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Acute and Chronic Effects of Adriamycin on Fatty Acid Oxidation in Isolated Cardiac Myocytes

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S. Abdel-aleem, M. M. El-Merzabani, M. Sayed-Ahmed, D. A. Taylor and J. E. Lowe. Acute and Chronic Effects of Adriamycin on Fatty Acid Oxidation in Isolated Cardiac Myocytes. *Journal of Molecular and Cellular Cardiology* (1997) **29**, 789–797. This study was designed to determine if acute (*in vitro*) or chronic (*in vivo*) adriamycin inhibits cardiac fatty acid oxidation and if so at what sites in the fatty acid oxidation pathway. In addition, the role of L-carnitine in reversing or preventing this effect was examined. We determined the effects of adriamycin in the presence or absence of L-carnitine on the oxidation of the metabolic substrates [1-^{14}C]palmitate, [1-^{14}C] octanoate, [1-^{14}C]butyrate, [U-^{14}C]glucose, and [2-^{14}C]pyruvate in isolated cardiac myocytes. Acute exposure to adriamycin caused a concentration- and time-dependent inhibition of carnitine palmitoyl transferase I (CPT I) dependent long-chain fatty acid, palmitate, oxidation. Chronic exposure to (18 mg/kg) adriamycin inhibited palmitate oxidation 40% to a similar extent seen *in vitro* with 0.5 mM adriamycin. Acute or chronic administration of L-carnitine completely abolished the adriamycin-induced inhibition of palmitate oxidation. Interestingly, medium- and short-chain fatty acid oxidation, which are independent of CPT I, were also inhibited acutely by adriamycin and could be reversed by L-carnitine. In isolated rat heart mitochondria, adriamycin significantly decreased oxidation of the CPT I dependent substrate palmitoyl-CoA by 50%. However, the oxidation of a non-CPT I dependent substrate palmitoylcarnitine was unaffected by adriamycin except at concentrations greater than 1 mM. These data suggest that after *in vitro* or *in vivo* administration, adriamycin, inhibits fatty acid oxidation in part secondary to inhibition of CPT I and/or depletion of its substrate, L-carnitine, in cardiac tissue. However, these findings also suggest that L-carnitine plays an additional role in fatty acid oxidation independent of CPT I or fatty acid chain length.

Key Words: Adriamycin; Fatty acid oxidation; L-Carnitine; Cardiotoxicity.

Introduction

Adriamycin (doxorubicin) is a commonly used antineoplastic agent (Carter, 1975). Its use, unfortunately, must be limited due to its specific dose-dependent cardiotoxicity (Lefrak *et al.*, 1973; Goormaghtigh and Ruysschaert 1984; Kantrowitz and Bristow 1984; Buzadar *et al.*, 1985). However, the exact mechanism by which adriamycin induces cardiomyopathy remains unknown. Several hypotheses have been postulated, including: the generation of free radicals (Singal *et al.*, 1987), mitochondrial dysfunction (Goormaghtigh *et al.*, 1982), calcium overload (Singal and Pierce, 1986), inhibition of several membrane bound molecules (Law *et al.*, 1985; Singal and Panagia, 1984) and altered fatty acid oxidation.

Free fatty acids represent the major source of energy in the normal adult working heart (Neely and Morgan, 1974), whereas inhibiting their metabolism results in the accumulation of toxic intermediates including long-chain fatty acyl-CoA

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thioesters and long chain acylcarnitine derivatives (Katz and Messineo, 1981; Corr *et al.*, 1985). Accumulation of these intermediates causes deleterious effects in cardiac tissue and decreased cardiac function similar to that reported in adriamycin cardiomyopathy. Interestingly, McFalls and coworkers (McFalls *et al.*, 1985) have demonstrated partial improvement of cardiac function in the presence of L-carnitine in hearts isolated from rats injected chronically with adriamycin. Unfortunately, in these experiments substrate utilization was not examined so the role of fatty acid oxidation cannot be addressed. However, decreased fatty acid oxidation has been implicated in several other cardiomyopathies (Kako *et al.*, 1984), but the mechanism by which this occurs remains to be determined. Studies by Kako *et al.* (1974) demonstrated severe inhibition of fatty acid oxidation in heart homogenates from cardiomyopathic hamsters which the authors concluded was unrelated to an effect on fatty acid β-oxidation, or the enzymes palmitoylcarnitine transferase I (CPT I), or acyl-CoA synthetase involved in fatty acid metabolism. In contrast to these findings, Barakat *et al.* (1978) showed inhibition of some β-oxidation enzymes including enoyl-CoA reductase, β-hydroxyacyl-CoA dehydrogenase, and thiolase in heart homogenates prepared from these cardiomyopathic hamsters. Similarly, Brady and Brady (1987) demonstrated an inhibition of CPT I, an enzyme involved in long-chain fatty acid oxidation, in isolated rat liver mitochondria in the presence of increasing concentrations of adriamycin. Based on these observations these authors suggested that adriamycin-induced cardiotoxicity may be, at least in part, due to the inhibition of CPT I. However, this hypothesis was challenged by Kashfi *et al.* (1990) who reported that the inhibiton of CPT I by adriamycin was unrelated to its cardiotoxicity because less toxic analogs produced more potent inhibition of this enzyme. Finally, Beanlands *et al.* (1994) have shown an initial depression of fatty acid oxidation in a working heart preparation from rats injected chronically with adriamycin but again no mechanism for this effect has been ascertained.

