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Fatty acids are important for the Frank–Starling mechanism and Gregg effect but not for catecholamine response in isolated rat hearts

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ABSTRACT

In some pathophysiological conditions myocardial metabolism can switch from mainly long chain fatty acid (LCFA) oxidation to mainly glucose oxidation. Whether the predominant fatty acid or glucose oxidation affects cardiac performance has not been defined. In a buffer perfused isovolumetrically contracting rat heart, oxidation of endogenous pool LCFA was avoided by inhibiting carnitine-palmitoyl-transferase I (CPT-I) with oxfenicine (2 mM). In order to restore fatty acid oxidation, hexanoate (1 mM), which bypasses CPT-I inhibition, was added to the perfusate. Three groups of hearts were subjected to either an increase in left ventricular volume (VV, +25%) or an increase in coronary flow (CF, +50%), or inotropic stimulation with isoproterenol (10^{-8} and 10^{-6} M). The increase in VV (the Frank–Starling mechanism) increased rate–pressure product (RPP) by 21 ± 2% under control conditions, but only by 6 ± 2% during oxfenicine-induced CPT-I inhibition. The contractile response to changes in VV recovered after the addition of hexanoate. Similar results were obtained in hearts, in which an increase in CF was elicited (the Gregg phenomenon). Isoproterenol caused a similar increase in contractility regardless of the presence of oxfenicine or hexanoate. In all groups, a commensurate increase in oxygen consumption accompanied the increase in contractility. The fatty acid oxidation is necessary for an adequate contractile response of the isolated heart to increased pre-load or flow, whereas the inotropic response to adrenergic β-receptor stimulation is insensitive to changes in substrate availability.

Keywords adrenergic agonists, contractile function, fatty acids, Frank–Starling mechanism, glucose, Gregg phenomenon, mitochondria, myocardial metabolism.

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Long chain fatty acids (LCFA) are the major oxidation fuel of the healthy heart *in vivo* and *in vitro*, while carbohydrates, especially lactate and glucose, provide the remaining energy source (Neely *et al.* 1969, Morgan *et al.* 1984, Jeffrey *et al.* 1995). However, in pathological conditions, such as myocardial ischaemia, heart failure and hypertrophic cardiomyopathy, cardiac glucose uptake is higher and fatty acid oxidation is likely to be reduced (Olson 1959, Wikstrom *et al.* 1997, Recchia *et al.* 1998, Osorio *et al.* 2002). Myocardial metabolism of normal hearts can also rapidly adapt to changes in substrate availability. For example, the heart shifts to preferential utilization of carbohydrates when the arterial concentration of free fatty acids falls below 0.3 mmol L^{-1} (Nuutila *et al.* 1994).

Numerous studies have aimed to establish whether the oxidation of a given type of substrate vs. another energy source results in a more or less advantageous environment for cardiac energetics. The prevailing idea is that the preferential oxidation of glucose improves cardiac efficiency of the ischaemic heart (Simonsen & Kjekshus 1978, Burkhoff *et al.* 1991, 1995, Korvald *et al.* 2000) and therefore glucose oxidation is considered beneficial during myocardial ischaemia (Pepine & Wolff 1999, Taniguchi *et al.* 2001). In fact, compared with fat oxidation, carbohydrate utilization increases the ratio between adenosine triphosphate (ATP) synthesis and consumed oxygen (Starnes *et al.* 1985). To date, however, it is not clear whether changes in the type of substrate also have a direct effect on contractile

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strength of the non-ischaemic heart. Likewise it is also not clear whether the preferential utilization of glucose could be part of the mechanisms leading to cardiac decompensation (Sack *et al.* 1996, Recchia *et al.* 1998, Osorio *et al.* 2002) or, vice versa, could improve cardiac performance as recently proposed by Sabbah *et al.* (2000).

As *in vivo* the Frank–Starling mechanism is partly dependent on neuro-hormonal activation (Thorén 1979, Levick 2000), and as mechanisms can be precisely separated and controlled in the rat Langendorff heart preparations, the present study was designed to test the hypothesis that alternating the utilization of glucose or fatty acids may influence the cardiac contractile response to mechanical and adrenergic stimuli. Mechanical stimulation was obtained by incrementing either ventricular volume (VV, Frank–Starling mechanism) or coronary flow (CF, Gregg phenomenon) (Gregg 1963, Rastaldo *et al.* 2001), while adrenergic β-receptors were stimulated by isoproterenol. The stretching of myocardial fibres caused by increments in VV or CF, improves the myofilament overlapping and/or increases the sensitivity of the molecular contractile apparatus to calcium (Schouten *et al.* 1992, Burkhoff *et al.* 1995), whereas β-adrenergic agonists increase contractility via elevation of the cytosolic Ca^{2+} concentration during systole (Hiraoka *et al.* 1980, Levick 2000).

