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LACTATE UTILIZATION BY AN *IN SITU* PERFUSED TROUT HEART: EFFECTS OF WORKLOAD AND BLOCKERS OF LACTATE TRANSPORT

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Summary

Lactate utilization was studied in an *in situ* perfused trout heart preparation that was capable of performing at levels similar to *in vivo* maximum cardiac performance. Hearts were perfused with modified Cortland saline containing $0.5\,\mathrm{mmol\,l^{-1}}$ iodoacetic acid (to block endogenous glycolysis) and varying amounts of lactate (1 or $10\,\mathrm{mmol\,l^{-1}}$). We confirmed previous observations that lactate utilization is limited by substrate availability. However, contrary to previous observations, exogenous fuel availability did not limit cardiac performance, even at the high workload. Furthermore, when plentiful (i.e. $10\,\mathrm{mmol\,l^{-1}}$), exogenous lactate was preferred over endogenous fuel and was able to supply the heart's energy requirements at both the low and high workloads. Pyruvate at 10-fold greater concentration, had no apparent effect on lactate utilization at the high workload. α-Cyano-4-hydroxycinnamate (α-CIN) ($2.5\,\mathrm{mmol\,l^{-1}}$) proved to be an unsuitable probe of lactate transport in the trout heart as it caused a reduction in both lactate utilization and cardiac performance. However, addition of $20\,\mu\mathrm{mol\,l^{-1}}$ isobutyl carbonyl lactyl anhydride or $100\,\mu\mathrm{mol\,l^{-1}}$ 4-acetamido-4′-isothiocyano-stilbene-2,2′-disulphonic acid (putative lactate transport blockers) to the perfusate virtually abolished lactate oxidation at the high workload without affecting cardiac performance. These observations suggest that lactate uptake by the *in situ* perfused trout heart is carrier-mediated.

Introduction

It is widely accepted that cardiac muscle is capable of oxidizing a variety of substrates for energy production, including glucose, lactate and fatty acids. Under aerobic conditions, the brook trout (*Salvelinus fontinalis*) heart will preferentially oxidize lactate as a metabolic fuel (Lanctin *et al.* 1980). During recovery from exhaustive exercise, blood lactate levels are elevated ($20\,\mathrm{mmol\,l^{-1}}$ is not uncommon; Milligan and Wood, 1986) at a time when cardiac demand is high. While

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trout hearts are rich in glycogen (10–20 μmol g^{-1} wet mass; C. L. Milligan, unpublished results), levels are not affected by exhaustive exercise (C. L. Milligan, unpublished results). Rather, the heart appears to take up lactate from the blood (Milligan and Wood, 1986). These observations suggest that blood lactate may be a significant fuel source to the heart during periods of increased demand. Thus, one goal of this study was to assess the role of exogenous lactate as a fuel source to the working heart.

Lactate availability appears to be the limiting factor in lactate oxidation, at least in isolated perfused hearts from sea raven (*Hemitripterus americanus*) and ocean pout (*Macrozoarces americanus*; Driedzic and Hart, 1984). However, in the isolated perfused brook trout heart, the relationship between lactate availability and oxidation appears to be complex; lactate utilization was the same at 2 and 10 mmol l^{-1} lactate, but markedly increased at 20 mmol l^{-1} lactate (Lanctin *et al.* 1980). Thus, an additional goal of the present study was to reassess the relationship between lactate utilization and substrate availability in a working trout heart.

In vivo, the accumulation of lactate in the heart during recovery from exhaustive exercise is associated with an alkalosis, suggesting that lactate is not entering the heart as the free acid but rather may be carrier-mediated (Milligan and Wood, 1986). Nothing is known about the mechanism of lactate transport by teleost cardiac muscle. However, in isolated cardiac myocytes from the guinea pig, lactate uptake is carrier-mediated (Poole *et al.* 1989). Having met the first two goals and established: (1) that lactate utilization is limited by substrate availability, and (2) that cardiac performance is not limited by exogenous lactate, we felt that with the *in situ* perfused trout heart we would be able to gain insights into the potential mechanism of lactate transport. We reasoned that blockade of lactate uptake would result in a reduction in lactate oxidation, since substrate is limited. Also, since cardiac performance was not limited by lactate availability, we were able to differentiate between a general effect on the heart (as revealed by a decrease in cardiac performance) and a specific effect on lactate uptake (as revealed by a decrease in lactate utilization with no change in cardiac performance). The *in situ* perfused heart preparation is a good model system to examine these questions as it mimics *in vivo* cardiac performance in both resting and swimming fish quite well (Farrell *et al.* 1986), especially if the pericardium is left intact (Farrell *et al.* 1988*a*).