Thus, the present study was designed to clarify the effect of chronic adriamycin on fatty oxidation in the heart, and to compare the site(s) at which acute (*in vitro*) and chronic (*in vivo*) adriamycin might act on cardiac fatty acid oxidation. In addition, these studies were designed to determine if L-carnitine could reverse the effects of adriamycin and if this occurred through CPT I-mediated or CPT I-independent mechanisms.

Materials and Methods

Animals

Male Sprague–Dawley rats, weighing 200–250 g, were obtained from Charles River Laboratory (Raleigh, NC, USA). Rats were allowed free access to a standard diet and water *ad libitum*. The animal protocol was approved by Duke University Institutional Care and Animal Use Committee. Animals were killed by decapitation.

Materials

[1-^{14}C]palmitate, [1-^{14}C]octanoate, [U-^{14}C]glucose [1-^{14}C]butyrate, [2-^{14}C]pyruvate, [1-^{14}C]palmitoyl-CoA, and [1-^{14}C]palmitoylcarnitine were purchased from New England Nuclear (Boston, MA, USA). Sigma was the source of bovine serum albumin (BSA, essentially fatty acid free). L-carnitine was a gift from Sigma-Tau Pharmaceuticals, Inc. (Gaithersburg, MD, USA). Adriamycin was a gift from Pharmacia, Inc. (Albuquerque, NM, USA). Joklik essential medium was purchased from Gibco Laboratories (NJ, USA). Collagenase type II was purchased from Worthington (NJ, USA).

Adriamycin administration protocol (chronic model)

Rats were divided into four groups: (1) normal control rats injected intraperitoneally (i.p.) with 0.9% sodium chloride solution (no mortality was observed in this group); (2) rats injected i.p. with 18 mg/kg of adriamycin according to a previously published protocol (Beanlands *et al.*, 1994). Briefly, adriamycin (18 mg/kg) was divided into six equal doses given over a period of 2 weeks. At the end of this period, signs of congestive heart failure including ascites and liver rigidity were evident. The mortality in this group was approximately 50% by end of injection protocol. (3) Rats injected i.p. daily with L-carnitine (500 mg/kg) over a period of 6 weeks (no mortality was observed in this group); and (4) rats injected with L-carnitine plus adriamycin. L-carnitine was injected i.p. (daily dose of 500 mg/kg of body weight) for 6 weeks, starting 2 weeks before and ending 2 weeks after adriamycin administration (in separate studies, this group mortality averaged between 10 and 25%). At the end of the injection protocols, substrate oxidation was determined in cardiac myocytes isolated from animals in each of these four groups.

Isolation of myocytes

Adult rat heart myocytes from each of the four groups were isolated using the method of Frangakis *et al.* (1980). Myocytes were isolated in Joklik essential medium containing 5.55 mM glucose, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$ and 0.5 mM $CaCl_2$. The viability of myocytes isolated by this procedure was 80–90% as determined by trypan blue exclusion.