Higgins *et al.* (1981) described the inhibitory action of *oxfenicine* on LCFA oxidation. Later on, Bielefeld *et al.* (1985) obtained the first evidence that this compound blocks carnitine-palmitoyl-transferase I (CPT-I), a mandatory enzyme for LCFA β-oxidation. In our experiments, we blocked CPT-I, with oxfenicine, to avoid degradation of endogenous pool triglycerides and to *force* the hearts to utilize the supplied glucose (Molaparast-Saless *et al.* 1987, Van De Velde *et al.* 1996, Kennedy *et al.* 2000). In the presence of oxfenicine, the utilization of fatty acids was made possible by infusing *hexanoate*, a medium chain fatty acid that bypasses the CPT-I inhibition (Madden *et al.* 1995). Under these various conditions, we measured oxygen consumption and quantified the contractile response to increases in VV, CF and to isoproterenol infusion, in three separate groups of isolated hearts. For comparative purposes we also evaluated cardiac performance in hearts perfused with glucose-enriched Tyrode solution only.

MATERIALS AND METHODS

The study was performed in 26 isolated hearts removed from 5 to 6-month-old male Wistar rats (450–550 g of body weight). Each animal was pre-treated with heparin (2500 U, i.m.), anaesthetized by an intra-peritoneal injection of 2 mL of urethane (0.25 g mL^{-1}) 10 min later and then killed by decapitation. The heart was rapidly excised and the aorta retrogradely perfused with oxygenated buffer solution at 37 °C (i.e. as a Langendorff preparation). The flow was kept constant (9 ± 1 mL min^{-1} g ww^{-1}) using one of the three heads of a Watson-Marlow pump (Watson-Marlow 313, Falmouth, Cornwall, UK). Each of the three heads was calibrated to generate identical flow rates (see 'Experimental protocol'). During the experiments the flow was titrated to reach a coronary perfusion pressure (CPP) of 85–90 mmHg. Perfusion and flow oscillations were limited using the *314 four roller pump-heads* of the Watson-Marlow pump and by inserting a 50-mL Windkessel device into the perfusion line. Constant flow was used to minimize alterations in contractility caused by the Gregg phenomenon (Gregg 1963, Dijkman *et al.* 1996, Rastaldo *et al.* 2001). A small aperture in the left ventricular wall allowed the drainage of the thebesian flow. Left ventricular pressure (LVP) was recorded by a polyvinyl chloride balloon placed in the left ventricle via the mitral valve and connected via a three-way stop-cock to an electromanometer (Monitoring Kit mk 5-02 DTBNVF, Abbott, Milan, Italy). The balloon was filled with saline to achieve an end-diastolic LVP of 0–5 mmHg and connected through a three-way stop-cock to a graduated syringe filled with the same fluid. The piston of the syringe was connected to a micromanipulator in order to change VV according to the experimental protocol. The volume–pressure relationship of the balloon was determined before insertion into the ventricle. The values of balloon pressure at each volume were then subtracted from LVP recorded during the experiments at corresponding levels of VV.

The heart rate was measured from individual cardiac contractions and was then increased 15–20% above the spontaneous beating frequency using a stimulator (Sane'ei Instrument Ltd, Tokyo, Japan) to avoid any secondary effects caused by changes in heart rate. The isovolumetrically paced model was selected as this provides a load- and heart rate-independent assessment of ventricular function (Dijkman *et al.* 1996, Rastaldo *et al.* 2001).

A small cannula inserted into the right ventricle via the pulmonary artery was used to remove samples of the coronary effluent for gas analysis.

Coronary perfusion pressure and CF were monitored with an additional electromanometer and an electromagnetic flow-probe (Pencar 107/1000, Austec, Milan, Italy), respectively, both placed in the perfusion line.

Left ventricular pressure, CF and mean CPP were recorded using a TEAC R-71 recorder (Tokyo, Japan), digitized at 1000 Hz and analysed off-line with a CODAS software (DATAQ Instruments, Inc., Akron, OH, USA), which allowed the determination of the maximum rate of increase of systolic LVP (dP/dt_{max}). Left ventricular end-diastolic pressure (LVEDP), left

ventricular end-systolic pressure (LVESP), and heart rate were obtained from the LVP curve, and the difference between LVEDP and LVESP was also calculated [left ventricular developed pressure (LVDP)]. The product of heart rate and LVDP served as an index of myocardial work [rate–pressure product (RPP)]. Measurements of heart rate and LVDP were performed during steady-state conditions (see below). The oxygen content of in- and out-flowing buffer solutions was measured using a Ciba-Corning 280 gas-analyser (Ciba-Corning, Halstead, Essex, UK). The myocardial oxygen consumption (MVO_2) was calculated by multiplying CF by the difference in oxygen content between in- and out-flowing buffer solutions. Animal use was in accordance with the University of Torino ethical committee guidelines and conformed to the Italian law.