Materials and methods

Experimental animals

Adult rainbow trout *Oncorhynchus mykiss* (formerly *Salmo gairdneri* Richardson) of both sexes (400–600 g) were obtained from West Creek Trout Farm, Aldergrove, BC. The fish were held indoors in 2000 l circular fibreglass tanks continuously supplied with aerated, dechlorinated tap water at 10 °C. During holding, fish were exposed to a photoperiod simulating 49 °N and fed commercial trout pellets *ad libitum* three times weekly.

In situ *perfused heart preparation*

The preparation of the *in situ* perfused heart is essentially as described by Farrell *et al.* (1986) and modified by Farrell *et al.* (1988*a*). In brief, fish were anaesthetized in a buffered solution of 0.02 % MS 222 (Sigma) and placed on an operating sling where the gills were irrigated with cooled, buffered and aerated anaesthetic solution (0.01 % MS 222). The fish were then injected with 60 i.u. heparin in 0.4 ml of modified Cortland saline (MCS, see below; Farrell *et al.* 1986) *via* the caudal vessels. Perfusate (see below) was delivered from a temporary reservoir to the sinus venosus *via* a stainless-steel input cannula placed in the hepatic vein. The ventricle pumped perfusate against the output pressure head *via* a stainless-steel cannula placed in the ventral aorta. The pericardium was left intact.

The fish were transferred to and fully immersed in a Cortland saline bath in the experimental apparatus (Fig. 1) which was maintained at 10°C. The input cannula was switched from the temporary reservoir to a constant pressure head receiving flow from a reservoir containing control perfusate (500 ml) maintained at 10°C and equilibrated with 0.5 % CO_2/99.5 % O_2. An in-line flow probe measured cardiac output. The heights of the input and output pressure heads were varied to change filling pressure and diastolic afterload, respectively. Input and output pressures were measured through saline-filled side-arms.

Experimental protocols

Six experimental series were performed (Table 1). Once the preparation had stabilized, it was switched from perfusion with the control perfusate to perfusion from the reservoir containing the experimental perfusate (100 ml). The composition of the perfusate varied, depending upon the experimental series, and is listed in Table 1. Each series consisted of 2–20 min experimental periods. During the first period, cardiac output was set to approximate that of a resting fish (Q=15 ml min^{-1} kg^{-1} fish mass) by manipulation of stroke volume *via* the filling

Table 1. *Summary of experimental series*

Series	Perfusate composition	
	Low work load	High work load
I (N=4)	1 mmol l^{-1} lactate	1 mmol l^{-1} lactate
II (N=6)	10 mmol l^{-1} lactate	10 mmol l^{-1} lactate
III (N=4)	1 mmol l^{-1} lactate	1 mmol l^{-1} lactate+10 mmol l^{-1} pyruvate
IV (N=5)	10 mmol l^{-1} lactate	10 mmol l^{-1} lactate+20 μmol l^{-1} iBCLa
V (N=6)	10 mmol l^{-1} lactate	10 mmol l^{-1} lactate+100 μmol l^{-1} SITS
VI (N=3)	10 mmol l^{-1} lactate	10 mmol l^{-1} lactate+2.5 mmol l^{-1} α-CIN

MCS, modified Cortland saline; IAA, iodoacetic acid; iBCLA, isobutyl carbonyl lactyl anhydride; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid; α-CIN, α-cyano-4-hydroxycinnamate.

The perfusate contained MCS, 0.5 mmol l^{-1} IAA and 0.2 μCi mmol^{-1} [U-^{14}C]lactate plus the listed ingredients.

