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Dual effects of dichloroacetate on cardiac ischaemic preconditioning in the rat isolated perfused heart

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1 Ischaemic cardiac preconditioning represents an important cardioprotective mechanism which limits myocardial ischaemic damage. The aim of this investigation was to assess the impact of dichloroacetate (DCA), a pyruvate dehydrogenase complex activator, on preconditioning.

2 Rat isolated hearts were perfused by use of the Langendorff technique, and were subjected to either preconditioning (3×4 or 3×6 min ischaemia) or continuous perfusion, followed by 30 min global ischaemia and 60 min reperfusion. DCA (3 mM) was either given throughout the protocol (pretreatment), during reperfusion only (post-treatment), or not at all. Throughout reperfusion mechanical performance was assessed as the rate-pressure product (RPP: left ventricular developed pressure × heart rate).

3 In non-preconditioned control hearts, mechanical performance was substantially ($P<0.001$) depressed on reperfusion (the RPP after 60 min of reperfusion ($RPP_{t=60}$) was $4,246\pm974$ mmHg beats min^{-1} compared to baseline value of $21,297\pm1,728$ mmHg beats min^{-1}). Preconditioning with either 3×4 min or 3×6 min cycles caused significant protection, as shown by enhanced recovery ($RPP_{t=60}=7,818\pm1,138$, $P<0.05$, and $11,123\pm587$ mmHg beats min^{-1}, $P<0.001$, respectively).

4 Addition of DCA (3 mM) to hearts under baseline conditions significantly ($P<0.001$) enhanced systolic function with an increased left ventricular developed pressure of 108 ± 5 mmHg compared to 88.3 ± 3.0 mmHg in the controls.

5 Pretreatment with 3 mM DCA had no effect on recovery of mechanical performance in the non-preconditioned hearts ($RPP_{t=60}=3,640\pm1,235$ mmHg beats min^{-1}) while the beneficial effects of preconditioning were reduced in the preconditioned hearts (3×4 min: $RPP_{t=60}=2,919\pm1,060$ mmHg beats min^{-1}; 3×6 min: $RPP_{t=60}=8,032\pm1,367$ mmHg beats min^{-1}). Therefore, DCA had increased the threshold for preconditioning.

6 By contrast, post-treatment of hearts with 3 mM DCA substantially improved recovery on reperfusion in all groups ($RPP_{t=60}=5,827\pm1,328$ (non-preconditioned), $14,022\pm3,743$ (3×4 min; $P<0.01$) and $23,219\pm1,374$ (3×6 min; $P<0.001$) mmHg beats min^{-1}).

7 The results of the present investigation clearly show that pretreatment with DCA enhances baseline cardiac mechanical performance but increases the threshold for cardiac preconditioning. However, post-treatment with DCA substantially augments the beneficial effects of preconditioning.

Keywords: Rat isolated perfused heart; cardiac ischaemic preconditioning; dichloroacetate (DCA); pyruvate dehydrogenase complex; myocardial ischaemia; reperfusion; cardiac intracellular metabolism

Introduction

Brief periods of transient cardiac ischaemia exert a protective effect on the myocardium, by limiting both ischaemic tissue damage and arrhythmogenesis, following prolonged ischaemia. This phenomenon has been termed cardiac preconditioning (Murry *et al*., 1986) and protects the heart in all species so far studied, including man (Yellon *et al*., 1993; Lawson & Downey, 1993; Ottani *et al*., 1995).

The mechanisms underlying cardiac preconditioning have been extensively investigated, and several regulatory systems have been suggested for different species. It has been shown that adenosine may cause protection via adenosine A_1-receptors, in a variety of species including the dog, pig and rabbit (see Lawson & Downey, 1993). There is further evidence that adenosine 5′-triphosphate (ATP)-sensitive potassium (K_{ATP}) channels, coupled to adenosine release via adenosine A_1-receptors, may also play a role (Parratt & Kane, 1994). Protein kinase C has been identified as an important second messenger in preconditioning mechanisms in several species, including the rat (Ytrehus *et al*., 1994; Mitchell *et al*., 1995).

Cardiac function is necessarily dependent on oxidative ATP regeneration since on reperfusion following ischaemia the recovery of cardiac function is negatively correlated with myocardial accumulation of glycolytic metabolites, such as lactate and protons and does not correlate with ATP levels (Neely & Grotyohann, 1984). Alterations in energy metabolism represent a possible mechanism of action of cardiac preconditioning and in this context there is a growing, albeit conflicting, literature describing alterations in energy metabolism which are associated with cardioprotection. On the one hand Janier *et al*. (1994) demonstrated in the rabbit heart, that ischaemic preconditioning stimulates anaerobic glycolytic ATP generation, which they argue decreases both contractile dysfunction and tissue necrosis by unidentified diverse means (Vanoverschelde *et al*., 1994). Conversely, others have shown in the isolated working rat heart, that preconditioning inhibits glycolysis and proton accumulation (Finegan *et al*., 1995). While in this preparation adenosine receptor activation has been shown to mimic preconditioning by preserving post-ischaemic cardiac performance and this was ascribed to inhibition of glycolysis and proton formation (Finegan *et al*., 1996). In the canine heart, Murray *et al*. (1990) found that