Metabolic studies using myocytes

Myocytes (2 mg cell protein) suspended in 0.9 ml of Joklik medium, containing 25 mM $NaHCO_3$, 5.55 mM glucose, 1.2 mM $MgSO_4$, 0.5 mM $CaCl_2$ and 10 mM HEPES (pH 7.4), were placed in a 25 ml Erlenmeyer flask. To acutely administer adriamycin, cells from control rats were preincubated with the desired concentrations of adriamycin for 10 min at 37°C prior to addition of metabolic substrate. Time course measurements were begun at this time. In myocytes isolated from rats treated chronically with adriamycin or L-carnitine, no exogenous adriamycin or L-carnitine was added to the myocyte suspensions. To monitor metabolic substrate use, 0.1 ml of a single labeled metabolic substrate was added to cell suspensions from each group, yielding a final concentration of 0.2 mM [1-^{14}C]-palmitic acid (2.2×10^5 d/min), 0.2 mM [1-^{14}C]octanoate (2.5×10^5 d/min), 0.2 mM [1-^{14}C]byturate (2.5×10^5 d/min), 5.55 mM [U-^{14}C]glucose (2×10^5 d/min), or 2 mM [2-^{14}C]pyruvate (2×10^5 d/min). The Erlenmeyer flask was then closed with a rubber septum containing a plastic center well. Incubation was continued during shaking at 37°C for 10, 20, 30 and 40 min. An injection of 0.3 ml of 1 M hyamine hydroxide was administered through the septum into the center wells to absorb released CO_2, and the metabolic reactions were terminated by injecting 0.4 ml of 7% perchloric acid through the septum into the incubation medium. The flasks were then shaken continuously for 2 h at 37°C, at which time the plastic center well was removed, and placed in a scintillation vial containing 10 ml of Scinti Verse BD, and counted in a liquid scintillation counter. Control experiments with $NaH^{14}CO_3$ added to the cell suspension showed that the release of $^{14}CO_2$ was complete 1 h after the addition of perchloric acid.

Stock solutions of the substrates were prepared by dissolving octanoate or pyruvate in the cell suspension buffer and by dissolving palmitate in a solution of defatted bovine serum albumin in the cell suspension buffer. The molar ratio of palmitate to albumin was 2:1.

Isolation of rat heart mitochondria

Rat heart mitochondria were isolated by the procedure of Chappel and Hansford (1969). The isolation buffer contained 0.21 M mannitol, 0.07 M sucrose, 5 mM Tris-HCl (pH 7.4), and 1 mM EGTA.

Oxidation of palmitoyl-CoA and palmitoylcarnitine in rat heart mitochondria

Substrate oxidation in mitochondria was measured by previously published methods (Yang *et al.*, 1987; Abdel-aleem *et al.*, 1992). These studies were performed in a reaction mixture contained in a final volume of 1.0 ml, 50 mM Tris-HCl (pH 7.4); 120 mM KCl; 0.5 mM EDTA-K_2 L-carnitine and 0.5 mM (pH 7.4), 2 mM KP_i, and 0.1 mg/ml BSA, and 40 μM [1-^{14}C]palmitoyl-CoA, or 100 μM [1-^{14}C]palmitoylcarnitine were placed in a 25 ml Erlenmeyer flask. Substrate oxidation was initiated by the addition of rat heart mitochondria (0.5–1 mg) which were preincubated with or without adriamycin for 5 min at room temperature. The rate of oxidation of palmitate, palmitoyl-CoA or palmitoylcarnitine was determined by measuring the release of $^{14}CO_2$ as above for myocytes.

Protein concentration was determined by the Bio-Rad protein assay (Bio-Rad, Richmond, VA, USA).

Statistical analysis

Data are presented as the mean $\pm$ S.D. of four separate experiments. Statistical significance was determined using the unpaired *t*-test and analysis of variance. Neuman–Keuls test was used to determine statistical difference between groups ($P<0.05$ was considered significant).

Results

Acute effects of adriamycin

Figure 1 shows the effects of increasing concentrations of adriamycin on the oxidation of palmitate, octanoate, and glucose in isolated cardiac

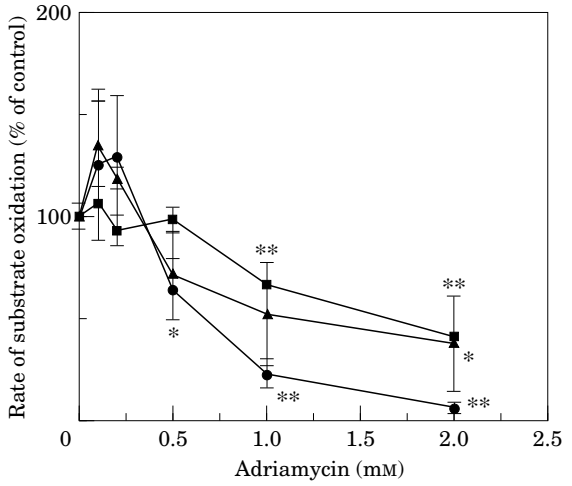

Figure 1 The effect of increasing concentrations of adriamycin on substrate utilization in myocytes. The control values for the oxidation of palmitate (●), octanoate (▲), and glucose (■) were 3.2±0.22, 13.94±0.26, and 3.16±0.2 nmol/mg protein/30 min, respectively. Values are presented as the mean±S.D. of four experiments. *,**Indicate a P value <0.05 and 0.01, respectively, of adriamycin *v* control.