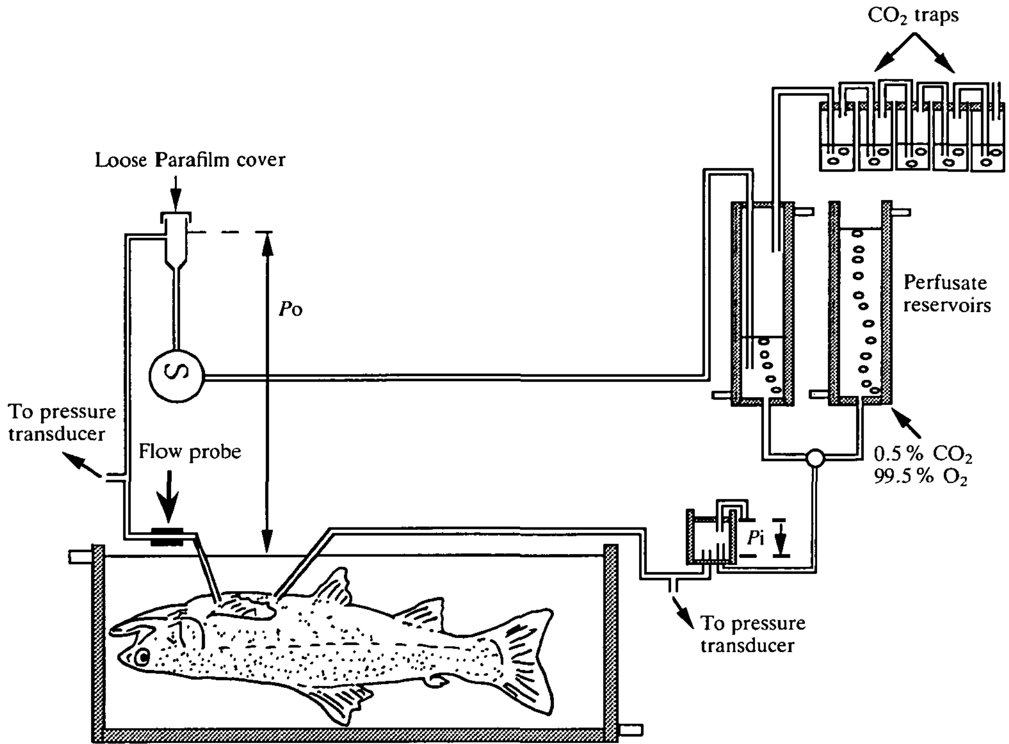

Fig. 1. A schematic diagram of the *in situ* perfused heart preparation. The heart maintained a steady intrinsic rhythm and the input (*P*i) and output (*P*o) pressure heads were altered to set cardiac output and diastolic afterload, respectively. Upon transfer to the experimental apparatus, the hearts were perfused on a flow-through basis with perfusate containing MCS, 0.5 $mmol\,l^{-1}$ IAA and either 1 or 10 $mmol\,l^{-1}$ lactate. Once the preparations had stabilized, perfusion was switched to the experimental perfusate (containing MCS, 0.5 $mmol\,l^{-1}$ IAA, 1 or 10 $mmol\,l^{-1}$ lactate and [^{14}C]lactate), which was recirculated by means of a peristaltic pump. The perfusate was continually gassed with 0.5% CO_2/99.5% O_2 which carried over into $^{14}CO_2$ traps placed in series. The switch from the control to experimental perfusate was achieved by turning a three-way stopcock. Samples were withdrawn anaerobically from the perfusate reservoir and $^{14}CO_2$ traps by means of a 500 μl gas-tight Hamilton syringe connected to the traps *via* a three-way stopcock.

pressure, since the heart rate was determined by the intrinsic pacemaker rhythm. The mean diastolic afterload was set so that the mean output pressure was about 4.9 kPa (see Table 2 for a summary of the cardiovascular variables). This period served as the control for each preparation. In the second 20 min experimental period, cardiac output was increased 3–4 times and diastolic afterload was increased so that mean output pressure increased to about 7.35 kPa, to simulate cardiac performance of swimming fish. These alterations led to a nearly fourfold increase in power output. (Power output is the product of cardiac output and pressure developed by the heart and is expressed in $mW\,g^{-1}$ ventricle mass.) During the second period, various potential blockers of lactate transport (e.g.

iBCLA, SITS, see Table 1) were added to the perfusate to the final concentrations shown in Table 1.

Tonic adrenergic stimulation, which is necessary to maintain performance of the *in situ* heart (Graham and Farrell, 1989), was provided by addition of adrenaline to the perfusate to a final concentration of 10^{-8} mol l^{-1}. Because the perfusate was recirculating, the adrenaline contained in it was degraded by biological means as well as by photo-oxidation. Therefore, to maintain a tonic level of adrenergic stimulation during the 40 min experiments, half-doses of adrenaline (i.e. sufficient alone to raise the adrenaline concentration in the perfusate to 5×10^{-9} mol l^{-1}) were added approximately every 10 min. An indication that this was an appropriate strategy was that, at the high workload, heart rate and thus diastolic flow decreased slightly with time (see Fig. 2C in particular). On addition of the half-dose of adrenaline, cardiac performance was restored to, but did not exceed, the former level. Given that adrenergic stimulation beyond the tonic 10^{-8} mol l^{-1} level increases cardiac performance substantially (Graham and Farrell, 1989), we were assured that we successfully titrated the adrenaline concentration reasonably near to 10^{-8} mol l^{-1}. In the worst case scenario, assuming no degradation of adrenaline, the maximum adrenaline concentration in the perfusate at the end of the experiment would have been 2.5×10^{-8} mol l^{-1}, which is still reasonable compared to *in vivo* resting levels (5×10^{-9} mol l^{-1}; Milligan and Wood, 1987).