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preconditioning resulted in reduced glycolysis and ATP degradation and increased the efficiency for contraction. The authors went on to propose that either the preservation of ATP or reduced catabolite generation are responsible for delaying ischaemic cell death. In apparent support of a reduction in energy demand of contraction being a protective mechanism it has been shown in the rat heart that preconditioning leads to inhibition of mitochondrial F_1F_0ATPase, thus conserving high energy phosphates (Vuorinen *et al*., 1995).

Clearly regulation of energy metabolism has an important role in the regulation of cardiac function and may indeed be a target for preconditioning mechanisms. Of particular interest, the pyruvate dehydrogenase complex (PDC) is of major significance as it dictates the fate of pyruvate during contraction and thereby may be critical in controlling the relative contribution made by anaerobic and oxidative carbohydrate disposal. The activity of the PDC is tightly regulated by a phosphatase-kinase system (Linn *et al*., 1969). The kinase and the phosphatase are both, in turn, subject to regulation (Linn *et al*., 1969) and obviously any alteration in the steady-state of the kinase/phosphatase system would have profound effects on cardiac energy metabolism and subsequently on cardiac function. In this context, there is evidence that PDC may influence post-ischaemic cardiac function, as activation of PDC with dichloroacetate (DCA) (a PDC kinase inhibitor; Whitehouse & Randle, 1973) improves cardiac function on reperfusion after prolonged ischaemia in the rabbit isolated perfused heart (Lewandowski & White, 1995) and the rat working heart (McVeigh & Lopaschuk, 1990). Similarly, it has been demonstrated that pyruvate administration increases PDC activation status in ischaemic hearts (Patel & Olson, 1984), the threshold for preconditioning in ischaemic hearts (Sargent *et al*., 1994) and the rate of functional recovery during reperfusion (Liedtke & Nellis, 1978). Given this important role for PDC in cardiac energy metabolism, the present investigation has been carried out to examine the effects of DCA on both the threshold for cardiac preconditioning and the functional recovery of preconditioned hearts, by administering the agent both before and after preconditioning. The concentration of DCA chosen was comparable to that used by McVeigh & Lopaschuk (1990) in isolated working hearts of the rat.

Methods

Preparation of the isolated Langendorff heart perfused at constant flow

Male Wistar rats (357 ± 5 g; $n = 78$) were treated with heparin (1,000 u kg^{-1}, i.p.) and anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.: Sagatal, Rhône Mérieux, Harlow, Essex). In each case, following a mid-line thoracotomy, the heart was rapidly excised and placed in ice-cold, oxygenated, modified Krebs-Henseleit solution (containing (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10, Na-pyruvate 2) to arrest cardiac contraction. The aortic stump was then cannulated and the heart perfused retrogradely by the Langendorff technique at a constant flow of 20 ml min^{-1} with oxygenated ($Po_2 = 550 - 600$ mmHg) Krebs-Henseleit buffer (Randall *et al*., 1997). A water-filled latex balloon catheter, coupled to a pressure transducer, was inserted through the pulmonary vein and advanced into the left ventricle, in order to measure left ventricular developed pressure (LVDP). In each case, left ventricular end diastolic pressure (EDP) was initially set at approximately 5 mmHg by adjusting the volume of fluid in the balloon. The pressure transducer was coupled to a MacLab 4e recording system (ADInstruments, New South Wales, Australia), and heart rate (HR) was derived from the pressure signal. Coronary flow was measured by means of a transit time ultrasonic flow meter (model T106, Transonic Systems Incorporated, Ithaca, New York, U.S.A.) coupled to an extracorporeal flow probe, placed in series with the aortic cannula. Coronary perfusion pressure was measured by means of a second pressure transducer connected to the aortic cannula and coupled to the recording system.

Experimental protocol

Following a 30 min equilibration period, baseline cardiac variables were recorded and the hearts were then either subjected to a preconditioning cycle or were continuously perfused (i.e. non-preconditioned) (Randall *et al*., 1997). To induce preconditioning, perfusion was stopped for either 4 or 6 min, while the heart was bathed in buffer at 37°C and the preparation was then reperfused for 6 or 4 min, respectively, after which the cycle was repeated twice more. At the end of the reperfusion phase of the third cycle, the hearts were subjected to 30 min of homeothermic global ischaemia, followed by 60 min of reperfusion. As recovery of cardiac function is optimized at reduced reperfusion flow rates (Takeo *et al*., 1995) the flow on reflow was, in all cases, reduced to 75% of the initial pre-ischaemic flow (i.e. 15 ml min^{-1}); throughout this time the cardiac variables were continuously monitored. The non-preconditioned hearts were treated in a similar manner to the preconditioned hearts, except that, instead of the 3 cycles of preconditioning, the hearts were perfused continuously for 30 min immediately before the ischaemic result.