Experimental protocol

The heart was allowed to stabilize for 20–30 min before baseline values were recorded. During this period, the heart was perfused with glucose-enriched Tyrode buffer [total solute concentration 10^{-3} mol L^{-1} made up from 154 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 11 D-glucose, 5 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (HEPES), pH adjusted to 7.35 with NaOH] along with 10 μg mL^{-1} lidocaine (Hare *et al.* 1998, Paolocci *et al.* 2000, Rastaldo *et al.* 2001). The buffer was oxygenated with 100% O_2 at a pressure of 640–650 mmHg.

The experimental manoeuvres (see below) were performed while the hearts were perfused with alternating buffer solutions of different composition. Perfusion A: Tyrode buffer perfusion supplying only glucose as substrate; Perfusion B: Tyrode buffer perfusion with the addition of 4-hydroxy-L-phenylglicine (oxfenicine, 2×10^{-3} mol L^{-1}), previously defined as a specific CPT-I inhibitor at this concentration (Molaparast-Saless *et al.* 1987, Van De Velde *et al.* 1996, Kennedy *et al.* 2000). Perfusion C: Tyrode buffer perfusion with oxfenicine and in addition hexanoic acid (10^{-3} mol L^{-1}) in order to bypass the block of CPT-I by oxfenicine. Note that in the presence of oxfenicine, the heart cannot oxidize LCFA derived from endogenous triglycerides (Morgan *et al.* 1984, Saddik & Lopaschuk 1991) and therefore must utilize glucose as the only fuel. Note also that hexanoic acid, a medium chain fatty acid, can enter into the mitochondria and then be metabolized bypassing the CPT-I inhibition (Madden *et al.* 1995). Perfusion buffers B and C were infused in a random sequence by switching from one head to the other on the Watson-Marlow pump.

Three different protocols were followed in three separate groups of hearts (Fig. 1).

Group 1: Increases in VV during heart perfusion with Tyrode buffers A, B and C (n = 6) Commencing with a VV of about 200 μL, increases in VV were achieved in two steps by adding 25 μL of fluid into the intra-ventricular balloon (total of 50 μL fluid added). After the second step of increased VV, LVP and CF were recorded during a steady-state period of 5 min. When the 5-min steady-state period ended, VV was reduced to the initial

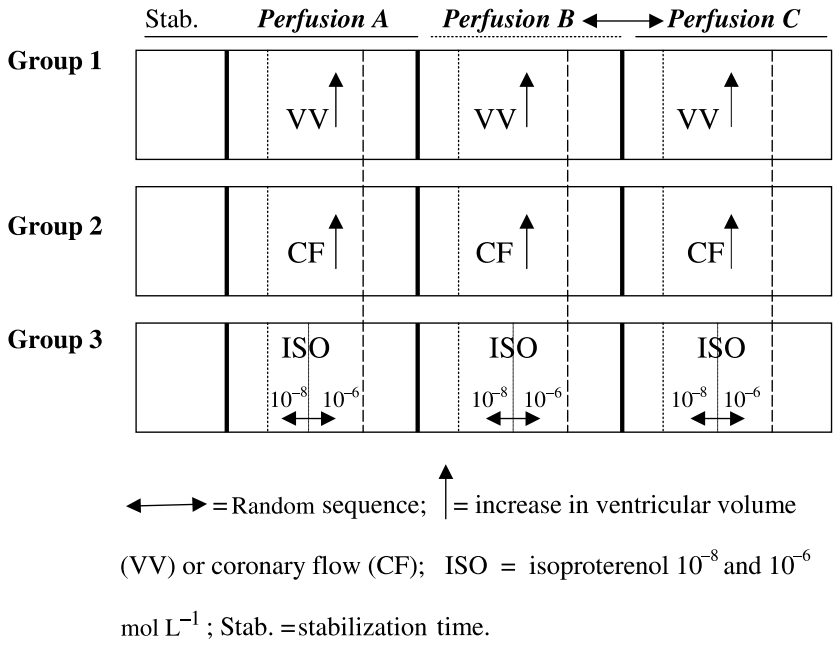

Figure 1 Experimental protocols. See also text for explanations.

control volume and after stabilization, recordings continued for a further 10-min period before the perfusion buffer was changed (Fig. 1).

A relatively low level of VV was chosen together with an upper limit of a 25% augmentation in VV in order to avoid over-stretching of the ventricle. This would have compromised the opportunities for comparing the effects of subsequent VV variations. In fact, an increase of VV in excess of 50% above its initial value (telediastolic LVP 0–5 mmHg) elicits an obvious reduction in contractility that persists for at least 30 min after returning VV to the baseline level (Pagliaro *et al.* 1998, Greyson *et al.* 2000).

Group 2: Increases in CF during heart perfusion with Tyrode buffers A, B and C (n = 10). Coronary flow was increased by 50% (from 9 ± 1 to 14 ± 1 mL min^{-1} g ww^{-1}) and after a steady-state period of 5 min the parameters were recorded. Coronary flow was then reduced to the initial control value and monitoring of variables continued for 10 min before the perfusion buffer was changed (Fig. 1).