The perfused heart preparation required that both the preload and afterload reservoirs were exposed to the atmosphere. This arrangement could potentially result in loss of labelled CO_2 from the system. In an attempt to minimize $^{14}CO_2$ loss, the opening of the input reservoir was *via* a 3 mm (i.d.) piece of glass tubing. Input pressure was adjusted such that a drop hung at the opening of the tube. Any perfusate that overflowed was collected in a beaker and promptly returned to the system. During an experiment, the outflow was collected in a reservoir (10 ml) which was loosely covered with Parafilm and returned to the main reservoir (nominal volume 100 ml) by means of a peristaltic pump (see Fig. 1). The pump was operated at a rate in excess of the cardiac output, thus minimizing the loss of [^{14}C]CO_2 from the system. [^{14}C]CO_2 produced during the experimental periods was collected in a series of five CO_2 traps each containing 10 ml of 10 mol l^{-1} KOH (Fig. 1). Preliminary experiments with [^{14}C]$NaHCO_3$ demonstrated that the [^{14}C]CO_2 trapping efficiency in this system was greater than 95 %. At the end of each of the 20 min experimental periods, samples (1 ml) from the CO_2 traps were taken and counted for ^{14}C-radioactivity. Perfusate was sampled (10 ml) and analyzed for [lactate] and total [^{14}C]CO_2. At the end of the experiment the volume of the entire system was determined by dye dilution.

Experimental series

The aim of the various experimental series was to examine factors affecting lactate utilization and, indirectly, transport by cardiac muscle under physiological conditions. High levels of lactate (10 mmol l^{-1}) were chosen to ensure that the performance of the heart preparation would not be limited by exogenous fuel

availability (Farrell *et al.* 1988*b*). To differentiate between a general metabolic effect and a more specific effect on lactate transport, hearts were challenged with a physiological high workload. The underlying assumption is that if the blocker was having a non-specific metabolic effect, it would be reflected in a change in cardiac performance (e.g. series VI), which would be exaggerated at the high workload. The first 20 min experimental period (low workload under control conditions; i.e. without added blockers) ensured that the preparations in each series were performing at the same level prior to blockade. Those preparations that did not perform adequately (i.e. were unable to maintain cardiac output) during the control period were discontinued.

Series I and II

These experiments examined whether exogenous fuel was limiting cardiac performance and lactate oxidation. Series II served as the control for series IV–VI.

Series III

This experimental series looked at the effect of 10-fold greater concentrations of pyruvate on lactate utilization. Pyruvate has been shown to inhibit lactate transport competitively in human erythrocytes (Halestrap, 1976).

Series IV

These experiments examined the effect of isobutyl carbonyl lactyl anhydride (iBCLA) on lactate utilization and cardiac performance at the high workload. iBCLA, a synthetic anhydride of lactic acid, has been shown to act as a specific inhibitor of lactate transport in Ehrlich ascites tumour cells and human erythrocytes (Johnson *et al.* 1980).

Series V

The effect of the stilbene derivative 4-acetomido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS) on lactate utilization and cardiac performance was assessed. SITS has been shown to inhibit inorganic anion transport in human erythrocytes (Deuticke *et al.* 1982; Halestrap, 1976).

Series VI

These experiments looked at the effect of α-cyano-4-hydroxycinnamate (α-CIN) on lactate utilization and cardiac performance. α-CIN has been shown to be a potent inhibitor of pyruvate mitochondrial transport as well as a competitive inhibitor of lactate transport in human erythrocytes (Halestrap, 1976).

Analytical techniques and calculations

Analyses of cardiac variables were based on 10 s samples of cardiovascular

signals taken at various intervals during the experimental series. Microcomputer analysis yielded average values for that period (see Farrell *et al.* 1986). Pressures were measured relative to the level of saline in the bath and were corrected for pressure drops across the input and output cannulae. Fish were weighed prior to the experiments and at the end the heart was removed, the ventricle dissected, blotted dry and the wet mass determined.

Samples (250 μl) from the base in the CO_2 traps were added to 10 ml of fluor (Scintiverse, Fisher Scientific) and counted on a Beckman LS9000 liquid scintillation counter. Samples were assayed in duplicate. Total [^{14}C]CO_2 in the perfusate was measured by acidifying 1 ml of perfusate with 1 ml of 1 mol l^{-1} HCl in a CO_2 trap containing filter paper wetted with 200 μl of 1 mol l^{-1} hyamine hydroxide. The traps were shaken for 1 h and the filter paper removed and counted in 10 ml of scintillation fluor and the counts were added to the ^{14}C-radioactivity from the CO_2 traps to yield total [^{14}C]CO_2 produced during the experimental periods. For analysis of lactate, 500 μl of perfusate was extracted with 500 μl of ice-cold 8 % perchloric acid. The samples were centrifuged at 10 000 *g* for 5 min, the supernatant neutralized with 3 mol l^{-1} K_2CO_3, re-centrifuged and the final supernatant assayed for lactate using Sigma reagents.