Dichloroacetate pre- and post-treatments

Some groups received DCA which was added to the perfusion fluid to achieve a concentration of 3 mM (comparable to 1 mM used by McVeigh & Lopaschuk, 1990). In some preparations DCA was added as a pretreatment at the start of perfusion and was therefore present throughout the entire protocol. When DCA was added as a post-treatment the agent was added to the perfusion fluid for the reperfusion period only. In some additional experiments DCA was added as a post-treatment at 10 mM.

Metabolite analysis

At the end of the experimental period, all hearts were snap-frozen with precooled tongs and immediately transferred to liquid nitrogen. Subsequently, tissues were freeze-dried and ATP, phosphocreatine (PCr), creatine and lactate concentrations were measured in powdered sections of the ventricular apex (Harris *et al*., 1974). Total creatine was calculated as the sum of PCr and creatine.

Quantitation and statistical analysis

All data are given as the mean ± s.e.mean. Baseline cardiovascular variables were compared by analysis of variance (ANOVA). Cardiac mechanical performance was quantified as the rate-pressure product (RPP, the mathematical product of LVDP and HR). Diastolic function was assessed as the EDP. The areas under the curves (AUCs) for the RPP- and

EDP-time plots were determined, by means of KaleidaGraph, in order to give a quantitative measure of the magnitude of the recoveries of cardiac performance during the 60 min reperfusion period (Randall *et al*., 1997). The derived AUCs were then compared by using ANOVA with significance differences being determined by Bonferroni's *post-hoc* test. Heart muscle metabolite concentrations are given as mmol kg^{-1} dry muscle (dm) and comparisons between treatments were made using ANOVA and Bonferroni's *post-hoc* test.

Drugs

Sodium dichloroacetate (Sigma Chemical Co., Poole, U.K.) was initially dissolved as a stock solution in saline before addition to the perfusion fluid.

Results

Baseline cardiac variables

In the hearts receiving DCA as a pretreatment there was a significant ($P<0.01$) increase in baseline mechanical performance as shown by the greater RPP ($28{,}820\pm1{,}649$ mmHg beats min^{-1}, $n=22$) compared with the controls (RPP = $24{,}192\pm825$ mmHg beats min^{-1}, $n=56$). This enhancement was entirely due to augmented systolic function as shown by the significantly ($P<0.001$) greater LVDP (108 ± 5 mmHg v 88.3 ± 3.0 mmHg) in the DCA-pretreated group, with no difference in heart rate (274 ± 4 beats min^{-1}, control; 268 ± 9 beats min^{-1}, DCA). DCA pretreatment did not significantly influence basal coronary perfusion pressure (87.8 ± 4.7 mmHg, control; 99.7 ± 8.9 mmHg, DCA).

Effects of DCA pre- and post-treatment in non-preconditioned hearts

In 9 control preparations, which were not preconditioned, the recovery of mechanical performance on reperfusion following the 30 min of ischaemia was limited (Figure 1a; Table 1), such that RPP after 60 min of reperfusion ($RPP_{t=60}$) was 20% of the baseline level (Table 1). The ischaemic period was also accompanied by an ischaemic contracture which was apparent as an increase ($P<0.01$) in EDP at the end of the ischaemic period (Table 1). While on subsequent reperfusion there was an impairment of diastolic function, as shown by the elevation of EDP above baseline throughout this perod (Table 1).

In the 6 hearts that were pretreated with DCA but were not preconditioned before ischaemia/reperfusion there was poor recovery of mechanical performance (Table 1) and RPP only recovered after 60 min reperfusion to 12% of baseline (Figure 1a), which was comparable to the recovery in non-preconditioned hearts not receiving DCA. The impairment of mechanical work production during the 60 min reperfusion period without preconditioning was reflected by the trend towards ATP ($P=0.06$) and PCr ($P=0.08$) concentrations being higher at the end of the reperfusion period in this group compared with both groups that were preconditioned (Figure 2).

In 9 hearts which were not preconditioned, but received DCA immediately at the start of reperfusion, the $RPP_{t=60}$ (Figure 1; Table 1) was comparable to the recovery in the non-preconditioned hearts with DCA pretreatment (Table 1). Although, the mechanical performance, at the onset of reperfusion (t = 1 min) and at 45 min of reperfusion, was enhanced compared to controls not receiving DCA (Figure 1a). In these hearts there was a substantial increase in EDP at the end of the ischaemic period (Table 1). Tissue lactate concentration was also elevated at the end of the reperfusion period (Figure 2).

