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Cysteine protects freshly isolated cardiomyocytes against oxidative stress by stimulating glutathione peroxidase

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Abstract

Cysteine has been implicated in myocardial protection, although this is controversial and constrained by limited knowledge about the effects of cysteine at the cellular level. This study tested the hypothesis that a physiologically relevant dose of L-cysteine could be safely loaded into isolated cardiomyocytes leading to improved protection against oxidative stress. Freshly isolated adult rat ventricular cardiomyocytes were incubated for 2 h at 37°C with (cysteine incubated) or without (control) 0.5 mM cysteine prior to washing and suspension in fresh cysteine-free media. Cysteine incubated cells had higher intracellular cysteine levels compared to controls (9.6 ± 0.78 vs. 6.5 ± 0.65 nmol/mg protein, $P < 0.02$, $n = 6 \pm SE$). Cell homeostasis indicators were similar in the two groups. Cysteine incubated cells had significantly higher glutathione peroxidase (GPx) activity (1.11 ± 0.23 vs. 0.54 ± 0.1 U/mg protein, $P < 0.05$, $n = 5 \pm SE$) and significantly greater expression of GPx-1 (5.01 ± 0.48 vs. 3.01 ± 0.25 OD units/mm², $P < 0.05$, $n = 4 \pm SE$) compared to controls. Upon exposure to H₂O₂, cysteine incubated cells generated fewer reactive oxygen species and took longer to show contractile changes and undergo hypercontracture. However, when cells were exposed to H₂O₂ in the presence of 0.05 mM of the GPx inhibitor mercaptosuccinic acid, this increased the control cells' susceptibility to H₂O₂ and completely abolished the cysteine mediated protection. These results suggest a new role for cysteine in myocardial protection involving stimulation of glutathione peroxidase.

Keywords

Cardioprotection; Cysteine; Glutathione peroxidase; H₂O₂

Introduction

The sulphhydryl containing amino acid, cysteine plays an important role in intermediary metabolism in the heart. In premature and new-born infants, cysteine is considered to be an

essential dietary component [1]. In adults cysteine is obtained from dietary sources, proteolysis and methionine catabolism [2]. Cysteine is involved in many reactions including: protein synthesis (e.g., coenzyme A [3], atriopeptin [4], and *S*-nitrothiols [5]), oxidative metabolism [6], sulphur provision [2] and the formation of phaeomalin and trichrome pigments [2]. The intracellular concentration of cysteine in the rat heart is 0.089 mM compared to 0.097 mM in the plasma [7].

In addition to the above roles, cysteine availability is thought to be one of the major rate limiting factors controlling the synthesis of glutathione (GSH) [8]. GSH, glutathione-*S*-transferase and glutathione peroxidase (GPx) form part of the myocardium's endogenous protection system against oxidative stress along with other enzymes such as superoxide dismutase and catalase [9]. The production of harmful reactive oxygen species (ROS) and their precursors including hydrogen peroxide, superoxide, peroxynitrite and hydroxyl radicals arises from oxidative stress. Oxidative stress has been implicated in ischaemia reperfusion injury, myocardial stunning and in the failing heart [9-11].

The relationship between cysteine availability and GSH synthesis has motivated strategies that test the protective potential of cysteine in the heart [5, 12, 13]. For example, we have shown that the addition of 0.5 mM cysteine improved recovery of the rate pressure product and preserved ATP levels in isolated and perfused adult rat hearts following ischaemia in comparison to non-cysteine perfused controls [13]. Yet despite these positive investigations, cysteine's role in myocardial protection remains controversial. At high non-physiological concentrations cysteine addition may compromise recovery from ischaemia [14] and auto-oxidation can generate thiyl and hydroxyl radicals [15]. The majority of these studies have been carried out on the whole heart [12-14] which is a complex organ that contains vascular, fibrous and muscular tissue. In contrast little is known about the effects of cysteine at physiologically relevant concentrations at the cellular level, particularly in the contractile unit, the working cardiomyocytes.

We have recently shown that several high affinity cysteine transporters are expressed and active in the heart [16, 17]. There were two main aims of this study. The first aim was to investigate whether cardiomyocytes could be safely loaded with a physiologically relevant dose of cysteine. The second aim was to investigate whether this loading led to improved protection against oxidative stress and, if so, to investigate the underlying mechanisms.