Lactate oxidation rate was calculated from the total [^{14}C]CO_2 produced, and the specific activity of the lactate in the perfusate. In calculating the rate of substrate utilization it was assumed that once decarboxylation was initiated, oxidation of substrate to CO_2 was complete. In initial experiments, total lactate and [^{14}C]lactate were measured at the beginning and end of each experimental period to calculate specific activity. [^{14}C]Lactate was isolated by column chromatography. A sample (100 μl) of the perchloric acid extract neutralized with 3 mol l^{-1} K_2CO_3 was layered onto a glass column (i.d. 5 mm) containing 0.5 g of Dowex 1 (Cl^- form; 100–200 mesh, Sigma). Lactate was eluted from the column with 5 ml of 2 mol l^{-1} acetic acid. This method yielded better than 95 % recovery of labelled lactate (Milligan and McDonald, 1988). It was found that specific activity did not change significantly during the experimental period, so in later experiments, lactate specific activity was determined only at the end of the experiment. Oxidation rates are expressed per unit wet mass of the heart.

The control perfusate was modified Cortland saline (MCS) which contained: 124 mmol l^{-1} NaCl, 3.8 mmol l^{-1} KCl, 1.9 mmol l^{-1} $CaCl_2$, 0.1 mmol l^{-1} NaH_2PO_4, 0.9 mmol l^{-1} $MgSO_4$, 10^{-8} mol l^{-1} adrenaline, and either 1 or 10 mmol l^{-1} lactate (see Table 1). Saline was equilibrated with 0.5 % CO_2/99.5 % O_2, then $NaHCO_3$ was added to a final concentration of 10 mmol l^{-1}. pH was adjusted to 7.85–7.9 at 10°C. Experimental perfusate consisted of MCS plus 0.5 mmol l^{-1} iodoacetic acid (IAA). IAA has been shown to block glycolysis, hence utilization of endogenous glucose/glycogen, in perfused heart preparations from teleosts (Driedzic *et al.* 1982; Driedzic, 1983). The rationale for using IAA was to eliminate endogenous glycogen as a fuel, thus putting the hearts in a situation which would maximize exogenous lactate utilization. Also, glycogen content of the trout heart can vary

considerably (C. L. Milligan, unpublished results), thus, by eliminating glycogen utilization by all hearts, we attempted to minimize potential variation in lactate utilization. In two preliminary experiments it was found that with $10\,mmol\,l^{-1}$ lactate as the sole exogenous substrate, $0.5\,mmol\,l^{-1}$ IAA had no effect on cardiac performance, at either the low or high workloads.

Chemicals were purchased from Sigma Chemical Co., St Louis, MO, except iBCLA which was a generous gift from Dr G. Tibbits, Department of Kinesiology, Simon Fraser University.

Means±1 S.E.M. are reported throughout, unless otherwise stated. Significant differences ($P<0.05$) were determined using a Student's *t*-test, paired or unpaired design.

Equipment

Pressures were measured with LDI-5 pressure transducers (Narco, Houston, Texas). Flow was measured with a 3.0 mm electromagnetic flow probe and associated SWF-4 meter (Zepada Instruments, Seattle, Washington). Pressure and flow signals were recorded continuously on a Gould 2400 chart recorder (Gould, Cleveland, Ohio). An Apple II microcomputer equipped with an analog-to-digital card was used to analyze the cardiovascular signals (Farrell and Bruce, 1987).

Results

Preparation stability

There were no significant changes in power output during the 20 min experimental period at either low or high workload, with the notable exception of series VI ($2.5\,mmol\,l^{-1}$ α-CIN; see Fig. 2D), indicating that the performance of the preparations was stable. The cardiac variables for each preparation were pooled and the mean values are shown in Table 2. In series VI the hearts failed rapidly after the addition of α-CIN at the high workload; representative mean values were therefore not obtainable.

In a typical experiment, cardiac workload was increased by increasing filling pressure and afterload to the heart. Increasing filling pressure by 0.1–0.2 kPa (from a subambient pressure to one slightly above ambient pressure) increased stroke volume to near its maximum. Stroke volume declined slightly to a new stable level when afterload was increased (Fig. 2A–D). Cardiac output remained constant at this new level throughout the 20 min experimental period, except in series VI (Fig. 2D).

