Pioglitazone Attenuates Acute Cocaine Toxicity in Rat Isolated Heart

Potential Protection by Metabolic Modulation


ABSTRACT

Background: The authors tested whether cocaine depresses mitochondrial acylcarnitine exchange and if a drug that enhances glucose metabolism could protect against cocaine-induced cardiac dysfunction.

Methods: Oxygen consumption with and without cocaine was compared in rat cardiac mitochondria using octanoylcarnitine (lipid) or pyruvate (nonlipid) substrates. Isolated hearts from rats with or without a pioglitazone-supplemented diet were exposed to cocaine.

Results: The 0.5 mM cocaine inhibited respiration supported by octanoylcarnitine (82 ± 10.4 and 45.7 ± 4.24 ngatomO min⁻¹ · mg⁻¹ · protein ± SEM, for control and cocaine treatment, respectively; P < 0.02) but not pyruvate-supported respiration (281 ± 12.5 and 267 ± 12.7 ngatomO min⁻¹ · mg⁻¹ · protein ± SEM; P = 0.45). Cocaine altered contractility, lusitropy, coronary resistance, and lactate production in isolated heart. These effects were each blunted in pioglitazone-treated hearts. The pioglitazone diet attenuated the drop in the rate-pressure product (P = 0.002), cocaine-induced diastolic dysfunction (P = 0.04), and myocardial vascular resistance (P = 0.05) compared with that of controls. Lactate production was higher in pretreated hearts (P = 0.008) and in ventricular myocytes cultured with pioglitazone (P = 0.0001).

Conclusions: Cocaine inhibited octanoylcarnitine-supported mitochondrial respiration. A pioglitazone diet significantly attenuated the effects of cocaine on isolated heart. The authors postulate that inhibition of acylcarnitine exchange could contribute to cocaine-induced cardiac dysfunction and that metabolic modulation warrants additional study.

COCAIN, a local anesthetic with potent sympathomimetic properties, is a common drug of abuse in the United States, and acute cocaine intoxication is a common cause of emergency department visits. Patients often present with hypertension, arrhythmias, and chest pain—a clinical equivalent to acute coronary syndrome. Cocaine induces ischemia by concomitant systemic and coronary arterial vasоconstriction, which causes imbalance in the myocardial oxygen supply-demand ratio.1,2 Severe cocaine toxicity is also associated with myocardial contractile depression, but the mechanisms underlying this effect are not clearly established.3 We previously have shown that bupivacaine, another cardiotoxic local anesthetic, impairs mitochondrial uptake of fatty acid substrates through inhibition of acylcarnitine exchange.4 This effect is postulated to contribute to bupivacaine-induced myocardial toxicity. In the current study, we...
tested the hypothesis that cocaine similarly impairs mitochondrial fatty acylcarnitine metabolism and ascertained whether a strategy of metabolic modulation could reduce the cardiotoxic effects of cocaine.

Pioglitazone is a member of the thiazolidinedione class of peroxisome proliferator-activated receptor-γ agonists currently used for treatment of type 2 diabetes. Peroxisome proliferator-activated receptor-γ activation plays a critical role in energy homeostasis by modulating insulin sensitivity in both adipose and muscle tissue. We previously showed that thiazolidinediones increase glucose consumption and lactate production in cultured astrocytes and exhibit cytoprotection against hypoglycemia-induced cell death. Thiazolidinediones have also been found to exert multiple transcription-independent effects, including regulation of mitochondrial function by altering complex I of the respiratory chain. The heart, like the central nervous system, is highly dependent on aerobic metabolism to maintain cell function and viability. We postulated that pretreatment with pioglitazone as a dietary additive could attenuate subsequent cocaine-induced cardiac toxicity by reducing sensitivity to mitochondrial metabolic challenges.

Materials and Methods

Rats
Adult male Sprague-Dawley rats, weighing between 450 and 550 g (3–4 months old) were used in all experiments. All protocols were approved by the Animal Care Committee of the University of Illinois Office for Protection of Research Subjects (Chicago, Illinois) and by the Institutional Animal Care and Use Committee of the Veterans Administration Chicago Healthcare System (Chicago, Illinois).

Pioglitazone Treatment
Rats were fed ad libitum either standard chow or chow containing pioglitazone, 100 ppm, equivalent to low micromolar serum concentrations for 1 week before the experiments.