Materials and methods

Materials

Fluorescent dyes were purchased from Molecular Probes (Invitrogen, Paisley, UK); assay kits for catalase and glutathione peroxidase activity, and the anti-GPx-1 antibody were purchased from Calbiochem (Merck Biochemicals, Nottingham UK); whilst Western Blotting reagents were purchased from Amersham. All other chemicals were purchased from either SIGMA (Poole, Dorset UK) or VWR International (Lutterworth, Leicestershire UK) and were of the best analytical grade available.

Cell isolation

All of the tissues used in this study were obtained from male Wistar rats (250–300 g body weight), and all of the procedures were approved by the Ethics Committee, University of Bristol. The rats were killed by stunning and cervical dislocation and the heart and brain dissected. Calcium tolerant ventricular cardiomyocytes were isolated by enzyme digestion and mechanical dispersion as described previously [18, 19]. At the end of this procedure approximately 70% of the cells were rod shaped and able to exclude Trypan Blue (not shown).

Cysteine incubation

Following isolation the cells were divided into two groups. One of the groups of cells was incubated with 0.5 mM cysteine (cysteine incubated). A stock solution containing 50 mM cysteine dissolved in cell isolation media was prepared on the morning of each experiment. This stock solution was added to the cell sample to yield a final cysteine concentration of 0.5 mM. The tube containing the sample was briefly shaken and then incubated in a gently shaking water bath at 37°C for 2 h. The other group of cells were treated identically but did not receive any cysteine (control). At the end of the incubation in order to wash the extracellular space the two groups of cells were centrifuged at 900 g for 2 min at room temperature. At the end of the centrifugation, the supernatant was discarded and the two cell pellets were suspended in fresh cysteine-free media.

Loading of fluorescent dyes and subsequent fluorescent measurements (ROS and calcium transients)

The production of reactive oxygen species (ROS) was measured using the fluorescent dye, 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H₂DCFda). The intracellular calcium concentration was measured using Fura-2AM. The cells were only loaded with a single dye according to procedures that we have described previously [18, 19], or according to the manufacturer's advice [20]. In brief the loading methods involved the preparation on the morning of the experiment of a stock solution containing either 1 mM Fura-2AM or 0.5 mM CM-H₂DCFda in dimethyl sulphoxide (DMSO). An aliquot of the stock solution was added to the cell suspension to give a final concentration of 2 μM Fura-2AM or 5 μM CM-H₂DCFda. The cell suspension was incubated at 37°C for 15 min in a shaking water bath prior to centrifugation and suspension of the resulting cell pellet in fresh dye-free Tyrode.

Fluorescence levels in dye loaded cardiomyocytes were measured using a photon counting system (Newcastle Photometric Systems [Newcastle UK]) that was attached to an inverted Nikon Diaphot microscope (Nikon, Surrey UK). The excitation and emission wavelengths for CM-H₂DCFda were 485 nm and 510 nm, respectively. Calcium measurements using Fura-2AM were calculated from fluorescence ratios with excitation at 340 nm and emission at 510 nm versus excitation at 380 nm and emission at 510 nm [20]. The calcium signals were calibrated, after subtraction of background fluorescence using a solution containing 2 μM Fura-2 and 0–39 μM calcium (Calcium Calibration Buffer Kit with Magnesium #2 *zero to 10 mM CaEGTA with 1 mM Mg²⁺, Cat. No. C-3722, Molecular Probes).

Perfusion protocols and exposure to oxidative stress

Cells were superfused in a chamber on the stage of an inverted fluorescence microscope at 37°C and field stimulated at 0.2 Hz as described previously [18, 19]. Contraction and morphology was visualised using a video camera and monitor [18, 19]. A single cell was chosen, which was rod shaped and contracted rhythmically in response to stimulation with no spontaneous contractions. Cells were initially superfused with Tyrode which contained (in mM): 137 NaCl, 5 KCl, 1.2 MgSO₄, 1.2 NaH₂PO₄, 16 glucose, 1 CaCl₂ and 20 HEPES (pH 7.4 with NaOH). After a period of equilibration and the collection of baseline data, the cardiomyocytes were exposed to oxidative stress by switching the normal Tyrode for an identical solution containing 0.2 mM H₂O₂. We have previously shown that these conditions lead to significant ROS production, disruption of contractile activity and eventual hypercontracture [19]. The superfusion with H₂O₂ was continued until the cell under observation had undergone hypercontracture, and then the experiment was terminated.