Of the three blockers used in this study, only α-CIN had a significant effect on cardiac performance (Fig. 2B, C *versus* D). The addition of α-CIN to a final concentration of $2.5\,mmol\,l^{-1}$ and 0.5 % ethanol resulted in a very rapid deterioration of cardiac performance, as indicated by the decline in cardiac output (Fig. 2D). To determine if this effect of α-CIN on cardiac performance was due to α-CIN itself or to the carrier (0.5 % ethanol), hearts were perfused with 0.5 %

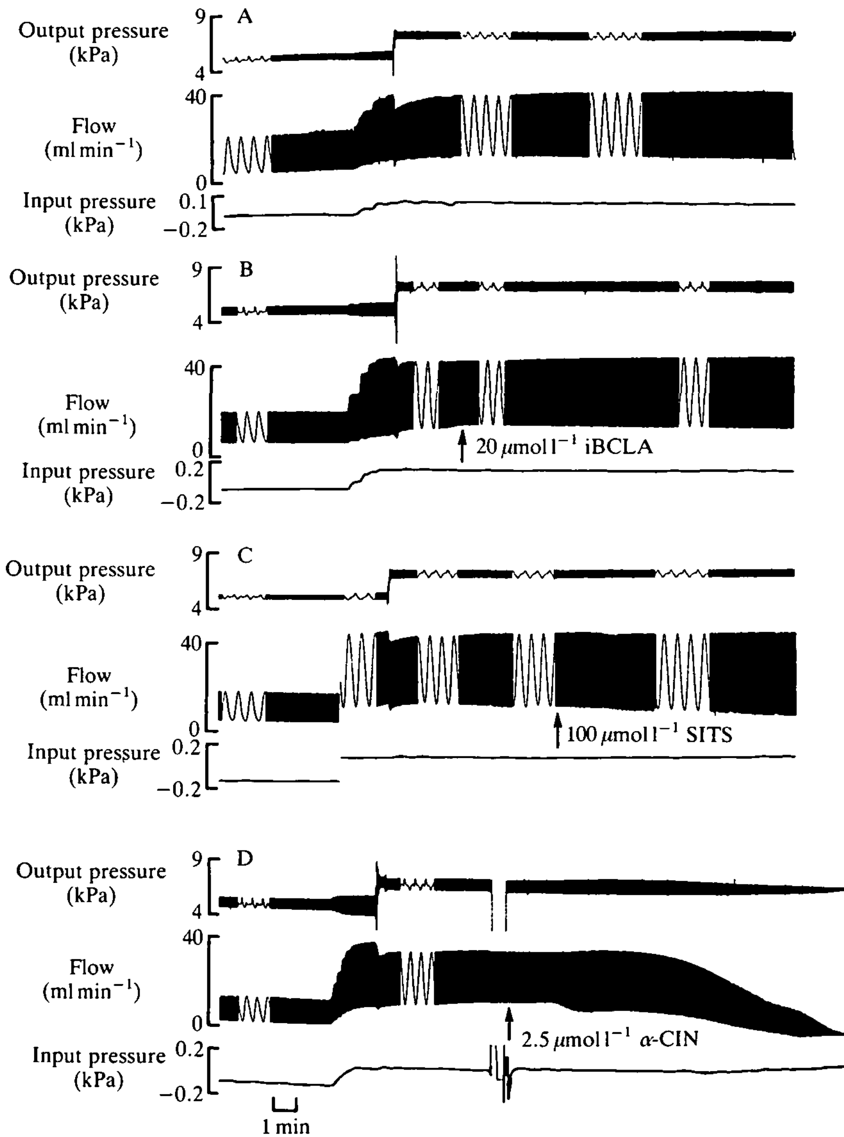

Fig. 2. A continuous record of cardiovascular variables at the low and high workload and the transition from low work to high work. (A) Series II, 10 mmol l^{-1} lactate; (B) series IV, 10 mmol l^{-1} lactate plus 20 μmol l^{-1} iBCLA at the high workload; (C) series V, 10 mmol l^{-1} lactate plus 100 μmol l^{-1} SITS at the high workload; (D) series VI; 10 mmol l^{-1} lactate plus 2.5 mmol l^{-1} α-CIN at the high workload. Arrows indicate time of blocker addition. Pressures are not corrected for cannula resistances. Note: the time scale of the expanded regions of the trace is decreased 100-fold.