Mitochondrial Studies
Cardiac interfibrillar mitochondria were prepared from a homogenate of rat cardiac ventricles by differential centrifugation according to the procedure of Palmer et al. Respiratory was measured at 30°C in a 0.5-ml chamber containing mitochondria in a final concentration of 1 mg protein/ml. After equilibration of the Clark oxygen electrode probe (YSI Clark Oxygen Probe, Yellow Springs, OH), endogenous mitochondrial substrates were depleted by addition of 0.1 mM adenosine diphosphate. Respiration was then initiated by the addition of substrate to the incubation medium. Mitochondrial oxygen use was monitored during pyruvate-stimulated respiration (n = 6) and octanoylcarnitine-stimulated respiration (n = 6) by measuring the rate of decrease of oxygen concentration in the chamber. Calculations of respiratory rates, in units of ngatmO min⁻¹ · mg⁻¹ · protein, were derived from the slope of oxygen concentration in the reaction chamber.

Isolated Heart System
Rats were anesthetized by intraperitoneal injection of 60 mg/kg sodium pentobarbital (Abbott Labs, Abbott Park, IL), and after systemic heparinization, hearts were removed, cannulated through the ascending aorta, suspended from a Langendorff apparatus and perfused at a constant rate of 16 ml/min with Krebs-Ringer bicarbonate buffer (KRB) containing 100.00 mM NaCl, 4.74 mM KCl, 1.18 mM KH₂PO₄, 1.18 mM MgSO₄, 1.00 mM CaCl₂, 25.00 mM NaHCO₃, 11.50 mM glucose, 4.92 mM pyruvate, and 5.39 mM fumarate with pH 7.40 via roller pump. KRB perfusate was warmed in the Langendorff apparatus by countercurrent flow from a 37°C water bath, and the temperature of the KRB was continuously measured just above the heart and maintained at 37°C. The heart was suspended inside a glass cylinder warmed by the same countercurrent. KRB was also equilibrated with a mixture of oxygen (95%) and carbon dioxide (5%) by passage through a membrane oxygenator.

Monitoring Cardiac Function
Pressure data from a latex balloon in the left ventricle connected to a pressure transducer were recorded, archived, and analyzed by Powerlab Data Analysis System using Chart 5.2.1 (ADInstruments, Colorado Springs, CO). A catheter was placed in the pulmonary artery to sample outflow from the coronary circulation for determining venous Po₂.

Metabolic and Functional Parameters
Heart rate, left ventricular developed pressure (systolic pressure – diastolic pressure), the maximum positive rate of change in left ventricular pressure (dP/dt max), rate-pressure product (RPP; RPP = heart rate × left ventricular developed pressure) and the left ventricular relaxation time constant (τ) were continuously monitored throughout the experiment. The perfusate was sampled above the heart from the pulmonary artery catheter to calculate oxygen consumption (oxygen consumption = coronary flow × 0.024 × [Po₂arterial – Po₂venous]).

Lactate Measurements
To assess lactate production, samples were incubated with 90 μl Diagnostic Lactate (Sigma–Aldrich Corp., St. Louis, MO) reagent, for 20 min at room temperature, and absorbance was read at 550 nm. In each assay, a standard curve was prepared in the range of 0–100 mg/100 ml D-glucose or 0–50 mg/100 ml L-lactate in Dulbecco’s Modified Eagle’s Media. Concentrations in each sample were calculated by interpolation from these standard curves.

Cell Culture
Rat ventricular myocytes (H9C2) were obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco’s Modified Eagle’s Media containing 10% fetal calf serum (GIBCO Life Technologies, Gaithers-
burg, MD) and antibiotics. Cell culture reagents were from Sigma–Aldrich Corporation.

**Cocaine Infusion Protocol**

After the hearts were stabilized for 20 min on the Langendorff apparatus, a solution of cocaine hydrochloride was infused through a port 2 cm above the heart at a rate calculated to achieve a final concentration of 10 μM in the buffer. The infusion was continued for 5 min then increased sequentially to attain final concentrations of 50 μM and 100 μM, each for 5 min. These concentrations are typical of in vitro cardiac studies and sodium channel studies of cocaine and blood concentrations at postmortem examination in fatal cocaine overdose.