Measurement of NAD/NADH ratio by autofluorescence

Basal NADH levels were measured using cell autofluorescence as described previously [18]. The cells were excited at a wavelength of 350 nm, and emitted light was collected at 450 nm. Maximal reduction was measured by superfusion with Tyrode containing 2.5 mM NaCN and no glucose, whereas maximal oxidation was measured using Tyrode containing 1 μ M carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). The NAD⁺/NADH ratio was calculated as described previously [18].

Measurement of metabolites by HPLC (cysteine, creatine and ATP)

ATP, creatine and cysteine were extracted from cardiomyocytes using perchloric acid precipitation and potassium carbonate neutralization as described previously [13, 18]. At the end of this procedure the supernatant was divided into two 100 μ l aliquots. 8 μ l of a 100 mM iodoacetic acid solution [21] was added to the sample that was to be used for measuring cysteine followed by 15 min incubation. 100 μ l of a 0.1 M HCl solution was then added to all tubes prior to vacuum drying and HPLC of the reconstituted samples as described previously [13, 18].

Measurement of selected metabolites by spectrophotometry

Cellular activities of glutathione peroxidase (GPx) and catalase following oxidative stress (15 min incubation at 37°C with 0.2 mM H₂O₂) were assayed using Calbiochem kits and were conducted according to the manufacturer's instructions. In order to measure the effect of cysteine on GPx activity in vitro 0.5 mM cysteine was added to the kit's "assay buffer" and the GPx sample provided in the kit as a positive control was used. In order to measure the effect of different concentrations of mercaptosuccinic acid (MSA) on GPx activity in the cardiomyocytes, the cells were incubated for 2 h at 37°C with 0–0.075 mM MSA. Protein concentrations were measured using Lowry's reagent. Bovine serum albumin was used to construct the standard curve.

Western blotting of GPx-1

The cells were prepared for Western Blotting as follows. First cysteine incubated and control cells were centrifuged at 900 g for 2 min at 4°C. The resulting cell pellet was homogenized on ice in a solution containing (in mM) 50 TRIS-HCl, 5 EDTA and 1 DTT (pH 7.5, buffer 1). The homogenate was centrifuged at 4°C. At the end of this centrifugation step, the supernatant was transferred to a clean tube and frozen at –80°C until required and the cell pellet was discarded.

Western Blotting was carried out as described previously [22] using a monoclonal anti-GPx1 antibody. For each control or cysteine incubated sample an identical amount of protein (50 μ g) was loaded onto the gel. Two positive controls were used. The first positive control tested for the correct protein. This control consisted of the sample of glutathione peroxidase isolated from bovine liver which is provided as a positive control in the kit for assaying glutathione peroxidase activity. The second positive control tested that the correct protein could be detected in the rat. The sample chosen was rat crude brain homogenate, because the brain is known to express high levels of glutathione peroxidase [23]. The crude brain homogenate was prepared by 30 s homogenisation of the appropriate rat tissues on ice in buffer 1.

In order to obtain a semi-quantitative comparison of the expression of GPx1 in control and cysteine incubated cardiomyocytes, X-ray films were photographed and analysed on a scanning densitometer with incorporation into custom software (Quantity One version 4.6, Bio Rad, Hertfordshire UK).

Data analysis

Fluorescence was measured in arbitrary units and normalized as described previously [19]. Results in Figs. 1, 2, 3, 4, and 5 are presented as means $\pm$ SE from at least four hearts. Results in Fig. 6 are from a single experiment which is representative of at least four replicates. Comparisons between datasets were made using student's *t*-test or ANOVA with a Tukey post-test. *P* values <0.05 were considered significant.

Results

Following incubation and subsequent washing the intracellular cysteine concentration in cysteine incubated cardiomyocytes was 9.6 ± 0.8 nmol/mg protein ($n = 6$ cell suspensions from 6 hearts $\pm$ SE), which was significantly higher than the 6.5 ± 0.7 nmol/mg protein measured in the control cells ($P < 0.02$, $n = 6$ cell suspensions from 6 hearts $\pm$ SE). There were no significant differences in the size of the calcium transient, the concentrations of ATP and creatine, and the NADH/NAD⁺ ratio in cysteine incubated cardiomyocytes compared to control (Table 1).