Group 3: Isoproterenol response during heart perfusion with Tyrode buffers A, B and C (n = 10). Two doses of isoproterenol (10^{-8} and 10^{-6} mol L^{-1}) were studied whilst the hearts were perfused with one of the three buffers. The lower isoproterenol concentration was chosen on the basis of preliminary studies which showed that this concentration typically induces an increase in developed LVP similar to that observed during the increase in VV and CF (see 'Results'). In this group 90% of the CF was generated by the Watson–Marlow pump and 10% by one of two microperfusion pumps (Terumo, Tokyo, Japan) attached to 50 mL syringes. One syringe contained buffer only and the other isoproterenol freshly diluted in the buffer solutions at a concentration ten times higher that the final desired concentration. These microperfusion pumps were used alternatively to add flow to that of the Watson-Marlow pump thus generating the total CF. Drug administration was performed by switching from the buffer-only syringe to the syringe containing isoproterenol. This procedure guaranteed that application of the drug was not associated with variations in CF.

Under each of the three buffer perfusion conditions (A, B and C), isoproterenol was randomly infused for 5 min at low (10^{-8} mol L^{-1}) and at high (10^{-6} mol L^{-1}) final concentration. After the second period of isoproterenol infusion, the perfusion was changed to the buffer-only syringe to allow a washout. The steady state was usually reached after 3–4 min of isoproterenol application and parameters were recorded during the fifth minute of drug infusion (Fig. 1).

Data analysis

In all groups, measurements were carried out in steady-state conditions either during baseline or during the fifth minute following stimulation and all results were averaged over 30-s periods. Data are presented as mean ± SEM. Changes in each variable were compared between conditions by two-way analysis of variance (ANOVA) for repeated measures followed by Student's *t*-test, using a Bonferroni correction for multiple comparisons (SigmaStat 2.0). Differences were considered statistically significant at $P \leq 0.05$.

Drugs and chemicals

The drugs used in these experiments were all freshly dissolved at the required concentration in the perfusion buffers. Most of the compounds were obtained from Sigma Chemical (St Louis, MO, USA), heparin from Roche (Milan, Italy) and lidocaine from Astra Farmaceutici (Milan, Italy).

RESULTS

Baseline values of end-diastolic LVP, RPP, dP/dt_{max} and MVO_2 for each group at different perfusion conditions are given in Table 1. Oxfenicine (Perfusion B) and hexanoate (Perfusion C) per se did not significantly alter the values of any of these parameters.

Group 1 (increase in VV)

Per cent changes from baseline are shown in Figure 2. Under Tyrode perfusion A, the increase in VV (+25%, Frank–Starling mechanism) induced significant ($P < 0.001$) increases in RPP and in dP/dt_{max}. Oxfenicine (Perfusion B) significantly limited these increases. In particular, RPP augmentation caused by the increase in VV was reduced by about 60% ($P < 0.01$) with respect to the Tyrode perfusion control (Perfusion A). During the addition of hexanoate to the perfusate (Perfusion C), contractile responses to changes in VV recovered and were not different from those observed in the control conditions (Perfusion A), in spite of the inhibition of CPT-I. The variations in MVO_2 paralleled the changes in force of contraction. Oxygen consumption increased more under control Tyrode perfusion conditions and during hexanoate infusion than during oxfenicine.

A significant ($P < 0.01$) increase in end diastolic LVP induced by the 25% increase in VV was observed. The LVEDP increase was similar in all the conditions of perfusion (A: +9.5 ± 2 mmHg; B: +13 ± 3 mmHg; C: +11 ± 3 mmHg).

Table 1 Baseline parameters

	Group 1 ($n = 6$)			Group 2 ($n = 10$)			Group 3 ($n = 10$)		
	Control (perfusion A)	OXI (perfusion B)	HEXA (perfusion C)	Control (perfusion A)	OXI (perfusion B)	HEXA (perfusion C)	Control (perfusion A)	OXI (perfusion B)	HEXA (perfusion C)
Diastolic LVP (mmHg)	−1.5 ± 1.1	−1.9 ± 0.6	1.3 ± 0.3	2.3 ± 1.4	3.2 ± 1.6	4.2 ± 1.4	1.3 ± 1.6	3.0 ± 1.5	2.4 ± 1.7
RPP (mmHg min^{-1})	19 958 ± 1000	19 858 ± 983	20 068 ± 1004	20 904 ± 1335	19 956 ± 1098	19 853 ± 1003	19 857 ± 1199	19 856 ± 1115	19 959 ± 1045
dP/dt_{max} (mmHg s^{-1})	2270 ± 79	2256 ± 79	2244 ± 88	2274 ± 91	2279 ± 78	2288 ± 79	2151 ± 66	2112 ± 68	2143 ± 108
MVO_2 (mL min^{-1} g^{-1})	0.046 ± 0.006	0.048 ± 0.006	0.048 ± 0.006	0.047 ± 0.004	0.049 ± 0.004	0.049 ± 0.001	0.039 ± 0.006	0.036 ± 0.004	0.041 ± 0.005

Data are mean ± SEM. OXI, oxfenicine; HEXA, hexanoate; LVP, left ventricle pressure; RPP, rate–pressure product; dP/dt_{max}, maximum rate of increase of LVP in systole; MVO_2, myocardial oxygen consumption.