**Statistical Analysis**

All data sets were imported and analyzed in GraphPad Prism 5 (GraphPad Software, San Diego, CA). A two-tailed unpaired t test with Welch’s correction was used to compare respiratory rates of different groups of mitochondria. Heart rate, rate-pressure product, left ventricular relaxation rate constant (τ), left ventricular end-diastolic pressure, oxygen consumption, and lactate production were each analyzed for the control and pioglitazone treatment groups at baseline using two-tailed, unpaired t test with Welch’s correction. During cocaine infusions, between-group differences in parameters were analyzed at 0, 10, 50, and 100 μM cocaine using repeated measures two-way ANOVA with Bonferroni posttests when P < 0.05.

**Results**

**Mitochondrial Studies**

We compared the effects of 0.5 mM cocaine on respiration supported by either pyruvate or octanoylcarnitine (n = 6 for both groups in all experiments except lactate concentration, for which n = 5 for both control and pioglitazone groups). Rates of oxygen consumption during pyruvate-supported respiration were the same for control and cocaine-treated groups (281 ± 12.5 and 267 ± 12.7 ngatomO min⁻¹ · mg⁻¹ · protein ± SEM, respectively; P = 0.45; fig. 1). However, the respiratory rates during octanoylcarnitine-supported respiration for control and cocaine-treated groups were 82 ± 10.4 and 45.7 ± 4.24 ngatomO min⁻¹ · mg⁻¹ · protein ± SEM, respectively (P < 0.02). Thus, at 0.5 mM, cocaine inhibits lipid-based respiration in cardiac mitochondria by roughly 50%.

**Isolated Heart Experiments**

**Functional Parameters**

Baseline values of RPP, line pressure, τ, and left ventricular end-diastolic pressure were not different between the control and pioglitazone-treated hearts (n = 6 for all experiments). Beat-to-beat contractility is inversely influenced by heart rate, so RPP was used as a rate-independent measure of contractility (fig. 2A; effects on rate and pressure are not shown separately in the figures). Cocaine infusion induced a dose-dependent reduction of RPP in both groups (P < 0.0001; F = 79) that was greater in the control group, where mean normalized RPP at 100 μM cocaine was reduced to 19% (95% CI: 13–25) of baseline. Pioglitazone treatment exerted a highly significant protective effect against cocaine-induced cardiac depression compared with the control group (P < 0.002; F = 18) because mean normalized RPP at 100 μM cocaine in this group declined only to 48% (CI: 20–77) of baseline (difference in mean normalized values, 95% CI: 29%, 11–46).

Because KRB perfusion rates were held constant throughout the experiments, the perfusion line pressure provides a
Measure of coronary vascular resistance. Line pressure was dramatically increased in a dose dependent manner by infusion of cocaine in both control and test hearts ($P < 0.0001$; $F = 163$) for the overall cocaine effect; (figure 2B). However, this effect was smaller in the pioglitazone group, for which overall line pressure was significantly less than in controls ($P = 0.05$; $F = 5.0$ for the difference between groups). At $100 \mu M$, cocaine mean line pressure in the pioglitazone group was $113 \text{ mmHg}$ (95% CI: $88 – 138$), a 59% increase over baseline, versus $148 \text{ mmHg}$ (95% CI: $136 – 60$), an 89% increase in the control group (difference in mean values, 95% CI: $0.066 – 0.078$) was prolonged 96% at $100 \mu M$ cocaine, and mean line pressures measured in the 2 min before and after infusion of phentolamine were indistinguishable (data not shown).

Lusitropy, as measured by the cardiac relaxation time constant ($\tau$), was strongly affected by cocaine infusion (fig. 3A). Cocaine caused a significant, dose-dependent increase in $\tau$ ($P < 0.0001$; $F = 31$ for overall cocaine effect in both groups). This effect was observed in control hearts at concentrations as low as $10 \mu M$ cocaine, and mean $\tau$ (0.072 s; 95% CI: 0.066–0.078) was prolonged 96% at $100 \mu M$ cocaine. However, the time constants in hearts from rats fed pioglitazone were significantly shorter than those of control hearts ($P = 0.017$; $F = 8.1$ for the difference between groups) across the range of tested cocaine concentrations. $\tau$ was increased from baseline values by only 40% at $100 \mu M$ cocaine in the pioglitazone-treated group (mean, 0.047 s; 95% CI: 0.031–0.063), giving a difference in mean values of 0.025 s (95% CI: 0.0117–0.038). Cocaine similarly increased left ventricular end-diastolic pressure in a dose-dependent manner (fig. 3B; $P < 0.0006$; $F = 7.7$). This effect was attenuated in the pioglitazone-treated hearts, for which overall end-diastolic pressures were lower than that of the controls ($P = 0.043$; $F = 5.4$), and at $100 \mu M$ cocaine were increased to a mean of $10.8 \text{ mmHg}$ (95% CI: 5.3–16.4) or an 11% increase over baseline values. However, the end-diastolic pressure in control hearts was increased 89% over baseline, to a mean of $19.8 \text{ mmHg}$ (CI: 14.1–25.6). The difference in means was $9.0 \text{ mmHg}$ (95% CI: 2.70–15.3).