myocytes. Adriamycin caused a concentration-dependent inhibition of all three substrates. However, the effect appeared greatest on palmitate oxidation. Palmitate was inhibited 40% whereas octanoate oxidation was decreased 30% by exposure to 0.5 mM adriamycin *in vitro*. Glucose oxidation was only decreased at higher concentrations of adriamycin (1 and 2 mM). At the highest concentration of adriamycin (2 mM) palmitate oxidation was essentially abolished, whereas glucose and octonoate oxidation were decreased to 50%.

The temporal effects of adriamycin on the oxidation of palmitate, octanoate, and butyrate in the presence of L-carnitine are shown in Figure 2. The oxidation of these fatty acids was significantly decreased by 0.5 mM adriamycin [Fig. 2(a), (b) and (c)] at times as early as 20 min. However, preincubation of myocytes with L-carnitine (5 mM) resulted in a complete reversal of the adriamycin-induced inhibition of fatty acid oxidation, except at 40 min where the inhibition of palmitate or octanoate oxidation persisted despite the presence of L-carnitine. Interestingly, in the absence of adriamycin, L-carnitine appeared to stimulate palmitate oxidation at 30 and 40 min but had no effect on octonoate or butyrate oxidation.

To investigate the effect of adriamycin on the carnitine palmitoyltransferase reaction, which is responsible for the transfer of long-chain fatty acids

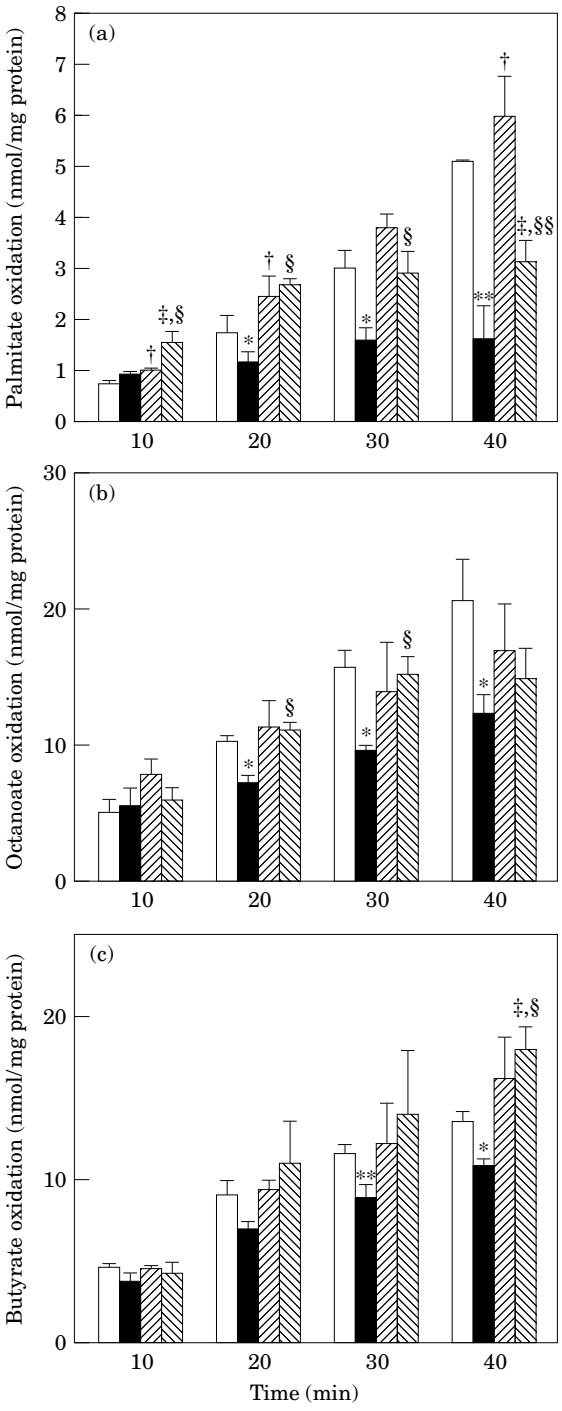

Figure 2 Comparison of the effect of acute adriamycin on substrate utilization in myocytes. The effect of adriamycin (0.5 mM) on the oxidation of (a) palmitate, (b) octanoate, and (c) butyrate is presented as function of incubation time. Values are presented as the mean±S.D. of four experiments. *, †, ‡, §, ¶ Indicate a P<0.05 of adriamycin (ADR) *v* control, Carn *v* control, Carn + ADR *v* control, ADR *v* Carn and ADR *v* ADR + Carn, respectively. Double and triple indicate a P value <0.01 and 0.001, respectively. (□), control; (■), ADR; (▨), Carn; (▧) ADR + Carn.