We have previously shown that the magnitude of CM-H₂DCFda fluorescence in isolated cardiomyocytes increases in proportion to the level of ROS production and the H₂O₂ concentration applied [19]. Figure 1 shows a time course of CM-H₂DCFda fluorescence during exposure of control and cysteine incubated cardiomyocytes to 0.2 mM H₂O₂. The level of fluorescence was similar in the two groups of cells for the first 13 min. After this point the fluorescence of both groups of cells increased; however, the fluorescence of the cysteine incubated cells was always significantly less than that of the control cells.

The lower fluorescence indicative of a smaller level of ROS production in the cysteine incubated cells compared to controls was mirrored by the effects on morphology and contractility. Cysteine incubated cardiomyocytes took 24.3 ± 4.5 minutes to become arrhythmic (visual change in the contractile response to stimulation such as cessation of contraction or asynchronous contraction in response to stimulation) and 40.5 ± 3.8 min to undergo hypercontracture during exposure to 0.2 mM H₂O₂. This was significantly longer than the 11.9 ± 0.6 min ($P < 0.01$, both groups $n = 23$ cells from 6 hearts $\pm$ SE) the control cells took to arrhythmia and the 26 ± 1.4 min ($P < 0.03$, both groups $n = 12$ cells from 5 hearts $\pm$ SE) the control cells took to undergo hypercontracture.

Figure 2 shows the activity of glutathione peroxidase and catalase following oxidative stress in cardiomyocytes incubated with or without cysteine. GPx activity was significantly higher in cysteine incubated cells compared to control cells (Fig. 2a), whilst there was no difference in catalase activity between the two groups of cells (Fig. 2b).

The importance of the relationship between cysteine incubation and GPx activity in the cardiomyocytes was investigated using the putative GPx inhibitor, mercaptosuccinic acid (MSA) [24]. Figure 3 shows the effect of different concentrations of MSA on GPx activity in control cardiomyocytes. Least squares analysis was used to fit the data in this figure to an asymmetric sigmoid function described by the equation: $Y = \text{Min} + (\text{Max} - \text{Min}) / (1 + ((X / X50)^{-P}))$ from which the X50 for MSA inhibition of 0.019 ± 0.002 mM was calculated.

Figure 4 shows how 2 h incubation with 0.05 mM MSA at 37°C affected the level of CM-H₂DCFda fluorescence after 15 min exposure to 0.2 mM H₂O₂ in control and cysteine incubated cardiomyocytes. Under these conditions, the highest level of fluorescence was measured in cells that had been exposed to MSA suggesting that these cells had the highest levels of ROS production. The lowest level of fluorescence was measured in the cysteine incubated group.

A similar pattern was apparent when the effect of MSA incubation upon cardiomyocyte contractility during oxidative stress was investigated. Figure 5 shows the time taken for each of the four groups of cells to become arrhythmic during exposure to 0.2 mM H_2O_2 . Cysteine incubated cardiomyocytes took the longest time to become arrhythmic, whilst the shortest time to arrhythmia occurred in groups of cells co-incubated with MSA. In control experiments measured under normal conditions without H_2O_2 , 0–0.1 mM MSA did not affect CM- H_2DCFda fluorescence and did not affect cardiomyocyte contractility and viability (not shown).

Finally the effect of cysteine incubation on the expression of GPx-1 was investigated by Western Blotting and scanning densitometry. These results are shown in Fig. 6. Monoclonal antibodies raised against GPx-1 recognized a protein of appropriate size as compared to molecular weight markers run on the same gel in samples of cardiomyocytes. A similar band was also detected in the two positive control samples comprising glutathione peroxidase and rat brain homogenate (Fig. 6a). Cysteine incubated cardiomyocytes were compared to controls by loading an identical amount of protein onto the gel, and the resultant bands following Blotting were analysed by densitometry. These results are shown in Fig. 6b and c. Cysteine incubated cells had significantly higher levels of GPx-1 compared to control cells.

Discussion

In summary, the main findings of this study were that freshly isolated cardiomyocytes could be loaded with 0.5 mM cysteine through incubation after isolation. This loading did not affect either calcium homeostasis or the cells' energetic status (Table 1). During oxidative stress the cysteine incubated cells generated significantly fewer ROS (Fig. 1) and maintained their morphologic and ionic homeostasis for a significantly longer period than the control cells. The underlying mechanism for this protection against H_2O_2 in the cysteine incubated cells may involve stimulation of GPx-1 expression (Fig. 6) and activity (Figs. 2, 4, and 5).