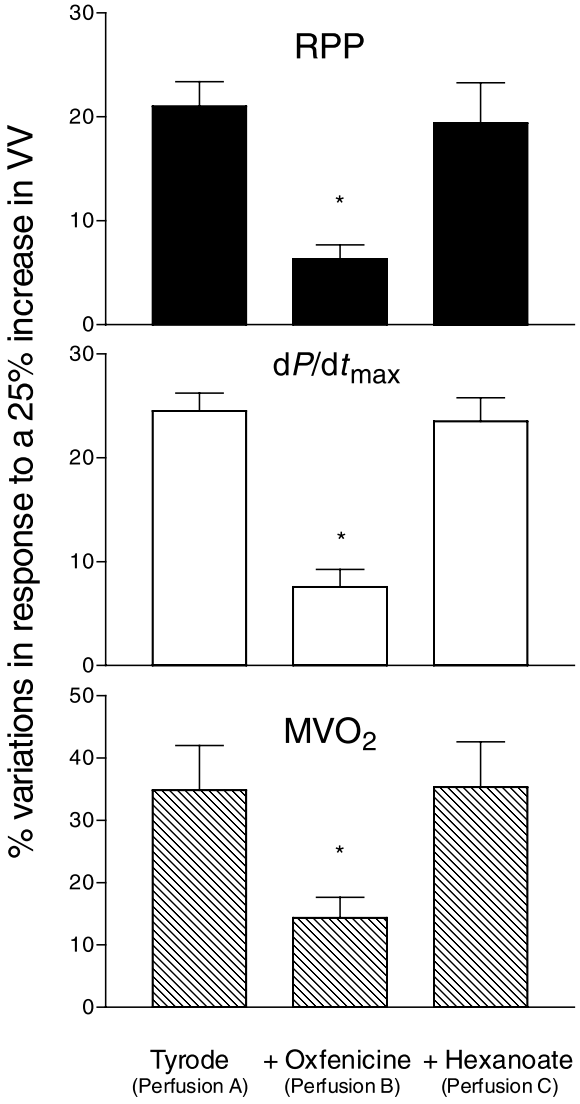

Figure 2 Per cent increase in rate–pressure product (RPP), maximum rate of increase of LVP in systole (dP/dt_{max}) and myocardial oxygen consumption (MVO_2) in response to an increase in ventricular volume (VV). The oxfenicine treatment resulted in blunting of RPP, dP/dt_{max} and MVO_2 responses. The response recovered after the addition of hexanoate. *$P < 0.02$: in comparison with Tyrode and hexanoate. Data are mean ± SEM; $n = 6$.

Group 2 (increase in CF)

Per cent changes from baseline are shown in Figure 3. Under control Tyrode perfusion conditions (Perfusion A), the increase in CF (+50%, Gregg phenomenon) induced significant ($P < 0.005$) increases in RPP and in dP/dt_{max}. Oxfenicine (Perfusion B) significantly limited these increases. In particular, RPP augmentation caused by CF increase was reduced by about 30% ($P < 0.05$) with respect to the control Tyrode perfusion (Perfusion A). During the addition of hexanoate to the perfusate (Perfusion C), contractile responses to

