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Ultrafine Particulate Matter Increases Cardiac Ischemia/Reperfusion Injury via Mitochondrial Permeability Transition Pore

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Abstract

Ultrafine Particulate Matter (UFP) has been associated with increased cardiovascular morbidity and mortality. However, the mechanisms that drive PM associated cardiovascular disease and dysfunction remain unclear. We examined the impact of oropharyngeal aspiration of 100 µg UFP from the Chapel Hill, NC air shed in Sprague-Dawley rats on cardiac function, arrhythmogenesis, and cardiac ischemia/reperfusion (I/R) injury using a Langendorff working heart model. We found that exposure to UFP was capable of significantly exacerbating cardiac I/R injury without changes to overall cardiac function or major changes in arrhythmogenesis. Cardiac I/R injury was attenuable with administration of Cyclosporin A (CsA), suggesting a role for the mitochondrial permeability transition pore (mPTP) in UFP associated cardiovascular toxicity. Isolated cardiac mitochondria displayed decreased Ca²⁺ buffering before opening of the mPTP. These findings suggest that UFP induced expansion of cardiac I/R injury may be a result of mPTP Ca²⁺ sensitization resulting in increased mitochondrial permeability transition and potential initiation of mPTP associated cell death pathways.

Keywords

Particulate Matter; Mitochondria; Reperfusion Injury; Cardiac; mPTP

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality in the United States, with acute myocardial infarction being a primary contributor to death (1). In recent years a strong correlation between exposure to environmental air pollutants such as particulate matter (PM) and exacerbations in CVD has been reported (2–4). Pulmonary exposures to different size classes of PM including fine, ultrafine, and nanoscale PM have been shown to

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result in: alterations in heart rate (3) and blood pressure (5) in human studies. Additionally, animal models investigating the effects of PM exposure on cardiovascular disease have observed acceleration of atherosclerosis (6) and expansion of myocardial infarction (7, 8). The epidemiological association between exposure to PM and CVD has been subject of numerous studies. Despite the many investigations codifying the associations between pulmonary exposure to PM and CVD the mechanisms by which they are linked remains unclear. There are several popular hypotheses regarding how pulmonary exposure to environmental toxicants such as PM can induce deleterious cardiovascular effects. These proposed mechanisms explain the link between air pollutants and CVD, and include: systemic inflammation, direct particle interactions with cells and tissues of the cardiovascular system, alterations in the autonomic nervous system, and mitochondrial dysfunction (9).

The heart is an organ with high metabolic demand; as such, delivery of oxygen to the myocardium via the coronary arteries in support of normal cardiac function is crucial. Occlusion of one or more coronary arteries leads to ischemia in areas distal to the blockage, whereby cardiac myocyte metabolism is compromised. The inability of the myocyte to generate adenosine triphosphate (ATP) via oxidative phosphorylation causes a shift to anaerobic glycolysis (10). Anaerobic glycolysis increases intracellular H^+ ion concentration inducing acidosis that then disturbs the Na^+/H^+ exchanger, while ATP depletion inactivates Na^+/K^+ ATPase. The combined effect results in Na^+ overload, and the Na^+-Ca^{2+} exchanger attempts to compensate for the Na^+ overload by pumping Ca^{2+} into the cytoplasm, however the Ca^{2+} overload induces myocardial stunning, contracture, and ultimately cell death (11). The restoration of blood flow, known as reperfusion, can inherently result in further myocardial injury. Reperfusion injury can encompass several processes including: arrhythmogenesis, myocardial stunning and contracture, Ca^{2+} mishandling, and induction of apoptotic or autophagic cell death pathways (10, 12, 13). Fluctuations in Ca^{2+} from the sarcoplasmic reticulum during reperfusion stimulate opening of the mPTP (14, 15). Opening of the mPTP results in the rapid dissipation of the membrane potential gradient required for synthesis of ATP, water enters via the open pore resulting in mitochondrial swelling and lysis triggering apoptosis and cell death (16).