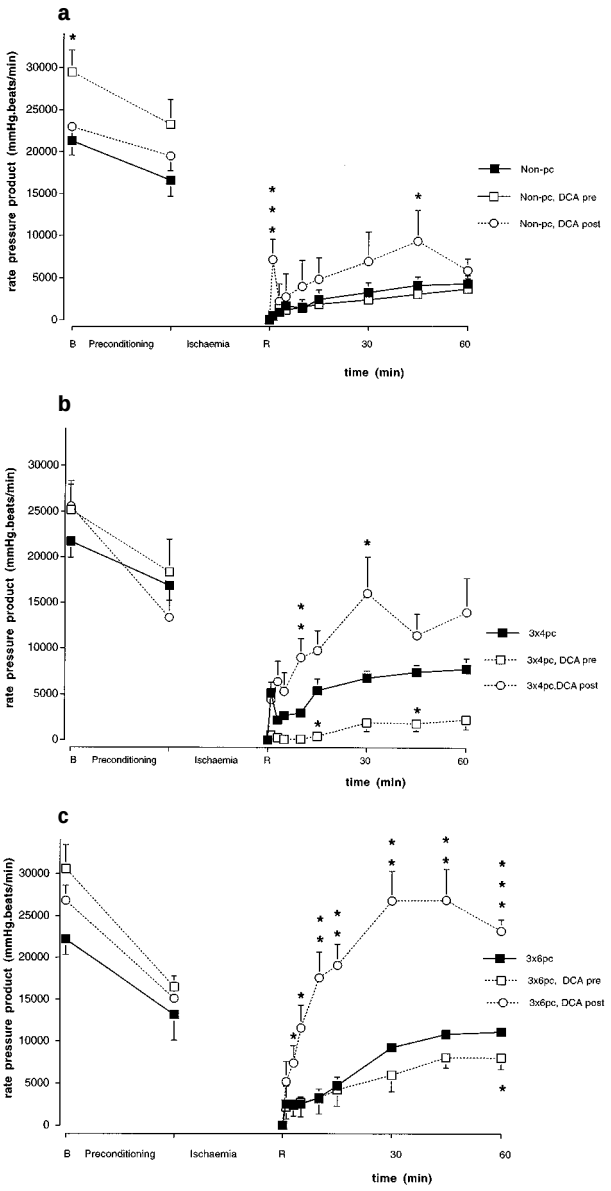

Figure 1 Recovery of mechanical performance, in terms of rate-pressure product (RPP), in control hearts, hearts pretreated and post-treated with DCA (3 mM). (a) The effects in non-preconditioned hearts, (b) the results in hearts subject to the 3×4 min preconditioning protocol and (c) the results in hearts subject to the 3×6 min preconditioning protocol. In each case, the baseline values (B) are given before and after the preconditioning (pc) cycle, before 30 min of global ischaemia. R indicates reperfusion after the ischaemic period and the time indicates the minutes after reflow. The values for the AUCs are given in Table 1. The data are given as mean± s.e.mean with the vertical lines indicating the errors. The statistically significant (ANOVA) differences between DCA-treated and non-treated hearts, with $*P<0.05$, $**P<0.01$ and $***P<0.001$.

Effects of DCA pre- and post-treatment on 3×4 min preconditioned hearts

In the 11 control preparations subjected to the 3×4 min preconditioning protocol, recovery of mechanical performance

Table 1 Effects of pre- and post-treatment with DCA on the recovery of cardiac mechanical performance on reperfusion in the absence and presence of preconditioning (pc)

		Non-pc			*3 × 4 min pc*			*3 × 6 min pc*	
	Control	*DCA pre*	*DCA post*	*Control*	*DCA pre*	*DCA post*	*Control*	*DCA pre*	*DCA post*
n	9	6	9	11	8	8	6	8	8
RPP_B (mmHg beats min^{-1})	21,297 ± 1,728	29,465 ± 2,614 *	22,981 ± 1,913	21,718 ± 1,787	25,177 ± 2,744	25,557 ± 1,297	22,191 ± 1,858	30,618 ± 2,810	26,682 ± 1,789
$RPP_{t=60}$ (mmHg beats min^{-1})	4,246 ± 974	3,640 ± 1,235	5,827 ± 1,326	7,818 ± 1,138 †	2,292 ± 1,060	14,022 ± 3,743 ††	11,123 ± 587 †††	8,032 ± 1,367 †	23,219 ± 1,374 ***†††
AUC for RPP_{0-60} ($\times 10^3$ mmHg beats)	175 ± 52	202 ± 79	396 ± 79	366 ± 37 ††	80 ± 36	773 ± 164 **	466 ± 16 †††	347 ± 90	1,321 ± 130 ***†††
ΔEDP (mmHg)	17.4 ± 4.0	33.7 ± 3.2 *	36.3 ± 4.5 **	14.9 ± 6.2	5.17 ± 4.04 †††	17.5 ± 1.4 †††	21.6 ± 0.5	7.1 ± 6.4 †††	9.78 ± 1.7 †††
AUC for EDP_{0-60} (mmHg min)	3,011 ± 359	3,886 ± 480	4,628 ± 274 ***	1,754 ± 375	5,031 ± 261 ***	2,429 ± 400 †††	2,634 ± 295	3,966 ± 465 *	1,040 ± 112 **†††