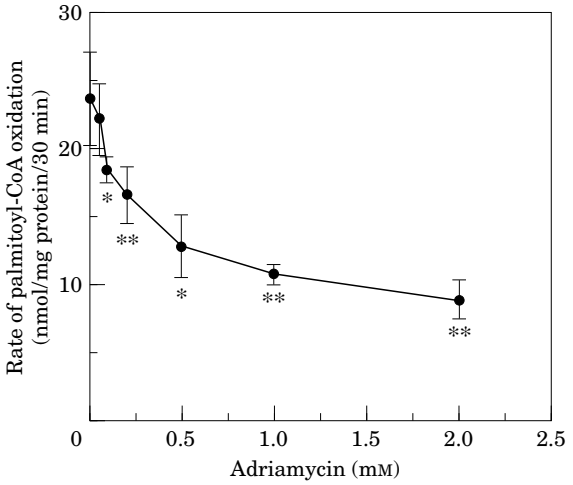

Figure 3 The effect of adriamycin on palmitoyl-CoA oxidation in isolated rat heart mitochondria. For more experimental detail see Material and Methods. Values are presented as the mean ± S.D. of three experiments. *, ** Indicate a P value <0.05 and 0.01, respectively, of adriamycin *v* control.

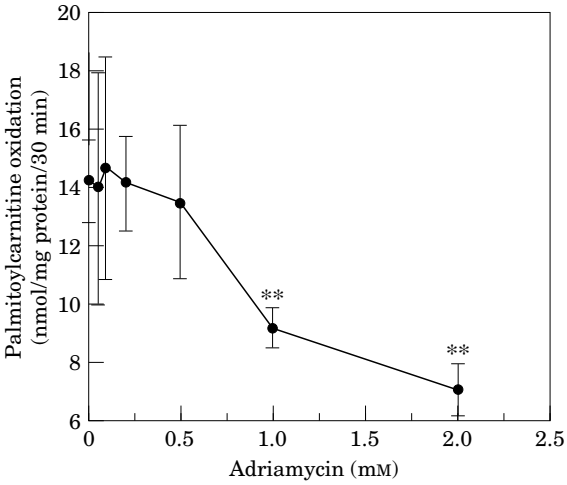

Figure 4 The effect of adriamycin on palmitoylcarnitine oxidation in isolated rat heart mitochondria. Values are presented as the mean ± S.D. of three experiments. *, ** Indicate a P value <0.05 and 0.01, respectively, of adriamycin *v* control.

into the mitochondria prior to β-oxidation, palmitoyl-CoA oxidation was determined in isolated rat heart mitochondria in the presence of adriamycin. As indicated in Figure 3 the oxidation of palmitoyl-CoA was decreased acutely by adriamycin in a concentration-dependent manner. Fifty percent inhibition of palmitoyl-CoA oxidation occurred at approximately 0.2 mM adriamycin.

To assess the effect of adriamycin on the β-oxidation pathway, its effect on palmitoylcarnitine oxidation was determined in isolated mitochondria. Acutely, adriamycin had no effect on palmitoylcarnitine oxidation except at higher concentrations, 1 and 2 mM, when palmitoylcarnitine oxidation was decreased by 35% and 50%, respectively (Fig. 4).

Chronic effects of adriamycin

These studies were performed with cardiac myocytes isolated from rats injected chronically with adriamycin, L-carnitine, or adriamycin plus L-carnitine. In each experiment, the oxidation of a single metabolic substrate was determined over 40 min in myocytes from each group ($n=4$). As shown in Figure 5, adriamycin significantly inhibited palmitate oxidation to 40% by 40 min. Chronic administration of L-carnitine in normal rats resulted in a significant stimulation of palmitate oxidation by 37% at 40 min. Interestingly, chronic administration of L-carnitine along with adriamycin