**Metabolism**

**Hearts**

Baseline rates of oxygen consumption were the same in both groups ($n = 6$ for all oxygen and lactate studies). However, the baseline (i.e., before cocaine infusion) effluent lactate concentration was greater in the pioglitazone-treated hearts than in the control group ($0.074 \pm 0.007 \mu M$; mean ± SEM; $P = 0.012$). Repeated measures two-way ANOVA showed that oxygen consumption was reduced in a dose dependent manner by cocaine infusion ($P < 0.0001$, $F = 140$ for the effect of cocaine in both treatment groups; fig. 4A). However, there was no overall between-group
Cocaine had no effect on lactate production at 30 minutes. Interventions that enhance myocardial oxygen delivery while increasing left ventricular after-load are traditionally viewed as a result of combined coronary and systemic vasoconstriction that would account for the observed differences in response to cocaine treatment. Coronary vascular tone is tightly regulated by metabolic activity: increased myocardial metabolism causes local coronary vasodilation, whereas reduced metabolism causes vasoconstriction. Therefore, cocaine-induced reductions in oxidative metabolism could contribute to coronary vasoconstriction and would be reversed by the insulin-sensitizing properties of pioglitazone. This prediction was confirmed by the observations that (1) pioglitazone treated hearts showed significant increases in lactate production after 4 h exposure to 20 μM pioglitazone (P < 0.0001; F = 119), whereas a 4-h exposure to cocaine had no effect on lactate production at 30 μM or 300 μM in either group (\(P = 0.008, F = 8.0\); fig. 4B).

**Difference in the Effect of Cocaine on Oxygen Consumption**

\[0.23\]

Although cocaine per se had no effect overall on lactate production (\(P = 0.52\)), there was an increase in lactate production in hearts from pioglitazone-treated rats compared with those of controls (\(P = 0.008, F = 8.0\); fig. 4B).

**Cell Culture**

H9C2 rat ventricular myocytes showed significant increases in lactate production after 4 h exposure to 20 μM pioglitazone (\(P < 0.0001; F = 119\)), whereas a 4-h exposure to cocaine had no effect on lactate production at 30 μM or 300 μM in either group (\(P = 0.52; F = 0.7\)).

**Discussion**

Our key findings are that cocaine selectively impairs metabolism of an acylcarnitine substrate and that a diet supplemented with pioglitazone, a peroxisome proliferator-activated receptor-γ agonist drug, reduces the adverse effects of cocaine on cardiac function in the isolated heart. Pioglitazone is an oral hypoglycemic agent that improves cellular glucose utilization. Attenuation of cocaine’s adverse effects on rate-pressure product, lusitropy, and coronary vascular resistance strongly suggests that pioglitazone also exerts an overall protective effect on myocardial metabolism and function. Increased lactate production in cultured myocytes and isolated hearts treated with pioglitazone indicates that increased glycolytic flux could explain its protective effect. We postulate that inhibition of mitochondrial acylcarnitine exchange could contribute to cocaine cardiac toxicity and infer that metabolic modulation designed to improve substrate utilization could improve cardiac function in cocaine overdose.

The contractile depression and diastolic dysfunction of severe cocaine intoxication are traditionally viewed as a result of combined coronary and systemic vasoconstriction that reduce myocardial oxygen delivery while increasing left ventricular after-load. Interventions that enhance myocardial metabolism (e.g., by increasing substrate delivery or utilization) should improve decrements in cardiac function caused by oxidative stressors. This prediction is supported by the finding that pioglitazone protected against cocaine-induced impairments in contractility and lusitropy, both energy-dependent elements of excitation-contraction coupling.