The data presented show the recovery of cardiac mechanical performance in control hearts and hearts receiving either a pretreatment (DCA pre) or post-treatment (DCA post) with 3 mM DCA. The RPP_B represents baseline rate-pressure product; $RPP_{t=60}$ = the rate-pressure product at the end of the 60 min of reperfusion; AUC for RPP_{0-60} = area under the curve for the rate-pressure product – time plot for the 60 min of reperfusion; the increase in ΔEDP = change in end diastolic pressure during 30 min of ischaemia; AUC for EDP_{0-60} = are under curve for the EDP – time plot for the 60 min of reperfusion. The data are mean ± s.e.mean. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ indicate significant differences between DCA-treated hearts and controls not receiving the drug, $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$ and $^{\dagger\dagger\dagger}P<0.001$ indicate significant differences between preconditioned hearts and the non-preconditioned hearts.

was significantly enhanced relative to that in the non-preconditioned hearts as shown by the greater AUC for $RPP_{0\text{-}60}$ ($P<0.01$) and the $RPP_{t=60}$ ($P<0.05$) (Figure 1b; Table 1). Therefore, at the end of the reperfusion period the RPP had returned to 36% of the baseline value.

In the 6 hearts which were pretreated with DCA before the 3 × 4 min preconditioning the recovery of mechanical performance was not significantly different from the non-preconditioned hearts (Figure 1a and b) but had lower AUC for $RPP_{0\text{-}60}$ and $RPP_{t=60}$ values (Table 1) and the hearts only recovered to 12% of baseline function. At t = 15 min and t = 45 min of reperfusion the RPP was less ($P<0.05$) than in the preconditioned hearts not receiving DCA. In these DCA pretreated hearts the ischaemic contracture (rise in EDP) was significantly ($P<0.001$) less than that in the non-preconditioned hearts receiving DCA (Table 1). Furthermore, lactate concentration at the end of the reperfusion period in the 3 × 4 min preconditioned hearts was 6 fold lower ($P<0.05$) compared with the non-preconditioned hearts receiving DCA (Figure 2).

Post-treatment with 3 mM DCA to hearts which had been subjected to the 3 × 4 min preconditioning regimen caused a substantial improvement in the recovery of mechanical performance as shown by AUCs for $RPP_{0\text{-}60}$ and $RPP_{t=60}$, which were significantly greater ($P<0.01$) than in the non-preconditioned hearts reperfused with DCA (Figure 1a and b; Table 1). The recovery of RPP in these hearts resulted in a $RPP_{t=60}$ which was 55% of baseline and at t = 10 min ($P<0.01$) and t = 30 min ($P<0.05$) of reperfusion the RPP was greater than in the preconditioned hearts not treated with DCA. The improvement in mechanical function in the hearts subjected to the 3 × 4 min preconditioning and DCA on reperfusion was accompanied by a greater than 2 fold reduction in tissue lactate accumulation when compared with the non-preconditioned hearts treated with DCA at the onset of reperfusion ($P<0.05$; Figure 2). Diastolic impairment during ischaemia in the hearts subjected to the 3 × 4 min preconditioning cycle in the absence or presence of DCA pretreatment was significantly less ($P<0.001$) than that in the non-preconditioned hearts receiving DCA only on reperfusion (Table 1).

Effects of DCA pre- and post-treatment on 3 × 6 min preconditioned hearts

In control hearts not receiving DCA the effects of preconditioning were further enhanced when the 3 × 6 min protocol was used, as shown by further increases in AUC for $RPP_{0\text{-}60}$ ($P<0.001$) and the $RPP_{t=60}$ ($P<0.001$) (Figure 1c; Table 1). Thus, in these hearts the RPP at the end of reperfusion was 50% of the baseline value. But the increase in EDP was not different from the non-preconditioned controls.

When DCA pretreated hearts ($n=8$) were subjected to the longer 3 × 6 min preconditioning cycle there was an enhancement in the recovery of mechanical performance relative to the non-preconditioned hearts receiving DCA, as shown by the greater AUC for $RPP_{0\text{-}60}$ and $RPP_{t=60}$ ($P<0.05$) (Figure 1c; Table 1). Thus in these hearts, RPP recovered to 26% of baseline performance, but this was not significantly different from the preconditioned hearts not receiving DCA. The ischaemic contracture was also significantly ($P<0.001$) less than that in the non-preconditioned hearts receiving DCA (Table 1). Lactate concentration in the preconditioned hearts at the end of the reperfusion period was 3 fold lower than in the non-preconditioned hearts (Figure 2b), but due to the high level of variability in the latter group this just failed to reach significance ($P=0.06$).