The cellular mechanisms that ultimately lead to cardiomyocyte death during ischemia and reperfusion injury are strongly tied to the mitochondria. Mitochondria are crucial in regulating normal cardiac metabolism and play a key role in the susceptibility of the heart to ischemia/reperfusion (I/R) injury (17, 18). It is hypothesized that PM induced changes in mitochondrial function may be driving adverse cardiovascular outcomes associated with exposure to PM. One study has linked fine PM exposure to multiple forms of mitochondrial dysfunction, including cardiomyocyte mitochondrial structure changes, changes in mitochondrial fusion/fission gene expression, and reduction in the activity of superoxide dismutase (19–21). Additionally, exposure to PM generated from mountain top removal mining was associated with increased susceptibility to opening of the mPTP (mitochondrial permeability transition pore) and thus induction of apoptotic pathways and cell death (22).

We hypothesize that exposure to ultrafine particulate matter (UFP) results in expansion of cardiac ischemia reperfusion injury, resulting from mPTP Ca^{2+} sensitization. In order to test

this hypothesis, we instilled male Sprague-Dawley rats with ultra-fine particulate (UFP) and 24 hours post instillation evaluated the *ex vivo* heart for cardiac function, arrhythmogenesis and ischemic injury via a Langendorff preparation. To more directly implicate mitochondrial dysfunction and increased mPTP opening we treated the isolated heart preparation with cyclosporine A, an inhibitor of opening of the mPTP (23), to determine if infarct expansion could be attenuated by preventing cell death associated with Ca²⁺-induced mPTP opening.

METHODS AND MATERIALS

Animals

Male Sprague-Dawley rats weighing between 270 – 300 grams were housed in the same room on a 12 hour light-dark cycle and provided food and water *ad libitum*. All animals were allowed a one week acclimatization prior to experiments. Aged matched rats were randomly assigned two to a cage, when received from the supplier, into two groups and exposed to either 100 µg UFP in 200 µl sterile saline (UFP) suspension or 200 µl saline vehicle control. All experiments were approved and conducted in accordance with the Institutional Committee on the Care and Use of Laboratory Animals at East Carolina University.

Ultra-Fine Particulate and Exposure

The ultrafine particulate matter utilized in this study was collected and characterized by the US EPA facility Chapel Hill NC. The particle characteristics have been previously described (24) and were collected from the October sampling date. All suspension aliquots were sonicated for 2 minutes after initial preparation and then vortexed immediately prior to delivery. Rats were anesthetized by inhalation of a 50:50 isoflurane propylene-glycol mixture in an induction chamber. After establishment of deep anesthesia as assessed by absence of limb withdrawal from a toe pinch, the rat was suspended on an inclined board and the tongue was anteriorly displaced using padded forceps, effectively closing the esophageal opening, and a 200 µL pipette tip containing the UFP suspension was placed into the laryngopharynx, just superior to the glottis. The animal was allowed to spontaneously aspirate the UFP suspension or vehicle control. Following the aspiration, the animal was returned to its cage and recovery was observed until normal grooming habits resumed. This form of oropharyngeal (OP) aspiration has been adopted by the members of the National Institute of Environmental Health Sciences Centers for Nanotechnology Health Implications Research (NCNHIR) and Nano GO consortiums in an efforts to minimize variability and is an accepted and previously published technique to achieve OP aspiration (25–27).

Myocardial Excision

Twenty-four hours following instillation of either saline or UFP animals were deeply anaesthetized using a ketamine/xylazine mixture (90/10 mg/kg, respectively). After the elimination of a toe pinch reflex hearts were removed by midline thoracotomy, briefly placed in ice-cold saline, and rapidly hung and retro-perfused on the cannula of a modified Langendorff apparatus.

Langendorff perfusion protocol

Following the removal of the heart and placement on the cannula, hearts were perfused with a modified Krebs buffer containing (mM) 118 NaCl, 24 NaHCO₃, 4.8 KCl, 2 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, and 10 glucose, equilibrated with 95 % O₂–5 % CO₂ at 37 °C (28). Following a 5 minute baseline period, animals were randomly selected to receive either this perfusate buffer alone or to receive the control buffer with the addition of 0.2 μM cyclosporine A (Sigma-Aldrich, St Louis, MO) administered throughout Langendorff perfusion. Perfusion pressure was maintained between 70 and 80 mmHg. A latex balloon was inserted through the left atrium into the left ventricle to monitor pressure and heart rate. ECG leads were placed in the warming bath surrounding the heart to monitor electrical activity in a pseudo-lead II configuration. After 10 minutes of stable recordings global ischemia was initiated by turning off the perfusate flow to the heart for 20 minutes. After the 20 minute ischemic period the flow was restored and reperfusion ensued for 2 hours.