We have previously shown that it is possible to load glutamate into cardiomyocytes through the addition of 6.4 mM glutamic acid during the enzyme digestion stage of cell isolation [18, 19]. This approach which takes advantage of changes to cellular morphology and physiology during enzyme digestion may have limited application in the clinical arena. In this study we show for the first time that it is possible to raise the intracellular concentration of an amino acid independently of the isolation process. In this case, the cells were loaded using the physiologically relevant cysteine concentration of 0.5 mM, which is consistent with the known transport characteristics of cysteine in cardiomyocytes [16, 17]. Cysteine loading did not significantly alter the intracellular concentrations of calcium, ATP, and creatine nor did it affect the NADH/NAD⁺ ratio compared to control. This suggests that under normal conditions incubation with 0.5 mM cysteine does not change calcium homeostasis and energy metabolism in the cardiomyocyte. These investigations were considered important because of the association between high nonphysiological cysteine concentrations and auto-oxidation [15].

Elevated levels of hydrogen peroxide are produced in the failing heart [10] and during ischaemia reperfusion in the heart [11]. Exposure to H_2O_2 is therefore a good and clinically relevant model for studying the effects of oxidative stress in isolated cardiomyocytes where studies have shown that low dose H_2O_2 induces hypertrophy whilst higher doses activate apoptotic pathways [25–27]. Unfortunately the majority of these studies have used either neonatal or cultured cells where developmental or electrophysiological differences limit the comparison with freshly isolated adult cardiomyocytes [25–27] and restrict the possible relevance to the failing heart.

Two possible explanations for the lower CM-H₂DCFda fluorescence (Figs. 1, 4) and longer maintenance of metabolic and ionic homeostasis (text and Fig. 5) during oxidative stress in cysteine incubated cells compared to control are reduced ROS production or increased ROS scavenging. Possible mechanisms involved in ROS scavenging include a direct effect mediated by the SH group of cysteine [12] or more indirect routes involving GSH and antioxidant enzymes. It is however unlikely that cysteine interacts with catalase since cysteine incubation did not alter catalase activity (Fig. 2).

The importance of GPx expression and activity in relation to myocardial protection has been suggested in transgenic mice where it was shown that GPx-1 knock-outs showed increased vulnerability to ischaemia reperfusion, whilst GPx-1 over-expression increased resistance to ischaemia reperfusion [28]. Glutathione peroxidase is also involved in protecting brain tissues against ischaemia reperfusion and has been shown to play an important role in the detoxification of hydrogen peroxide in oligodendrites and astrocytes [23]. It was for this reason that rat brain homogenate was considered to be an appropriate positive control in the first experiments investigating GPx1 expression in isolated cardiomyocytes. This was also considered necessary as the glutathione peroxidase sample provided in the kit for measuring glutathione peroxidase activity was isolated from bovine liver (i.e., a different species). Detailed examination of the effects of hydrogen peroxide upon GPx-1 expression in brain was beyond the scope of this study. The central role that GPx-1 plays during oxidative stress in cardiomyocytes was apparent from the increased vulnerability to H₂O₂ demonstrated in the presence of the GPx inhibitor mercaptosuccinic acid (MSA) and by the abolition of cysteine's protective effect by MSA (Figs. 4, 5). These findings suggest that GPx activity can be stimulated simply by cysteine incubation rather than the more technically demanding approach of utilizing transgenic animals.

One possible route by which cysteine may enhance GPx activity in cardiomyocytes might involve the triggering of as yet unidentified signalling events culminating in phosphorylation of GPx-1 by the non-receptor tyrosine kinases, c-Abl and Arg [29]. Cysteine incubation also increased GPx-1 expression (Fig. 6). This raises the possibility that cysteine may be facilitating a form of molecular modulation. One possibility could be post-transcriptional stabilization of GPx-1 as has previously been shown in primary cultures of human ventricular tissue following treatment with 0.2 mM α -tocopheryl acetate (vitamin E) or 1 mM ascorbic acid (vitamin C) [30]. The effects of cysteine are likely to be limited to GPx-1 since MSA is not known to affect other antioxidant enzymes such as the non-selenocysteine glutathione peroxidases [24].

In conclusion this study presents evidence of a new role for cysteine at physiologically relevant doses involving stimulation of glutathione peroxidase activity and expression leading to increased protection against oxidative stress. The clinical relevance of these findings is illustrated by the good correlation that exists between GPx-1 activities and the incidence of future cardiovascular events [31].

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Fig. 1.