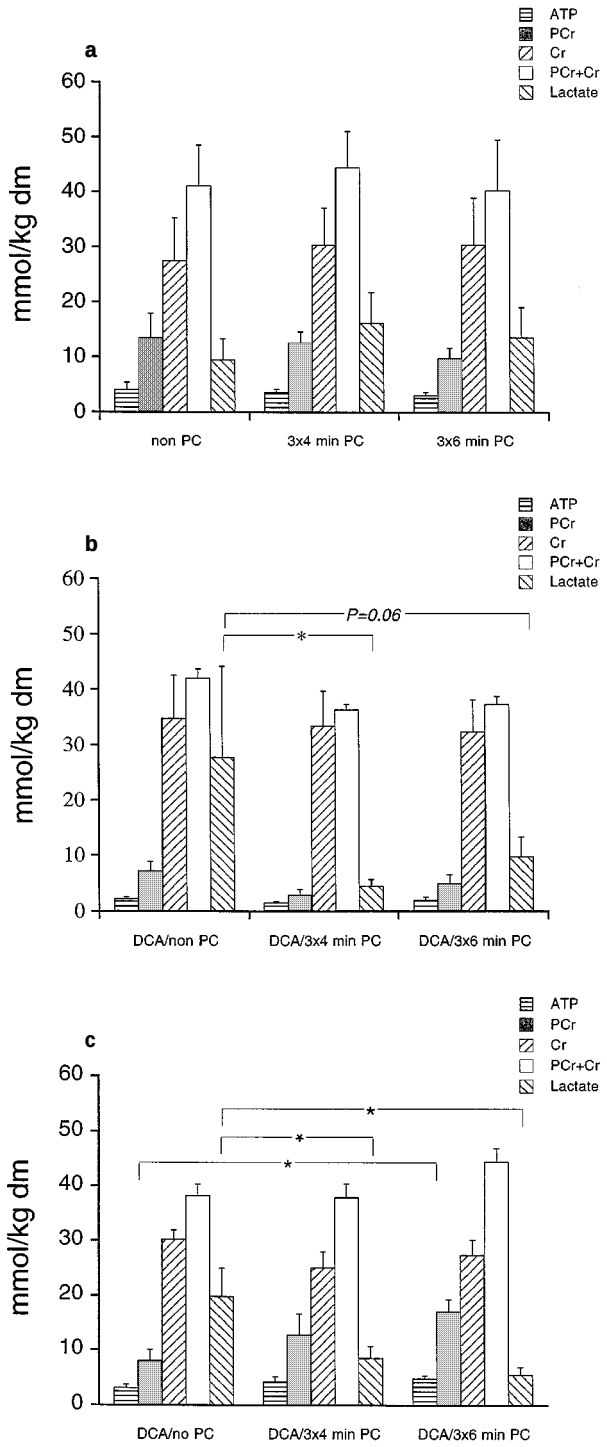

Figure 2 Muscle metabolite concentrations in snap frozen hearts subjected to 30 min of homeothermic global ischaemia and followed by 60 min of constant flow reperfusion. Before the global ischaemia and reperfusion the hearts were either preconditioned (PC; 3×4 min or 3×6 min) or not (non-PC). (a) Data from hearts not receiving DCA; (b) results from hearts pretreated with DCA; (c) results from hearts post-treated with DCA. Values represent mean $\pm$ s.e.mean. *$P < 0.05$ indicates significant differences between the groups.

In hearts which were subjected to the 3×6 min preconditioning protocol and then received DCA on reperfusion there was almost complete recovery of mechanical performance on reperfusion, such that the $RPP_{t=60}$ was 86.6% of the baseline value and AUC for $RPP_{0\text{-}60} = (1{,}321 \pm 130) \times 10^3$ mmHg beats (Figure 1c; Table 1). Thus $RPP_{t=60}$ and AUC for $RPP_{0\text{-}60}$ were both significantly ($P < 0.001$) greater than the 3×6 min preconditioned hearts not receiving DCA. This marked increase in contractile function was accompanied by a further reduction in tissue lactate accumulation ($P < 0.05$) and a preservation of muscle PCr stores ($P < 0.05$), when compared with the non-preconditioned hearts treated with DCA at the onset of reperfusion ($P < 0.05$; Figure 2c). The diastolic dysfunction during ischaemia was also significantly less than that of the non-preconditioned hearts post-treated with DCA ($P < 0.001$) and the 3×4 min preconditioned hearts which were also post-treated ($P < 0.01$; Table 1).