Electrophysiology and Arrhythmia Scoring

Arrhythmogenesis data was collected throughout the Langendorff protocol. Premature ventricular contractions were counted during the 10 minute stable baseline period prior to induction of ischemia. The first and second hours of reperfusion were scored for arrhythmia severity utilizing criteria set forth by Curtis and Walker (29) and is summarized in Supplemental Table 1. Time to ventricular tachycardia (VT) or ventricular fibrillation (VF) was defined as the duration of time from initiation of reperfusion and onset of VT/VF. Heart rate (HR) was recorded during a baseline period of 5 minutes, ischemia and reperfusion. Heart rate data was reported as the mean HR within groups during baseline, ischemia and the 1st and 2nd hours of reperfusion.

Measures of Cardiac Function

All physiological parameters of cardiac function from the isolated hearts of saline or UFP exposed hearts were continuously monitored and stored on a computer using Lab-Chart (AD Instruments, Colorado Springs, CO, USA).

Quantification of Myocardial Infarct

Following reperfusion, the heart was cut off the cannula just below the atria and the right ventricle removed. The left ventricle was then sliced into 4 approximately equal sections from the base to apex and weighed for the determination of infarct size (30). Each slice was then placed in a 0.1 % triphenyltetrazolium chloride solution and incubated at 37 °C for 10 minutes in a shaking water bath. Following incubation both sides of each slice were photographed with a digital camera attached to a dissecting microscope. Images were quantified using Image J software where total area (TA), lumen area and infarcted area (IA) were measured. The area at risk (AAR) was considered to be the entire left ventricle and was calculated by taking the TA and subtracting the lumen area. The infarcted area was determined by measuring the white appearing tissue in the AAR. Areas were converted to weights by multiplying the mean of the AAR and IA of both sides of the slice by the weight of each slice. The total weight of the IA from all sections was then divided by the total

weight of the AAR from all sections to obtain a percentage of the heart that was infarcted (25, 31, 32).

Isolation of Cardiac Mitochondria

Left ventricular cardiac mitochondria were isolated as described in Sloan *et al.* (33). Briefly, hearts were excised and immersed in 4 °C isolation solution (IS) containing: 300 mM sucrose, 10 mM sodium-hepes and 0.2 mM EDTA. The left ventricle was excised, weighed, and rinsed in fresh IS buffer. The ventricular tissue was minced and subjected to trypsin (1.25 mg) digestion (2 min) in 10 ml of IS (pH = 7.2). Following digestion, 10 ml of IS buffer containing 6.5 mg of trypsin inhibitor and BSA (1 mg/ml) was added and the suspension was then homogenized with a teflon Potter-homogenizer. The homogenate was centrifuged at $600 \times g$ for 10 min, followed by centrifugation of the supernatant at $8000 \times g$ for 15 min. The pellet was then re-suspended in IS buffer + BSA, re-centrifuged and the final mitochondrial fraction stored on ice in IS buffer. Mitochondrial protein content was determined using a BCA protein assay.

Ca²⁺ Retention Capacity

Calcium retention protocols were modified from Sloan *et al.* (33) where 0.5 mg mitochondria were suspended in an assay buffer containing: 125 mM KCl, 5 mM HEPES, 2 mM KH₂PO₄, 1 mM MgCl₂ and (25 °C, pH = 7.3). The fluorescent Ca²⁺ indicator, calcium green 5 N salt, was utilized to track changes in extra-mitochondrial calcium levels. Fluorescence was measured using a fluorescence spectrophotometer (Photon Technology International, Birmingham, NJ, USA), with excitation and emission wavelengths set to 506/532 nm, respectively. Calcium induced mPTP opening experiments were performed under state 2 respiration conditions (5 mM glutamate/5 mM malate). Mitochondria were subject to sequential 50 nmol CaCl₂ pulses every 3 minutes, which causes repeated decreases in the fluorescent signal as Ca²⁺ is taken up by the mitochondria. Induction of mPTP was denoted by a sharp increase in extra mitochondrial Ca²⁺ fluorescence, representing the release of the accumulated Ca²⁺ from the mitochondrial matrix. Calcium retention capacity was quantified as the amount of calcium needed to induce PTP opening (nmol CaCl₂/mg mitochondria). Because of the terminal nature of the Ca²⁺ Retention Capacity protocol the experiments were designed whereby the isolated mitochondria from the hearts of exposed animals were split into two groups: one that received Cyclosporine A (CsA) treatment and one that did not.

