antimony exposure readily produces overt cardiac damage.

Lu and Liu (1963) estimated that 70–90% of deaths resulting from antimony therapy were due to cardiac intoxication. Human pathological data are scarce, but Honey (1960) reported the finding of a myocardial infarct in addition to cardiac edema and fragmentation of myocardial fibril structures in a 21-year-old woman who died during treatment with sodium antimonyl tartrate. Comparable pathological findings were noted in experimental animals exposed to antimony (Bradley and Fredrick, 1941). Furthermore, Honey (1960) noted that antimony administration to one individual elicited Stokes–Adams syndrome, which is a third-degree or complete cardiac block that results in loss of consciousness and can be fatal. Such severe functional effects of acute antimony treatment have been observed in experimental animals, where a single bolus of PAT causes a progressive decrease in contractile force, fibrillation, and cessation of contraction (Bromberger-Barnea and Stephens, 1965). These effects were observed in both intact animals and isolated hearts leading the authors to conclude that the myocardium is directly affected by antimony.

The mechanism responsible for PAT-induced oxidative stress in cardiac myocytes is not known. PAT may directly generate activated oxygen species by reducing oxygen or indirectly by modifying cellular functions which in turn lead to the generation of activated oxygen species. Exposure of cardiac myocytes to activated oxygen species is known to cause lipid peroxidation and cytotoxicity (Massey and Burton, 1990; Janero *et al.*, 1991; Toraason *et al.*, 1994). Although the present study suggests that PAT induces oxidative stress in cardiac myocytes, we cannot determine from our findings what role oxidative stress contributes to the process of cell death. The ability of antioxidants to protect against lipid peroxidation and cell death after a 4-hr exposure but not after an 18-hr exposure to PAT suggests that the mechanism of PAT-induced cell death may be dependent on the concentration and time of exposure to PAT.

Other mechanisms may also contribute to PAT-induced cell death. There is evidence that antimony can combine with certain thiol groups of respiratory enzymes and inter-

fere with cellular metabolism (Stokinger, 1981). This in turn could lead to depletion of ATP and contribute to cell death. In addition, antimony could bind to other key cellular macromolecules and thereby disrupt normal cellular function. Studies are presently underway in our laboratory to further characterize the effects of PAT on cultured cardiac myocytes. Addressing the mechanism of PAT-induced cell death will enhance our understanding of antimony toxicity and provide insight into the extent to which the myocardium may be compromised by occupational exposures to antimony.

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