Effects of post-treatment with 10 mM DCA in preconditioned hearts

In a group of 5 hearts which were preconditioned with the 3×6 min protocol, post-treatment was carried out with a higher concentration of DCA (10 mM). In these hearts the recovery of both systolic and diastolic function was comparable with that seen in hearts post-treated with 3 mM DCA. In this respect the AUC for $RPP_{0\text{-}60}$ was $(1{,}291 \pm 109) \times 10^3$ mmHg beats, $RPP_{t=60} = 23{,}933 \pm 2{,}139$ mmHg beats min^{-1} (73% of baseline) and the AUC for $EDP_{0\text{-}60}$ was $1{,}415 \pm 268$ mmHg min and therefore not different from the hearts post-treated with 3 mM DCA. Similarly, the metabolites measured at the end of the reperfusion period with 10 mM DCA were indistinguishable from those measured in hearts post-treated with 3 mM DCA (i.e. ATP 5.2 ± 1.0, PCr 15.3 ± 2.8, Cr 28.0 ± 3.3, total Cr 43.3 ± 1.0 and lactate 7.0 ± 2.6 mmol kg^{-1} dm).

Discussion

The findings of the present investigation clearly identify a dual action of DCA on cardiac preconditioning. Pretreatment with DCA increases the threshold for cardiac preconditioning, while treatment on reperfusion has pronounced functional and metabolic beneficial effects which appear additive to the cardioprotection afforded by preconditioning.

The first important finding was that DCA caused an increase in baseline mechanical performance due to enhanced systolic function. This was not due to vascular effects as DCA had no effect on coronary resistance. It is possible that by enhancing the oxidative metabolism (through PDC activation) the agent has improved the metabolic status of the hearts, leading to enhanced contractile performance.

In the present investigation the efficacy of cardiac preconditioning has been confirmed and it has been further confirmed that preconditioning is not an 'all or nothing' phenomenon (Randall *et al.*, 1997), because the degree of protection appears to be related to the length of the preconditioning cycle.

When DCA was administered before preconditioning there were some important effects. Specifically, pretreatment with DCA abolished the cardioprotection provided by 3×4 min preconditioning, but when the longer cycle of 3×6 min preconditioning was used a degree of cardioprotection remained, but only to a level comparable with that in the 3×4 min preconditioned hearts not treated with DCA. Therefore, pretreatment with DCA increases the threshold for cardiac preconditioning by approximately 50%, such that a longer preconditioning cycle was required to produce the same effect as that in the absence of the drug. However, pretreatment with DCA did reduce tissue lactate accumulation and the development of ischaemic contracture in both the

3×4 min and 3×6 min groups, and to the same extent. This suggests, therefore, that the improvement in the RPP in the 3×6 min DCA treated hearts occurred independently of any effect of DCA on tissue lactate accumulation, which is in contrast the supposition of Janier *et al*. (1994) that lactate production during ischaemic preconditioning will decrease contractile dysfunction. However, it should be noted that the extent of improvement in the recovery of RPP in the 3×6 min DCA treated hearts was relatively modest. Furthermore, these data suggest that the reduction in lactate observed in the 3×4 min and 3×6 min preconditioned hearts treated with DCA may have been more a reflection of the reduction in ischaemic contracture that occurred in both groups during the ischaemic period, rather than any effect of DCA on lactate production *per se* (Table 1).

It is not possible to determine the mechanism responsible for the increase in the preconditioning threshold observed following DCA in the present experiment, especially as tissue samples were analysed at the end of the reperfusion period. However, it is unlikely that this functional effect of DCA was due to vascular effects of DCA as the drug did not influence coronary vascular resistance. Sargent *et al*. (1994) showed that pyruvate, the substrate for PDC, similarly increases the threshold for cardiac preconditioning in the rat heart by approximately 40%. Pyruvate and DCA have a similar chemical structure and both activate the PDC by inhibiting the kinase responsible for phosphorylation and therefore inactivation of the PDC (it has been shown that the concentration of DCA used in the present experiment fully activates PDC; Stacpole, 1989). It is possible, therefore, that pyruvate and DCA exert effects on the preconditioning threshold by maximizing flux through the PDC reaction and thereby minimizing the deleterious functional effects of lactate accumulation. However, as outlined above, DCA had no effect on function in the 3×4 min hearts in the present study despite a similar reduction in lactate accumulation as the 3×6 min hearts, which makes this explanation unlikely. Similarly, neither DCA (in the present study) nor pyruvate (Sargent *et al*., 1994) maintained tissue ATP content any better than in the non-preconditioned state, which also goes against this hypothesis.