Statistical analysis

Heart rate was analyzed using repeated measures two-way ANOVA followed by Tukey post hoc test. Values were reported as mean $\pm$ SEM. Differences in: infarct size between groups, hemodynamic variables, and arrhythmia data were analyzed using two-way ANOVA with Bonferonni post-hoc test. Calcium retention differences were calculated by two-way ANOVA with Tukey post hoc test. In all cases a p value of <0.05 was considered to indicate statistical significance. All data were analyzed and graphed using GraphPad Prism software (La Jolla, CA).

RESULTS

Cardiac Electrical Activity Following UFP Exposure

Pulmonary exposure to UFP had no impact on baseline HR compared to saline instillation. Likewise, administration of CsA had no impact on mean HR during a baseline assessment (Table 1). Following 20 minutes of ischemia and restoration of perfusate buffer, there were no differences in HR between groups during the reperfusion following stabilization of the ECG (Table 1). Following OP aspiration of UFP there was a modest increase but no statistically significant differences in the number of PVCs observed during a baseline period compared to saline control. Likewise, although statistically insignificant, there was a minor reduction in baseline PVCs in the presence of CsA in hearts from rats having aspirated either saline or UFP (Figure 1A). Aspiration of UFP resulted in a slight but non-statistically significant decrease in time to VT/VF compared to saline controls. However, the presence of CsA tended to lengthen the time before reperfusion associated VT/VF (Figure 1B). Overall, neither UFP nor CsA had a significant impact on arrhythmogenesis as there were no differences in arrhythmia scores during the first (Figure 1C) or second (data not shown) hours of reperfusion.

Cardiac Function Following UFP Exposure

Baseline assessment of cardiac function, defined as a 10 minute period prior to the onset of ischemia, revealed no statistically significant differences in left ventricular developed pressure (LVDP), left ventricular contractility (LV +dP/dt), and left ventricular relaxation (LV -dP/dt), between hearts from UFP and saline exposed rats. Likewise, CsA had no impact on baseline cardiac function (Table 2). Following twenty minutes of ischemia and during the first hour of reperfusion, there were no statistically significant differences in cardiac function between groups. However, overall the administration of CsA resulted in a moderate improvement in mean LVDP within the first hour of reperfusion, regardless of aspirate exposure (Table 3). While no statistically significant differences in mean LVDP, LV +dP/dt, or LV -dP/dt were observed between exposure groups during the final hour of reperfusion, the hearts from rats that had aspirated saline did exhibit a modest decrease in mean LVDP, LV +dP/dt and LV -dP/dt (Table 4) relative to all other treatment groups.

Expansion of Myocardial Ischemia/Reperfusion injury following UFP Exposure

Exposure to UFP significantly increased cardiac ischemia reperfusion injury by nearly 20 % compared to saline control (Figure 2). Addition of cyclosporine A reduced the overall expansion in reperfusion injury associated with UFP exposure by 15 % (Figure 2). Cyclosporine A only reduced expansion of ischemia reperfusion injury by approximately 5 % in hearts of rats that had aspirated saline.

UFP and Ca²⁺ Retention in Isolated Cardiac Mitochondria

Mitochondria isolated from the left ventricle of rats 24 hours following aspiration of UFP required significantly less Ca²⁺ to induce mPTP opening compared with saline controls, as measured by sustained Ca²⁺ fluorescence (Figure 3). Administration of CsA significantly improved Ca²⁺ uptake in isolated mitochondria from rats that had aspirated UFP, resulting in

attenuation of UFP associated impaired Ca^{2+} retention capacity. Administration of CsA had no statistically significant impact on calcium retention in mitochondria isolated from LV of rats that had aspirated with saline.