Time course of CM-H₂DCFda fluorescence during exposure to oxidative stress. Control and cysteine incubated cardiomyocytes were loaded with the fluorescent indicator, CM-H₂DCFda prior to superfusion with 0.2 mM H₂O₂. Data are presented as a percentage of the starting fluorescence in each condition. Open squares and dashed line: control; open circles and continuous line: cysteine incubated, * $P < 0.05$ versus control. Data shown are means $\pm$ SE of $n = 10$ cells isolated from six hearts

Fig. 2. Effect of cysteine incubation on the activities of antioxidant enzymes in cardiomyocytes. Measurements of GPx and catalase activity in control and cysteine incubated cardiomyocytes following oxidative stress evoked by 15 min incubation with 0.2 mM H_2O_2 . Open bars: control; closed bars: cysteine incubated; * $P < 0.05$ versus control. Data shown are means $\pm$ SE of $n = 5$ cell suspensions from five hearts

Fig. 3.

Dose response curve of the effects of mercaptosuccinic acid (MSA) on GPx activity in isolated cardiomyocytes. Control cardiomyocytes were incubated for 2 h at 37°C with 0–0.075 mM MSA prior to washing and measurement of GPx activity. Least squares analysis was used to fit the data to an asymmetric sigmoid curve described by the equation: $Y = \text{Min} + (\text{Max} - \text{Min}) / (1 + (X / X50)^P)$ ($r^2 = 0.99$) from which the X50 was calculated. Data shown are means $\pm$ SE of $n = 4$

Fig. 4.

Effect of a GPx inhibitor on ROS production in control and cysteine incubated cardiomyocytes during oxidative stress. Control and cysteine incubated cardiomyocytes were incubated with or without 0.05 mM MSA and loaded with the fluorescent indicator, CM-H₂DCFda prior to superfusion with 0.2 mM H₂O₂. Shown here is the level of fluorescence in each group of cardiomyocytes measured 15 min after exposure to H₂O₂. The data are presented as a percentage of the starting fluorescence in each group. Open bars: control; horizontally striped bars: control + MSA, solid bars: cysteine incubated, and cross-hatched bars: cysteine + MSA incubated. * $P < 0.01$ versus all other conditions, ** $P < 0.01$ versus control. Data shown are means $\pm$ SE of $n = 6$ cells isolated from five hearts

Fig. 5.

Effect of combining a GPx inhibitor and cysteine incubation on cardiomyocyte ionic homeostasis during oxidative stress. Time taken for control and cysteine incubated cardiomyocytes incubated with or without MSA to become arrhythmic during superfusion with 0.2 mM H₂O₂. Open bars: control, horizontally striped bars: + MSA, solid bars: cysteine incubated, and cross-hatched bars: cysteine and MSA incubated. * $P < 0.01$ versus all other conditions, ** $P < 0.01$ versus control. Data shown are means $\pm$ SE of $n = 12$ cells isolated from five hearts

Fig. 6. Expression of GPx-1 protein in cardiomyocytes. **a** Representative Western Blot using an antibody raised against GPx-1 in samples of isolated adult rat cardiomyocytes, rat brain homogenate and GPx. **b** Representative Western Blot comparing GPx-1 expression in four separate cardiomyocyte isolations that had been incubated for 6 h at 37°C with or without 0.5 mM L-cysteine. The bar to the right of these figures indicates the approximate molecular weight as determined by standards run on the same gel. Experiments shown in **a–b** were repeated 4–5 times with similar results. **c** Relative level of GPx-1 protein expression in the samples shown in figure **b** as determined by densitometry. * $P < 0.05$ versus control. Data shown are means $\pm$ SE of $n = 4$

Table 1

Comparison of cell homeostasis indicators in control and cysteine incubated cells. The parameters shown were measured in freshly isolated ventricular cardiomyocytes following incubation with/without 0.5 mM cysteine and subsequent washing and suspension in fresh cysteine-free media. Data shown are means $\pm$ SE of $n = 5$ cells isolated from four hearts

Indicator	Control	Cysteine incubated
Calcium transient size (nM)	155 $\pm$ 14	167 $\pm$ 39
ATP (nmol/mg protein)	20.09 $\pm$ 4	19.92 $\pm$ 5.08
NADH/NAD ⁺ ratio	0.429 $\pm$ 0.02	0.5 $\pm$ 0.04
Creatine (nmol/mg protein)	43.07 $\pm$ 9.14	42.12 $\pm$ 9.58