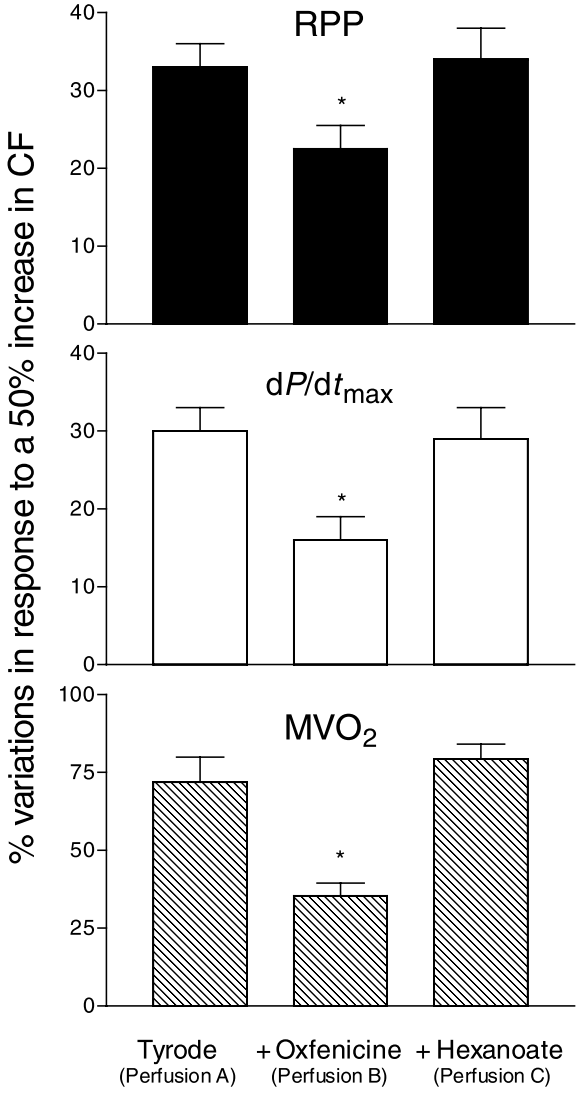

Figure 3 Per cent increase in rate–pressure product (RPP), maximum rate of increase of LVP in systole (dP/dt_{max}) and myocardial oxygen consumption (MVO_2) in response to an increase in coronary flow (CF). The oxfenicine treatment resulted in blunting of RPP, dP/dt_{max} and MVO_2 responses. The response recovered after the addition of hexanoate. $^*P < 0.05$: in comparison with Tyrode and hexanoate. Data are mean ± SEM; $n = 10$.

changes in CF recovered and were not different from those observed in the control conditions (Perfusion A). Similarly to group 1 the variations in MVO_2 paralleled the changes in force of contraction. Oxygen consumption increased more under control Tyrode perfusion and during hexanoate than during oxfenicine.

A significant ($P < 0.05$) increase in LVEDP induced by the 50% increase in CF was observed. The LVEDP increase was similar in all the conditions of perfusion (A: +3 ± 0.5 mmHg; B: +4 ± 1 mmHg; C: +5 ± 1 mmHg).

Group 3 (isoproterenol response)

Per cent changes from baseline are shown in Figure 4. Stimulation of β-receptors by isoproterenol (10^{-8} or 10^{-6} mol L^{-1}) resulted in a similar dose-dependent increase in contractility during the different conditions of perfusion (buffers A, B and C). The increases in RPP and dP/dt_{max} showed the same trend regardless of the different types of perfusing buffer. The increase in contractility was also accompanied by an increase in oxygen consumption but this was not significantly different between the three conditions of perfusion.

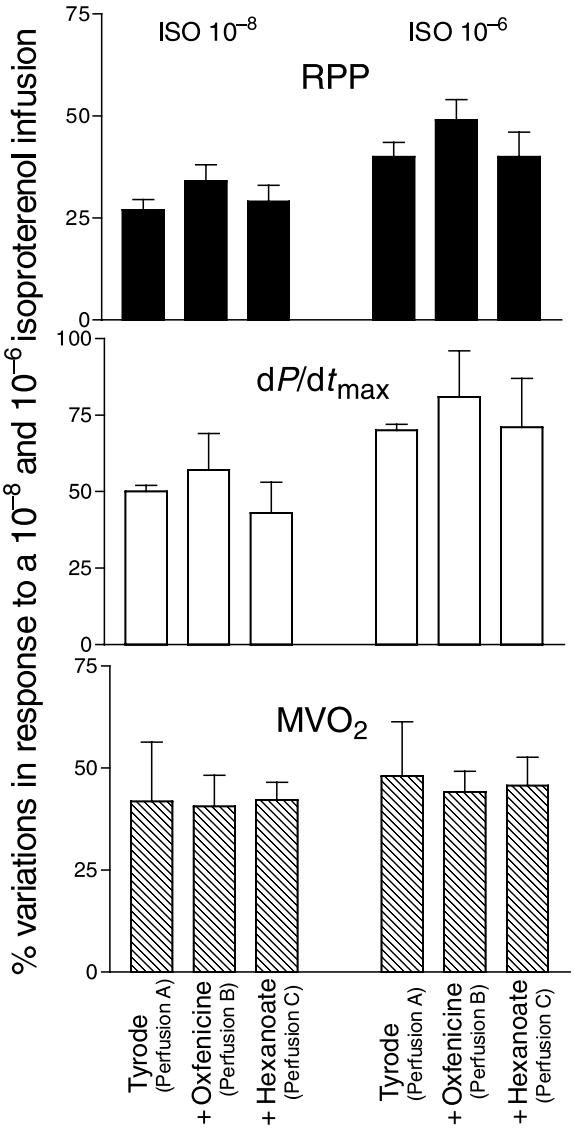

Figure 4 Per cent increase in rate–pressure product (RPP), maximum rate of increase of LVP in systole (dP/dt_{max}) and myocardial oxygen consumption (MVO_2) in response to isoproterenol (ISO, 10^{-8} and 10^{-6} mol L^{-1}). In all conditions of buffer perfusion (A, B and C) the per cent variation of the parameters was of similar magnitude, both at low and high ISO concentration. Data are mean ± SEM; $n = 10$.