DISCUSSION

The rise in the incidence and prevalence of CVD is concerning. In the face of worsening worldwide air quality, mechanisms linking pulmonary exposure to PM and CVD will become increasingly important in understanding that interaction and its impact on the severity and progression of CVD. Herein, we examined the impact of OP aspiration of UFP or saline on multiple aspects of cardiac function and expansion of myocardial injury including: HR, arrhythmogenesis, LVDP, +LV dP/dt, -LV dP/dt, and quantification of cardiac ischemia reperfusion injury. Given the multiple hypotheses regarding potential mechanisms that drive UFP to exacerbate cardiovascular disease, including inflammation, oxidative stress, autonomic dysfunction, or mitochondrial dysfunction (9), we hypothesized that UFP was able to exacerbate cardiac ischemia/reperfusion injury by promoting the opening of the mPTP. Our study supports previous studies linking detrimental cardiovascular endpoints with exposure to UFP (34, 35), and demonstrates a novel target of UFP toxicity within the mitochondria (20, 36), the mPTP. These findings are important for two reasons: 1) these data demonstrate the ability for UFP to exacerbate cardiac injury in the face of a secondary stressor, *i.e.* myocardial ischemia and reperfusion, 2) administration of CsA abrogates the observed increased I/R injury, indicating that UFP may be influencing mitochondrial function via the mPTP.

In order to assess impact of UFP on CVD, we first examined if OP aspiration of UFP had any impact on basal cardiac function. As there were no differences in baseline HR or cardiac function between saline and UFP exposed hearts, it was determined that a single acute UFP exposure alone was insufficient to induce gross changes in function of an isolated heart preparation or incidence of electrocardiographic ectopy. These observations are congruent with previous experimental findings whereby exposure to PM alone induced minor or no observable changes in cardiac function. However, when PM exposure is paired to a secondary insult or stressor, PM is capable of exaggerating latent electrophysiological dysfunction (37) or worsening cardiac injury (25). Furthermore, it is possible that any changes associated with UFP induced modulation of ion channels leading to increased susceptibility to arrhythmogenesis would likely need greater than 24 hours to fully manifest (38). Following exposure to UFP and the additional stress of ischemia and reperfusion, we observed minor increased susceptibility to VT/VF, although no significant differences in overall arrhythmia score were quantified, and may simply reflect extent of infarct induced by UFP in a global model of ischemia. We recognize a limitation of this study was the choice of a high dose delivered as a mass unit and therefore does not reflect a realistic occupational exposure. However, the results provided still provide clues to the mechanism through which particulate matter exposure may contribute to cardiovascular dysfunction.

Mitochondria are critical for the proper mechanical and electrophysiological function of the cardiomyocyte, playing roles that extend beyond bioenergetics and metabolism. Proper function is required to meet the high energetic demand of the cardiomyocyte, as well as

playing an important role in managing oxidative stress and Ca^{2+} handling (39). Exposure to UFP has been associated with both oxidative stress (40–44) and alterations in Ca^{2+} handling (45–47). Furthermore, mitochondria play a significant role in mediating the extent of injury during ischemia and reperfusion (48). This raises the possibility that the mitochondria may be a potential target for attenuating some of the toxicological endpoints associated with UFP exposure.

This study evaluated the role of UFP exposure on cardiac mitochondrial Ca^{2+} handling, and how subsequent alterations in tolerance to Ca^{2+} loading may contribute to cardiovascular dysfunction and injury through mitochondrial function. Using a paradigm of global cardiac ischemia reperfusion injury, we observed expansion of myocardial infarction in the presence of UFP. Administration of CsA was able to attenuate the expansion of I/R injury associated with UFP compared to saline in the isolated heart model. Cyclosporine A is hypothesized to reduce reperfusion injury by preventing the opening of the mPTP and inhibition of the resultant cell death pathways associated with mitochondrial permeability transition during reperfusion (49). Exposure to UFP was associated with a decrease in the number of Ca^{2+} pulses required to induce opening of the mPTP, an effect that was prevented by CsA. Given these results, UFP exposure appears to be associated mPTP Ca^{2+} sensitization.