Table 2. *Cardiac variables for each experimental series at low and high workloads*

Series	f_H (beats min^{-1}) Low	f_H (beats min^{-1}) High	$\dot{Q}$ (ml min^{-1} kg^{-1}) Low	$\dot{Q}$ (ml min^{-1} kg^{-1}) High	Filling pressure (kPa) Low	Filling pressure (kPa) High	Afterload (kPa) Low	Afterload (kPa) High	Power output (mW g^{-1}) Low	Power output (mW g^{-1}) High
I (N=4)	54.43±1.25	60.10±1.61	14.36±0.27	40.88±2.67	−0.12±0.03	0.05±0.02	4.91±0.03	7.06±0.03	1.54±0.10	6.41±0.36
II (N=6)	66.01±3.11	64.90±4.00	15.71±0.98	46.42±3.21	−0.25±0.04	0.07±0.06	4.92±0.03	7.12±0.06	1.65±0.09	6.47±0.28
III (N=4)	55.34±1.34	61.12±0.23	14.67±0.34	41.23±2.12	−0.16±0.02	0.04±0.02	5.03±0.02	6.97±0.05	1.75±0.03	6.47±0.28
IV (N=5)	59.58±1.89	61.0±1.84	14.05±0.16	43.82±3.21	−0.28±0.05	0.05±0.05	4.94±0.03	7.20±0.07	1.45±0.08	6.43±0.41
V (N=6)	61.20±3.34	56.21±4.64	15.02±0.96	43.49±2.41	−0.25±0.03	0.16±0.04	4.91±0.04	7.11±0.05	1.63±0.22	6.14±0.63
VI† (N=3)	54.89±3.14		13.14±1.27		−0.20±0.03		4.93±0.04		1.54±0.12	

† All preparations in this series deteriorated within 5–10 min of exposure to 2.5 mmol l^{-1} α-CIN, so representative cardiac variables were not obtainable.

Values are mean±1 S.E.M.

f_H, heart rate; $\dot{Q}$, cardiac output.

ethanol in series I perfusate. At the start of the high workload, prior to ethanol addition, power output was 6.96 mW g^{-1}; 15 min after the addition of ethanol to a final concentration of 0.5 %, power output had fallen to 6.28 mW g^{-1}. This result suggests that while 0.5 % ethanol may have had a detrimental effect on cardiac performance, it cannot, by itself, explain the deterioration of the α-CIN preparations. To determine if the α-CIN effect was lactate- specific, an experiment was performed substituting glucose for lactate and without IAA in the media. Cardiac performance declined in a fashion similar to that shown in Fig. 2D, suggesting that the negative effect of α-CIN on cardiac performance was not lactate-specific, but rather a generalized effect on the heart. In contrast, pyruvate, iBCLA and SITS had no effect on cardiac performance.

Lactate oxidation and cardiac work

Lactate utilization was dependent upon the external lactate concentration. At the low workload, the rate of lactate utilization in series I and III (1 mmol l^{-1} lactate; Fig. 3A) was about 10 % of the oxidation rate observed in series II (10 mmol l^{-1} lactate; Fig. 3A). Increasing power output led to a significant increase in lactate oxidation in series II, but not in series I (Fig. 3A *versus* Fig. 3B).

Series III, IV, V and VI examined the effect of several putative lactate transport blockers on lactate utilization and cardiac performance. Since series I and II demonstrated that: (1) lactate oxidation was dependent upon lactate availability; (2) lactate utilization increased with increasing workload at high external [lactate]; and (3) cardiac performance was not limited by lactate availability, it was reasoned that limiting lactate availability by blocking uptake would be reflected in a reduction in oxidation rate, without a concomitant reduction in cardiac performance. Thus, monitoring cardiac performance provided a means of determining whether a reduction in lactate utilization was due to cardiac failure (e.g. series VI) or to limited availability because uptake was blocked.

Increasing power output about fourfold led to a near doubling of lactate utilization in series II. However, in the presence of both 20 μmol l^{-1} iBCLA and 100 μmol l^{-1} SITS (series IV and V, respectively), lactate oxidation was significantly reduced, to levels even lower than those observed at the low workload (Fig. 3B *vs* A). However, cardiac performance, as indicated by power output, was not affected by either 20 μmol l^{-1} iBCLA or 100 μmol l^{-1} SITS (Fig. 3B; Table 2). The addition of 10 mmol l^{-1} pyruvate, a potential competitor of lactate transport, to perfusate containing 1 mmol l^{-1} lactate, did not alter either performance or lactate utilization rates at the high workload (Fig. 3B, series I *versus* III).

As previously mentioned, heart preparations deteriorated rapidly in the presence of 2.5 mmol l^{-1} α-CIN (series VI). Typically, the preparation lasted about 5–8 min and, while there was detectable lactate utilization (1.16±0.54 μmol g^{-1} min^{-1}; N=3), it cannot be correlated with performance because of the deleterious effect of α-CIN on the heart preparation (Fig. 2D).