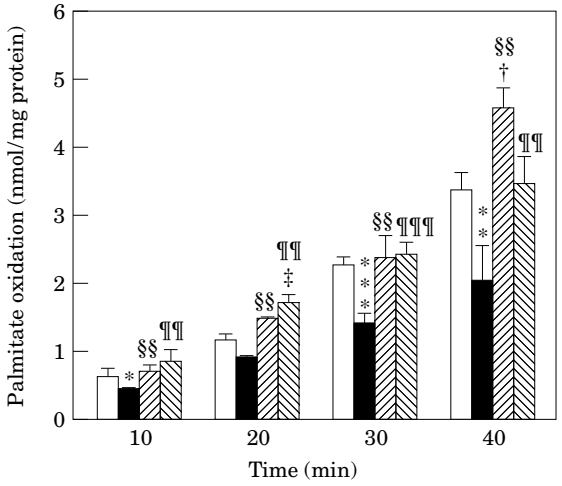

Figure 5 The effect of chronic administration of adriamycin, L-carnitine, or adriamycin plus L-carnitine on palmitate oxidation in cardiac myocytes. Values are presented as the mean ± S.D. of four separate experiments. *, †, ‡, §, ¶ Indicate a P<0.05 of adriamycin (ADR) *v* control, Carn *v* control, Carn + ADR *v* Carn and ADR *v* ADR + Carn, respectively. Double and triple symbols Indicate a P value which is less than 0.01 and 0.001, respectively. (□), control; (■), ADR; (▨), Carn; (▧), ADR + Carn.

resulted in complete reversal of adriamycin-induced inhibition of palmitate oxidation similar to the effects seen *in vitro*.

To determine whether administration of chronic adriamycin specifically affected long chain fatty acid oxidation or if other substrates such as pyruvate and octanoate oxidation were also affected, use of

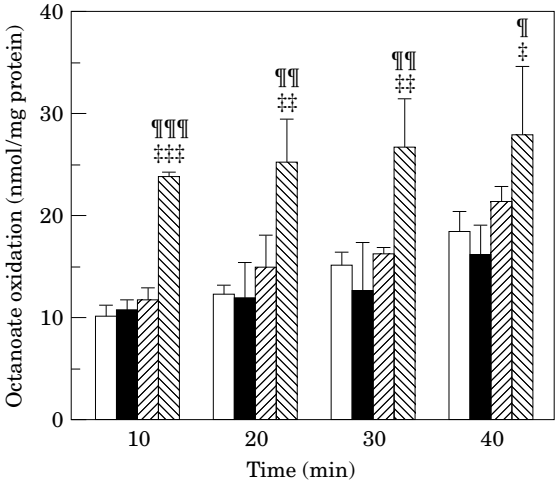

Figure 6 The effect of chronic administration of adriamycin, L-carnitine, or adriamycin plus L-carnitine on octanoate oxidation in myocytes. Values are presented as the mean ± S.D. of four separate experiments. *, †, ‡, §, ¶; Indicate a $P<0.05$ of adriamycin (ADR) *v* control, Carn *v* control, Carn + ADR *v* control, ADR *v* Carn and ADR *v* ADR + Carn, respectively. Double and triple symbols indicate a *P* value <0.01 and 0.001, respectively. (□), control; (■), ADR; (▨), Carn; (▧), ADR + Carn.

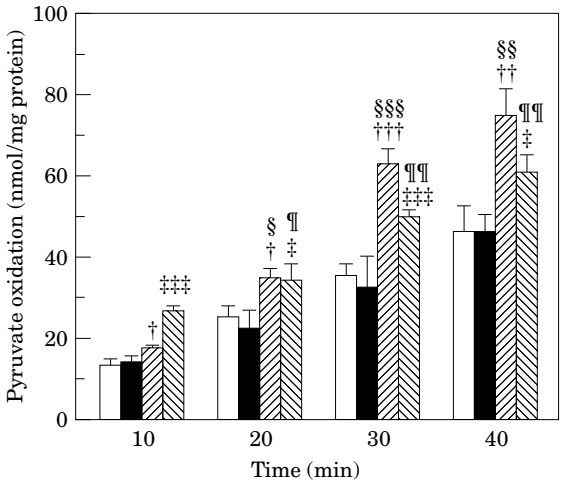

Figure 7 The effect of chronic administration of adriamycin, L-carnitine, and adriamycin + L-carnitine on pyruvate oxidation in myocytes. Values are presented as the mean ± S.D. of four separate experiments. *, †, ‡, §, ¶ Indicate a $P<0.05$ of adriamycin (ADR) *v* control, Carn *v* control, Carn + ADR *v* control, ADR *v* Carn and ADR *v* ADR + Carn, respectively. Double and triple symbols Indicate a *P* value <0.01 and 0.001, respectively. (□), control; (■) ADR; (▨), Carn; (▧), ADR + Carn.

each substrate was measured in isolated myocytes. Effects of chronic administration of adriamycin, L-carnitine, or adriamycin plus L-carnitine on octanoate oxidation are shown in Figure 6. Chronically administered adriamycin or L-carnitine had no effect on the oxidation of octanoate in isolated myocytes. Surprisingly, chronic administration of L-carnitine along with adriamycin caused a nearly two-fold increase in octanoate oxidation.