It is interesting to note that the inability for CsA to completely abrogate expansion of reperfusion injury associated with UFP exposure suggests the I/R injury is not solely tied to mitochondrial dysfunction but may be due to UFP associated oxidative stress. Additionally, despite UFP exposure being capable of generating significant infarct expansion there are no differences in cardiac function between groups during reperfusion. This, however, may reflect the infarct size experienced by the myocardium irrespective of treatment were large enough to generate cardiac dysfunction. Although not measured in this study, exposure to UFP has been associated with increased levels of oxidative stress (40–43) which may promote opening of the mPTP as a result of reactive oxygen species induced reactive oxygen species release (50). This mechanism is not effectively attenuated by CsA (51), suggesting a potential additional role for other mitochondrial ion sensitive channels. Furthermore, it is important to note that centrifugation of mitochondria may result in a separation of healthy and unhealthy mitochondria. If this is the case, then this study may have only evaluated decreased calcium buffering in the healthy mitochondria following exposure to you UFP. It is possible that CsA is only capable of attenuating injury by preventing mitochondrial transition in healthy mitochondria following UFP exposure and explaining why CsA was unable to fully attenuate UFP induced expansion of myocardial I/R injury.

As the Langendorff model is used only to evaluate changes that are intrinsic to the heart there still exist alternate potential mechanisms for the exacerbation of cardiac injury by UFP which have been previously studied, but not addressed in this study: autonomic imbalance, altered vascular reactivity, and increased thrombosis leading to vascular occlusion (9). The lack of total attenuation of UFP associated expansion of cardiac I/R injury suggests that despite the mPTP playing an important role in UFP cardiac toxicity, it is unlikely to be the only target of UFP. These previously investigated mechanisms of UFP toxicity may drive expansion of cardiac I/R injury in addition to this new contribution of impaired calcium buffering and mPTP activation in the mitochondria. The precise mechanism by which UFP

may influence the mitochondrial permeability transition pore and influence ischemia reperfusion injury is certainly elusive. A direct action of UFP on the mitochondrial permeability transition pore is unlikely but more likely to act through oxidant burden or modulating immune responses and by which CsA may be contributing and effect. This would be predicated on the notion that UFP was able to generate an oxidant stress which may have an additive effect in the presence of reperfusion zone where oxidant stress or a strong immune response element to PM toxicity was present. In general, CsA does not have any inherent antioxidant capacity but through its action on the mitochondrial permeability transition pore can reduce ischemic induce free radical production in the myocardium (52).

CONCLUSION

Air pollution, particularly PM has been strongly associated with increasing prevalence, incidence, and severity of CVD. We investigated the impact of OP aspiration of a UFP bolus on cardiac function, arrhythmogenesis, and cardiac ischemia/reperfusion injury, using a Langendorff model of the working heart. We observed no differences in overall cardiac function; the susceptibility to arrhythmogenesis was marginally increased by exposure to UFP but ischemia/reperfusion injury was significantly increased in hearts from UFP exposed rats, which was attenuated by administration of CsA. Furthermore, isolated mitochondria demonstrated impaired calcium buffering before opening of the mPTP. Together these findings suggest that UFP exposure is associated with mPTP Ca^{2+} sensitization resulting in the promotion of mitochondrial transition and contribution to an expanded cardiac reperfusion injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cardiac Electrical Activity

Ex vivo electrocardiogram recordings captured throughout the duration of the Langendorff protocol. Open bars represent values obtained from control hearts and solid bars represent values from age matched hearts treated with 0.2 μ M CsA. Parameters measured were number of PVCs during a 10 minute baseline period (A), Time to VT/VF following reperfusion (B), and arrhythmia scoring for the 1st hour of reperfusion (C). Statistical significance considered as $p < 0.05$ by two-way ANOVA with Bonferroni post-hoc test. Data are reported as mean $\pm$ SEM with $n = 4-6$.

Myocardial Infarct Size

Figure 2. Myocardial Ischemia/Reperfusion Injury

Following a baseline period *ex vivo* hearts were subjected to 20 minutes of ischemia followed by 2 hours of reperfusion. Exposure to UFP was associated with expansion of cardiac ischemia/reperfusion injury 24 hours following the oropharyngeal aspiration. Open bars represent values obtained from control hearts and Solid bars represent values from hearts treated with 0.2 μ M CsA. Statistical significance considered at $p < 0.05$ vs saline and indicated by (*) as calculated by two-way ANOVA with Bonferroni post-hoc test. Data are reported as mean $\pm$ SEM with $n = 6-7$.