A slight but non-significant reduction in LVEDP induced by isoproterenol (10^{-6} mol L^{-1}) was observed. The reduction was similar under all conditions of perfusion (A: -1 ± 0.5 mmHg; B: -1 ± 0.7 mmHg; C: -0.5 ± 0.7 mmHg).

It is noteworthy that under control conditions (Perfusion A) and during hexanoate (Perfusion C) the low concentration of isoproterenol (10^{-8} mol L^{-1}) induced an increase in rate–pressure product similar to that induced by the increase in VV (Group 1) and in flow (Group 2). Yet during forced glucose utilization by oxfenicine (Perfusion B), the VV- and flow-induced increase in RPP was attenuated, whereas the isoproterenol-induced increase in RPP was not. Hence, the extent of LVP response does not affect the influence of perfusion buffer on contractile response.

DISCUSSION

In the present study we demonstrate for the first time that by impeding fatty acid oxidation the Frank–Starling mechanism and the Gregg phenomenon cannot be adequately evoked. However, the positive inotropic effect of adrenergic β-receptor stimulation is insensitive to alterations in substrate metabolism.

The importance of fatty acid utilization, for an adequate contractile response of the isolated heart to mechanical stimuli (acute increase in VV or CF), is shown by the fact that increases in work load and dP/dt_{max} were markedly attenuated when LCFA oxidation was prevented by oxfenicine and fully recovered when fatty acid oxidation was restored by hexanoate. It is noteworthy that the contractile response to β-receptor stimulation was preserved during the blockade of LCFA oxidation. This observation suggests that the reduced response to pre-load and flow increase cannot be attributed to the cardiac substrate starvation.

It has previously been shown that CPT-I blockade does not change baseline cardiac performance both in conscious dogs (Van De Velde *et al.* 1996) and in isolated rat hearts (Kennedy *et al.* 2000) which was confirmed to be the case under conditions utilized in the present study. Moreover, in the present study the baseline cardiac performance was also unaffected by addition of hexanoate. It seems that only sustained stimuli applied to the ventricle, in combination with oxfenicine infusion, could unmask the effect of fatty acid utilization on contractile force. Although it is not clear why there is no difference in function under baseline conditions, these findings may explain why differences played by myocardial metabolic substrate in the complex regulation of both cardiac oxygen consumption and also contractile strength have not been evidenced by previous studies *in vitro* (e.g. Burkhoff *et al.* 1991, Goodwin *et al.* 1998). In such studies, isolated hearts were supplied with different types of substrate whilst kept at constant VV or were challenged by mechanical or pharmacological stimuli without blocking LCFA oxidation. Moreover, *in vivo* this complex regulation is further complicated by the fact that VV variations may be sensed by intra-chamber receptors which, by nervous reflex, may influence the cardiac contractile response (Thorén 1979). As we found that the positive inotropic action of isoproterenol was not affected by oxfenicine, it may be speculated that LCFA oxidation becomes important, *in vivo* only when ventricular performance relies heavily on the Frank–Starling mechanism, and less on adrenergic activation. This occurs typically in chronic heart failure (Weil *et al.* 1998, Levick 2000).

It is well known that an increase in CF in isolated hearts induces an increase in force of contraction and oxygen consumption (Gregg phenomenon) (Gregg 1963). However, this phenomenon has not yet been ascribed a full and complete explanation (Dijkman *et al.* 1996, Rastaldo *et al.* 2001). A 'garden hose'-like effect that causes sarcomere stretch has been proposed to explain the positive inotropic effect following an increased CF. Here, we report, for the first time, that the increase in heart performance brought about by increases in CF or VV is similarly affected by the inhibition of fatty acid oxidation.

The different sensitivity of LVP to inhibited LCFA oxidation during mechanical vs. adrenergic stimulation may allow explanations on the basis of existing reports of cardiac metabolism. In the absence of exogenous fatty acids in the perfusate, the isolated working heart derives more than 50% of the energy used from the oxidation of endogenous triglyceride reserves of LCFA (Morgan *et al.* 1984, Saddik & Lopaschuk 1991). In addition to LCFA, isolated hearts utilize endogenous glucose from the glycogen pool and exogenous glucose contained in the perfusion buffer (Goodwin *et al.* 1998). An increase in left ventricular workload stimulates fatty acid oxidation (Neely *et al.* 1969) as well as endogenous and exogenous glucose utilization (Neely *et al.* 1969, Goodwin *et al.* 1998). However, in the present study we demonstrate that only the portion of energy obtained from oxidation of LCFA supports an adequate enhancement of left ventricle force of contraction following increases in VV or in CF. It is possible that under these conditions, the rate of endogenous and exogenous glucose utilization is not sufficient to meet the augmented energy requirements. This hypothesis is also consonant with the well-documented notion that at unlimited oxygen supply LCFA oxidation is both preferred and more efficient than glucose in normal (Neely *et al.* 1969, Morgan *et al.* 1984) as well as in post-ischaemic heart (Van De Velde *et al.* 1996).