Effects of chronic administration of adriamycin, L-carnitine or adriamycin plus L-carnitine on pyruvate oxidation in myocytes are shown in Figure 7. Pyruvate oxidation was unaffected in myocytes isolated from adriamycin injected rats. However, pyruvate oxidation was significantly increased by 60% at 40 min in myocytes isolated from rats injected chronically by 60% at 40 min in myocytes isolated from rats injected chronically with L-carnitine. Also, chronic administration of L-carnitine along with adriamycin resulted in a significant 44% increase in pyruvate oxidation at 40 min.

Discussion

Acute effects of adriamycin

Data from these studies show that adriamycin inhibits fatty acid oxidation *in vitro* regardless of the chain length, at concentrations where glucose oxidation is unaffected. However, the inhibition of palmitate oxidation by adriamycin is greater than that of octanoate and butyrate suggesting that adriamycin acts additionally on long chain fatty acid oxidation presumably through its effect on CPT I. The inhibition of both fatty acids and glucose oxidation at higher concentrations of adriamycin (1–2 mM) may represent a non-specific toxicity of this drug, because at this concentration, adriamycin severely inhibited oxidation of all substrates tested in myocytes and mitochondria. The concentration of adriamycin which inhibited fatty acid oxidation in this study, 0.5 mM, may be considered a supra-pharmacological dose when compared to the adriamycin doses used clinically. However, this acute concentration (0.5 mM) may also acutely reflect the cumulative toxic effect seen *in vivo*. This is supported by the data demonstrating similar effects on substrate utilization in cardiac myocytes after either 0.5 mM acute or 18 mg/kg chronic adriamycin administration.

Long-chain fatty acid, or palmitate, oxidation encompasses a series of events that starts in the plasma membrane and ends in the mitochondrial matrix. These events include: the transport of palmitate across the plasma membrane, conversion of palmitate into palmitoyl-CoA by acyl-CoA synthetase in the cytosol, transport across the inner

mitochondrial membrane mediated by the carnitine palmitoyltransferase system (CPT I, CPT II and translocase), and finally oxidation of palmitoyl-CoA in the mitochondrial matrix through the β-oxidation cycle. Medium- and short-chain fatty acids, such as octanoate and butyrate, cross the inner mitochondrial membrane independent of CPT I activity and are also oxidized through the β-oxidation cycle.

The inhibition of palmitoyl-CoA oxidation and concomitant lack of inhibition of palmitoylcarnitine oxidation by adriamycin in isolated rat heart mitochondria suggests that CPT I, which promotes the mitochondrial transport of long-chain fatty acids, is inhibited by this compound. This observation is also consistent with previous studies demonstrating that CPT I and CPT II can be inhibited by adriamycin in isolated liver and heart mitochondria (Brady and Brady, 1987; Kashfi *et al.*, 1990). One possible mechanism by which adriamycin may inhibit CPT I is by depleting its cofactor, L-carnitine. This is supported by the data demonstrating that L-carnitine administration reverses the inhibition of palmitate oxidation by adriamycin in isolated cardiac myocytes.

The inhibition of octanoate and butyrate oxidation by adriamycin is surprising, because their transport across mitochondrial membranes is not dependent upon CPT I. Previous studies in this laboratory have shown that octanoate oxidation remains unchanged during complete blockage of CPT I (Abdel-aleem and Schulz, 1987). Therefore, inhibition of octanoate and butyrate oxidation suggests that adriamycin acts separately possibly to inhibit the fatty acid β-oxidation pathway. If this hypothesis was correct, then the oxidation of palmitoylcarnitine, another CPT I-independent β-oxidation substrate, should be decreased by adriamycin. However, oxidation of palmitoylcarnitine was unaffected by adriamycin (except at higher concentrations 1–2 mM) in isolated mitochondria suggesting that the β-oxidation pathway *per se* is not the target of adriamycin. This may in part be explained by further examining the role of L-carnitine. Reversal of adriamycin-induced inhibition of octanoate oxidation by L-carnitine suggests that this compound may play a role in enhancing fatty acid metabolism in addition to its role as a cofactor for CPT I. One possible mechanism for this is suggested by previous studies which demonstrated adriamycin interacts with cardiolipin, a specific structural phospholipid of the inner mitochondrial membrane in heart (Nicolay *et al.*, 1984; Battelli *et al.*, 1992) presumably to decrease mitochondrial integrity. L-Carnitine may reduce this adriamycin-cardiolipin interaction, preserve the integrity of inner mitochondrial membrane, and thereby enhance fatty acid oxidation. Future studies will be necessary to further evaluate this extra-CPT I effect of L-carnitine.