Calcium Retention Capacity

Figure 3. Isolated Mitochondria Ca^{2+} Sensitivity

Quantification of calcium retention capacity following 50 nmol pulses of CaCl_2 in isolated mitochondria from hearts of rats that aspirated saline or UFP 24 hours prior to their isolation. Isolated mitochondria were treated with either 0.2 μM CsA (solid bars) or control (open bars). Statistical significance at $p < 0.05$ vs saline indicated by (*), or vs +CsA indicated by (#), were calculated by two-way ANOVA with Bonferroni post hoc test. Data are reported as mean $\pm$ SEM with $n = 3-4$ experimental groups.

Table 1**Heart Rate**

	Baseline (bpm)	Reperfusion (bpm)
Saline n = 5	303.7 ± 15.0	389.2 ± 41.5
Saline + CsA n = 6	271.6 ± 9.8	351.7 ± 92.8
UFP n = 4	273.3 ± 20.5	403.0 ± 66.6
UFP+CsA n = 8	269.8 ± 16.4	351.7 ± 92.8

Data presented as calculated mean heart rate recorded during baseline period and during reperfusion, following stabilization of the ECG. Values are expressed as mean ± SEM. Evaluation for statistical significance ($p < 0.05$) was calculated by repeated measures two-way ANOVA with Tukey post hoc test within a time period.

Table 2

Cardiac Function - Baseline

	LVDP (mmHg)	LV +dP/dt (cm H ₂ O/sec)	LV -dP/dt (cm H ₂ O/sec)
Saline n = 4	77.8 ± 2.3	2953 ± 158	-2023 ± 152
Saline + CsA n = 6	80.9 ± 8.1	2716 ± 304	-2061 ± 156
UFP n = 4	80.7 ± 10.6	3329 ± 595	-2109 ± 467
UFP+CsA n = 8	70.5 ± 8.1	2480 ± 374	-1763 ± 153

Calculated mean left ventricular developed pressure (LVDP), left ventricular contractility (LV +dP/dt), and left ventricular relaxation (LV -dP/dt) recorded during baseline. Values as expressed as mean ± SEM. Evaluation of statistical significance (p<0.05) was calculated by two-way ANOVA with Bonferroni post hoc test within a time period.

Table 3**Cardiac Function – 1st Hour of Reperfusion**

	LVDP (mmHg)	LV +dP/dt (cm H ₂ O/sec)	LV –dP/dt (cm H ₂ O/sec)
Saline n = 5	1.6 ± 0.1	319.6 ± 63.3	–237.1 ± 31.2
Saline + CsA n = 6	3.1 ± 0.9	468.4 ± 88.5	–329.0 ± 57.2
UFP n = 4	1.8 ± 0.1	413.6 ± 118.5	–317.5 ± 86.6
UFP+CsA n = 8	2.9 ± 1.0	515.8 ± 98.0	–375.3 ± 61.6

Calculated mean left ventricular developed pressure (LVDP), left ventricular contractility (LV +dP/dt), and left ventricular relaxation (LV –dP/dt) recorded during the 1st hour of reperfusion. Values are expressed as mean ± SEM. Evaluation for statistical significance (p<0.05) was calculated by two-way ANOVA with Bonferroni post hoc test within a time period.

Table 4Cardiac Function 2nd Hour of Reperfusion

	LVDP (mmHg)	LV +dP/dt (cm H ₂ O/sec)	LV -dP/dt (cm H ₂ O/sec)
Saline n=4	7.7 ± 1.8	330.1 ± 63.4	-213.4 ± 30.0
Saline + CsA n=6	16.4 ± 4.6	587.3 ± 113.1	-409.1 ± 83.0
UFP n=4	13.9 ± 6.6	559.6 ± 210.7	-369.3 ± 137.0
UFP+CsA n=8	16.9 ± 3.2	600.8 ± 92.3	-416.1 ± 65.3

Calculated mean left ventricular developed pressure (LVDP), left ventricular contractility (LV +dP/dt), and left ventricular relaxation (LV -dP/dt) recorded during the 2nd hour of reperfusion. Values are expressed as mean ± SEM. Evaluation for statistical significance (p<0.05) was calculated by two-way ANOVA with Bonferroni post hoc test within a time period.