Our data suggest a metabolic component for both the cocaine-induced reductions in myocardial performance and their attenuation with thiazolidinedione pretreatment. This connection is supported by the observation that coronary vascular resistance was much less affected by cocaine infusion in hearts from pioglitazone-fed rats than those from controls. Cocaine is an indirect vasoconstrictor and increases, in a dose-dependent manner, coronary perfusion pressures in control hearts under constant flow conditions. This effect was significantly reduced by pioglitazone. Pioglitazone treatment typically reduces systolic blood pressure in patients by a few Torr but has not been reported to exert direct or indirect effects on coronary vessels. Buchanan et al. reported that pioglitazone blunted the contractile response of aortic rings to norepinephrine in vitro but did not alter the resting tension of intact or denuded rings. Moreover, baseline values of line pressure were not different in our two groups (\(P = 0.32\) by two-tailed t test, \(n = 6\) for both groups). Therefore, it is unlikely that a direct, dilatory effect of pioglitazone on coronary arteries would account for the observed differences in response of line pressure to cocaine treatment. Coronary vascular tone is tightly regulated by metabolic activity: increased myocardial metabolism causes local coronary vasodilation, whereas reduced metabolism causes vasoconstriction.

Therefore, cocaine-induced reductions in oxidative metabolism could contribute to coronary vasoconstriction and would be reversed by the insulin-sensitizing properties of pioglitazone. This prediction was confirmed by the observations that (1) pioglitazone treatment blunted cocaine-induced vasoconstriction, and (2) α-adrenergic blockade by phentolamine did not prevent cocaine-induced increases in line pressure. These findings suggest an alternative explanation for the well-described phenomenon of cocaine-induced coronary vasoconstriction.

Cocaine is reported to inhibit electron transport and reduce mitochondrial transmembrane potential. A recent study also reported that chronic cocaine-induced cardiac dysfunction may be caused by an uncoupling effect on oxidative phosphorylation. Inhibiting any of these components of oxidative phosphorylation would reduce adenosine triphosphate concentrations in metabolically active tissues such as the heart and therefore result in the same phenotype of poor contractility and delayed or incomplete left ventricular relaxation that results from acute ischemia. Although reoxygenating ischemic tissues is a primary goal of acute surgical or medical intervention in coronary occlusion, optimizing metabolic efficiency is another potential target for treating oxidative stress caused by lack of oxygen or substrate utilization. Thiazolidinediones potently improve glycolytic flux, and we postulate that pioglitazone treatment effectively...
protects against the functional myocardial depression caused by these metabolic deficits.

Metabolic strategies for improving cardiac performance in ischemia have been highly effective in both experimental and clinical settings. For instance, inhibiting β-oxidation of fatty acids is useful in reducing signs and symptoms of ischemia and heart failure because the heart switches to carbohydrate substrate as fuel, which is more efficient in terms of moles of adenosine triphosphate produced per mole of oxygen consumed than is fatty acid oxidation. A converse metabolic approach seeks to increase adenosine triphosphate synthesis from glycolysis when it is limited by lack of oxygen or inhibition of substrate transport or oxidation. This represents the biochemical rationale behind insulin and glucose infusion for the treatment of myocardial infarction, a therapeutic strategy dating back four decades and still being investigated.

Thiazolidinediones represent a chronic insulin-sensitizing pharmacologic intervention that parallels the physiologic mechanism underlying acute administration of glucose and insulin. This therapeutic equivalence has been demonstrated by reports that pioglitazone enhances functional recovery and attenuates ventricular remodeling after myocardial infarction in a murine model and that rosiglitazone can protect the heart from ischemia/reperfusion injury.

We previously showed that thiazolidinediones increase glucose uptake and lactate production in cultured rodent glioma cells. They also increased the mitochondrial transmembrane potential and exhibited cytoprotective effects during substrate deprivation. In the current study, we similarly found that treatment with pioglitazone increased lactate production in cultured cells and isolated hearts. This could result from chronically increased glycolytic flux caused by enhanced glucose uptake, which would provide additional adenosine triphosphate through substrate-level phosphorylation. It is also possible that secondary mechanisms not mediated by direct peroxisome proliferator-activated receptor-γ activation or insulin sensitivity contribute to enhanced lactate production.

This study identifies an alternative hypothesis for the cardiotoxic effects of cocaine, namely inhibition of mitochondrial lipid substrate utilization. This hypothesis is further supported by the protective effect of pretreatment with a drug that improves carbohydrate metabolism. Thiazolidinediones are widely used as oral hypoglycemic agents for treating type 2 diabetes. This study points to the potential benefit of metabolic strategies for modulating substrate oxidation and adenosine triphosphate synthesis in cocaine overdose.

References
20. Devi BG, Chan AW: Impairment of mitochondrial respiration...