Of relevance to the present investigation, McVeigh & Lopaschuk (1990) showed that when DCA was administered before the onset of ischaemia to hearts not subject to preconditioning, the recovery on reperfusion was compromised. They attributed this effect to enhanced glycolytic product accumulation during ischaemia, due to enhanced glucose utilization before the onset of ischaemia. Partly in agreement, DCA administration in the absence of preconditioning in the present study resulted in a marked accumulation of lactate, and it is likely that the magnitude of this increase was associated with the increase in ischaemic contracture in this group. However, in contrast with McVeigh & Lopaschuk (1990), heart function during recovery did not appear to be compromised any more than in the absence of both DCA and preconditioning. This finding also lends support to the supposition outlined above that lactate accumulation *per se* was not directly responsible for the changes in heart function observed following pretreatment with DCA in the present series of experiments. It would appear, therefore, that changes in energy metabolism as a consequence of DCA administration may not have been responsible for the increase in the threshold for preconditioning. An alternative explanation could be that, like pyruvate, DCA has the capacity to reduce the extent of free radical induced damage during ischaemic contraction (Deboer *et al*., 1993). The authors demonstrated that when 2 mM pyruvate was added to hearts during reperfusion following ischaemia, recovery of function was markedly increased in parallel with a reduction in free radical generation, measured by electron spin resonance.

When DCA was administered as a post-treatment at the end of ischaemia and immediately before reperfusion there were improvements in the recovery of mechanical performance, especially in the case of the 3×6 min hearts. In the case of this latter group there was nearly complete recovery of mechanical performance to baseline level and the hydrolysis of ATP and PCr and accumulation of lactate at the end of reperfusion were lower than in all other groups. Clearly, in contrast to the pre-treatment experiments, DCA on reperfusion either enhanced preconditioning or had independent effects which were additive with the protection afforded by preconditioning.

The PDC complex plays a pivotal role in dictating the fate of pyruvate to either lactate or acetyl-CoA. Others have shown in both the isolated perfused (McVeigh & Lopaschuk, 1990) and the working heart of the rat (Liu *et al*., 1996) that administration of DCA on reperfusion to ischaemic hearts promotes the recovery of mechanical performance. In both cases this enhanced recovery was attributed to increased oxidative glucose utilization and improved balance between anaerobic glycolysis and glucose oxidation. In the latter study this improved metabolic balance was associated with reduced proton production, perhaps consistent with DCA reducing acidosis. The ability to reduce glycolytic formation of protons may indeed be the key to the beneficial effects of DCA post-treatment, as adenosine agonists have been shown to inhibit glycolysis, and also proton formation, and this is associated with enhanced recovery of mechanical performance on post-ischaemic reperfusion in the rat heart (Finegan *et al*., 1996). It has been calculated from stable-isotope studies (Patel & Olson, 1984) and determined from biochemical analysis (Kobayashi & Neely, 1983) that PDC activation status is reduced by about 45% in the post-ischaemic heart. Furthermore, several groups have demonstrated that the addition of DCA and pyruvate on reperfusion can augment carbohydrate oxidation and heart recovery during reperfusion (McVeigh & Lopaschuk, 1990; Lewandowski & White, 1995). It is possible that DCA, by activating PDC on reperfusion in the present experiment, increased pyruvate oxidation to acetyl-CoA and by doing so reduced free ADP accumulation and thereby the reliance on anaerobic energy provision from PCr hydrolysis and glycolysis. Whether the increase in mechanical function during reperfusion under these conditions resulted from a decrease in lactate, proton accumulation and/or an increase in oxidative phosphorylation is unclear. Recent evidence favours the improvement in carbohydrate oxidation being more important than the reduction in anaerobic energy delivery (McVeigh & Lopaschuk, 1990; Lopaschuk *et al*., 1990).

The substantial improvement in mechanical function during reperfusion with DCA following preconditioning is a novel finding. Clearly, the changes in the PCr and lactate concentrations point to a metabolic mediated improvement in heart function but the exact mechanism underlying this response cannot be resolved from the present data. Recent evidence indicates that preconditioning, via protein kinase C activation, exerts its beneficial effects on function by activating intracellular proton pumps, thereby reducing the acidosis associated with ischaemia (Asimakis *et al*., 1992; Vuorinen *et al*., 1995; Gottileb *et al*., 1996). In support of this finding, the highest lactate concentrations in the present experiment were observed in hearts that were not subjected to preconditioning, particularly in the presence of DCA, and were lowest in preconditioned hearts which were also treated with DCA.

Therefore, one potential action of preconditioning may be to attenuate acidosis due to ischaemia and this may be additive to any beneficial effects of DCA on anaerobic glycolysis and/or oxidative energy production.

It is noteworthy that there was no relationship between ATP content and recovery. The values we obtained did not differ between preconditioned and non-preconditioned and are comparable with previous findings of Neely & Grotyohann (1984), which dissociated reperfusion ATP levels from recovery of mechanical performance. In this context, it has recently been demonstrated that preconditioning results in a reduction in mitochondrial ATPase activity, thereby preserving the high energy phosphate pool and possibly tissue viability (Vander Heide *et al*., 1996).

The results of the present study clearly indicate dual actions for DCA, with pretreatment opposing the effects of preconditioning and post-treatment either augmenting or having additive beneficial effects on the recovery of mechanical performance. These important results therefore suggest that PDC activators may have a therapeutic potential in ischaemic heart disease, but such agents would only confer benefit after a major ischaemic episode as pretreatment may interfere with preconditioning.

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