Chronic effects of adriamycin

Chronic administration of adriamycin resulted in inhibition of palmitate oxidation in isolated myocytes which increased with the duration of the acute study. These results are consistent with previous studies which demonstrated inhibition of palmitate oxidation both at short and long times after administration of adriamycin (Brady and Brady, 1987; Kashfi *et al.*, 1990; Beanlands *et al.*, 1994). The inhibition of palmitate oxidation after chronic adriamycin was reversed by L-carnitine suggesting that one mechanism by which adriamycin alters fatty acid oxidation is through depletion of L-carnitine in cardiac tissue. L-carnitine is an essential cofactor for the mitochondrial transport of long-chain fatty acids. Its reduction and subsequent inhibition of long-chain fatty acid oxidation could result in an accumulation of cardiotoxic fatty acid intermediates. This could explain our results as well as those of other laboratories where an apparent decrease in tissue carnitine was associated with cardiomyopathies caused by both adriamycin and secondary to other causes (Tripp *et al.*, 1981; York *et al.*, 1983; McFalls *et al.*, 1985; Whitmer, 1987). Further supporting this are the data where the use of L-carnitine has also previously been shown to preserve cardiac structure and improve cardiac function in adriamycin-induced cardiomyopathy (Maccari and Ramacci, 1981; Paterna *et al.*, 1984; Neri *et al.*, 1986; Battelli *et al.*, 1992; Carter *et al.*, 1992).

Stimulation of palmitate oxidation by chronic administration of L-carnitine may be secondary to increased mitochondrial transport of palmitate and/or increased mitochondrial CoA-SH/acetyl-CoA which would increase fatty acid β-oxidation by stimulating 3-ketoacyl-CoA thiolase. It is known that L-carnitine increases mitochondrial CoA-SH by decreasing mitochondrial acetyl-CoA in skeletal and cardiac muscle (Lysiak *et al.*, 1988; Uziel *et al.*, 1988). Reducing mitochondrial acetyl-CoA may also secondarily activate the β-oxidation of fatty acids by dis-inhibition of 3-ketoacyl-CoA thiolase which controls the final reaction in the β-oxidation pathway (Schulz, 1985).

The lack of inhibition of octanoate, whose oxidation is independent of CPT I activity, further

suggests an extra mitochondrial or extra CPT I inhibition of fatty acid oxidation by adriamycin. Also, the absence of an inhibitory effect on pyruvate oxidation, suggests that adriamycin does not affect the Krebs cycle, the final common pathway by which carbohydrates and fatty acids are ultimately oxidized. Why adriamycin acts differently on octanoate oxidation in the acute and chronic model remains unclear but suggests that the intact organ may compensate in ways unavailable to the isolated myocyte.

The stimulation of pyruvate oxidation in myocytes isolated from rats which were injected chronically with L-carnitine and L-carnitine plus adriamycin, could also be due to the effects of L-carnitine on reducing mitochondrial acetyl-CoA/CoA-SH ratio. This may lead to activation of the pyruvate dehydrogenase complex (PDH), a key enzyme in oxidative glucose metabolism, which may in turn stimulate pyruvate oxidation.

It should be noted that the cardiotoxic role of adriamycin cannot apparently be entirely explained by inhibition of long chain fatty acid oxidation through carnitine depletion in that previous investigators have demonstrated that L-carnitine can only partially improved cardiac function (McFalls, *et al.*, 1985), despite the complete reversal of the inhibition of fatty acid oxidation observed in this study.

Conclusion

These data demonstrate the inhibition of palmitate oxidation by acute and chronic administration of adriamycin with no effect on pyruvate utilization. L-Carnitine completely reversed adriamycin-induced inhibition of fatty acid oxidation regardless of the fatty acid chain length. These data suggest that adriamycin inhibits fatty acid oxidation in part by depleting L-carnitine and/or inhibiting CPT I. However, these findings also suggest that L-carnitine has an additional role in maintaining fatty acid oxidation in the heart regardless of chain length.

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