The explanation of isoproterenol stimulation is different from the above. It is possible that β-receptor stimulation per se favours glucose oxidation. Catecholamines activate the pyruvate dehydrogenase complex (PDC) (Hiraoka *et al.* 1980, Pepe *et al.* 1999) by promoting calcium entry into the cell and then into the mitochondria (Shah *et al.* 1994, Pepe *et al.* 1999). The increased cytosolic calcium is responsible for the augmented force of contraction, while mitochondrial calcium activates pyruvate phosphatase, which mediates PDC activation by dephosphorylation (Shah *et al.* 1994, Pepe *et al.* 1999). It is likely that the indirect stimulation of the carbohydrate oxidative pathway caused by oxfenicine (Higgins *et al.* 1981) was not as efficacious as PDC activation by isoproterenol in supporting ventricular function during increased workloads. It has been reported that catecholamines can also increase fatty acid oxidation by decreasing the levels of malonyl-CoA, which is a potent inhibitor of CPT-I (Hamilton & Saggerson 2000). Moreover, β-adrenergic stimulation may also activate triglyceride lipase (Manhta & Deshaies 1998). This activation could lead to enhanced fatty acid availability. In our study, the inotropic effect of β-receptor stimulation was not significantly different from the control conditions when the heart was forced to utilize glucose only. This suggests that increased oxidation of glucose is sufficient to support the augmented workload and that an additional increase in fatty acid oxidation is not essential during β-receptor stimulation. Besides, it must be kept in mind that high concentrations of catecholamines (e.g. noradrenaline 10^{-6} mmol L^{-1}) may lead to a reduction of myocardial ATP content and to an increase in CK leakage (Waldenstrom *et al.* 1978, Tipnis *et al.* 2000). Thus it cannot be excluded that sympathetic stimulation longer than that used in our model may be deleterious, regardless of substrate oxidized by the heart.

It is well known that the fraction of potential cross-bridges that are activated by a given concentration of calcium increases with stretch (length-dependent activation). This is also caused by an increase in the sensitivity of the contractile proteins to calcium with stretch. The unaltered diastolic compliance and the unaltered baseline function during inhibition of fatty acid oxidation are against the hypothesis that the reduced response to stretch may be due to an alteration of cross-bridge formation and/or of length-dependence of calcium sensitivity.

Critique of the model and methodological considerations

By reducing the number of independent variables to a reasonable minimum, the isolated crystalloid-perfused heart model appears suitable for studying the effects of mechanical and adrenergic stimuli on cardiac performance. Temperature is strictly controlled (±0.5 °C) and a constant balloon volume eliminates differences in loading conditions. Any interference by neuro-hormonal factors is limited, and we cannot exclude that part of the cardiac responses observed in this study were dependent on the particular model of denervated heart used.

The non-use of perfusate containing LCFA, lactate and/or pyruvate as additional substrates may be a limitation of the protocol. However, historically, many studies on contractile function in isolated hearts have been performed by supplying glucose as the sole exogenous substrate. Moreover, during acute transition from low to high workload, isolated hearts can still derive part of their energy requirements from endogenous reserves of LCFA (Neely *et al.* 1969, Morgan *et al.* 1984, Saddik & Lopaschuk 1991). For these reasons and to keep the number of variables to a reasonable minimum we did not add other substrates and/or insulin to the perfusate.

Finally, our study is limited by the absence of measurements of cardiac metabolites and high energy phosphates. The classical method to determine the rate of substrate utilization is based on isotopic tracer infusion. Although we did not use isotopic tracking of metabolic fate of substrates, cardiac metabolism of endogenous and exogenous carbohydrates and fatty acids in isolated hearts, as well as oxfenicine effects on CPT-I, have been widely investigated and are already well defined (e.g. Molaparast-Saless *et al.* 1987, Saddik & Lopaschuk 1991, Van De Velde *et al.* 1996, Goodwin *et al.* 1998, Kennedy *et al.* 2000). Moreover, in the present study, in the absence of any exogenous LCFA supply, the effects of oxfenicine were necessarily caused by inhibition of endogenous LCFA oxidation and, in fact, they were reversed by adding hexanoate to the perfusate. As isotopic tracers cannot allow measurements of the rate of oxidation of endogenous substrate, they would have not been useful in our study unless we had adopted challenging protocols of endogenous pool priming with labelled substrate. This was beyond our aims.

In conclusion, in the isolated rat heart, the Frank–Starling mechanism and Gregg effect are sensitive to the type of metabolic substrate used. Fatty acid oxidation seems to be necessary for an adequate contractile response of the isolated heart to myocardial stretch by increasing VV or CF, but not to β-receptor stimulation.

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