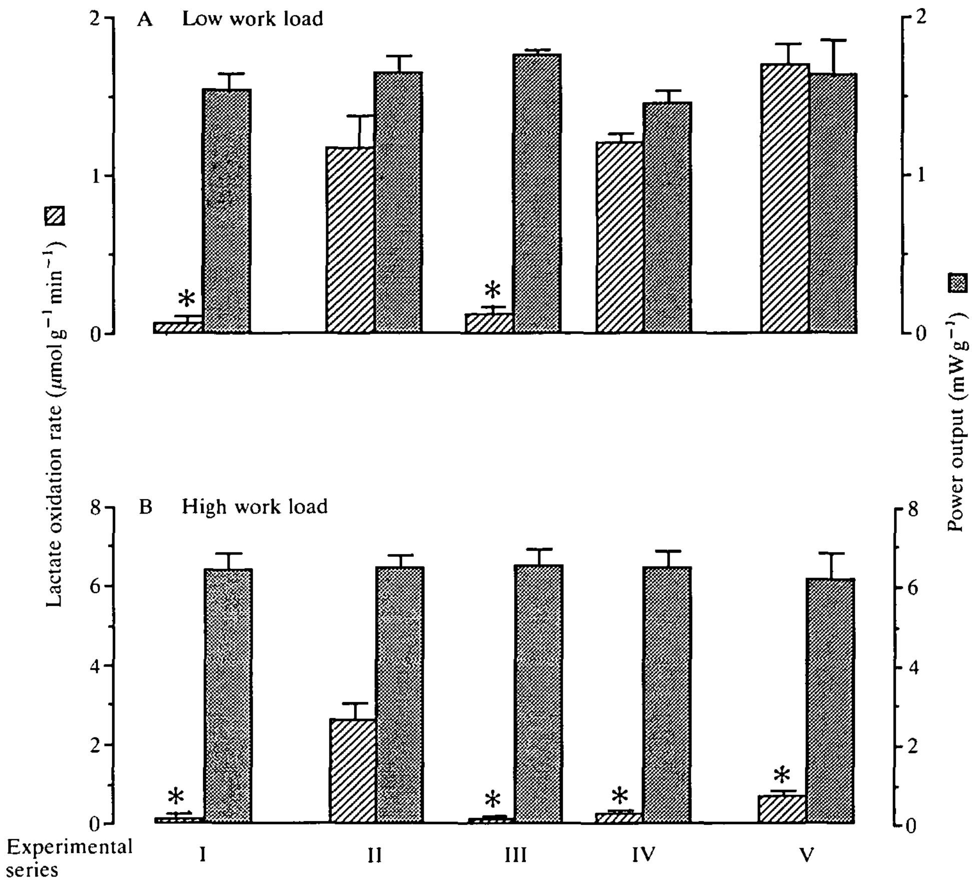

Fig. 3. Lactate oxidation rates and power output of the *in situ* perfused trout heart at (A) low and (B) high workloads. For details of experimental series see Table 1. * indicates a significant difference ($P<0.05$) from corresponding series II value.

Discussion

The *in situ* perfused trout heart preparation, employed here for the first time to study cardiac metabolism, was used since it mimics *in vivo* cardiac performance reasonably well. The cardiac variables at the low and high workloads are very similar to *in vivo* values obtained from trout at rest and at maximum sustainable swimming speed (Kiceniuk and Jones, 1977). Therefore, we were able to span most of the physiological range of cardiac performance with the advantage of being able to manipulate perfusate composition. An additional benefit provided by a perfused heart preparation is that effects of various agents on lactate utilization can be separated from more general effects on cardiac performance (e.g. series VI), thus permitting comment on the mechanism of lactate transport into cardiac muscle.

Fuel availability and cardiac performance

Iodoacetic acid ($0.5\,\mathrm{mmol\,l^{-1}}$) effectively blocks glycolytic metabolism in a number of perfused heart preparations (e.g. rat, rabbit, Rovetto, 1981; sea raven, ocean pout, Driedzic *et al.* 1982; Driedzic, 1983), presumably at the level of glyceraldehyde-3-phosphate dehydrogenase. While we have no direct evidence of the effectiveness of glycolytic blockade in the present study, there is no *a priori* reason to suspect it would act differently in a trout heart.

It is clear from the present study that the rate of lactate utilization by the *in situ* perfused trout heart is limited by extracellular lactate availability (series I *versus* II; Fig. 3A) but cardiac performance is not (series I *versus* II; Fig. 3B). The former observation is consistent with the data reported by Driedzic *et al.* (1985) for isolated, perfused ocean pout and sea raven hearts, which indicated that the most important limit on lactate oxidation is substrate availability. However, the latter observation contradicts those of Driedzic and Hart (1984) on isolated sea raven and skate hearts and those of Farrell *et al.* (1988*b*) on the *in situ* perfused trout heart. Both studies suggest that exogenous fuel availability was limiting to cardiac performance. In the study of Driedzic and Hart (1984), $0.5\,\mathrm{mmol\,l^{-1}}$ IAA caused a rapid decline in performance of the sea raven heart which was only partially offset by the addition of $2\,\mathrm{mmol\,l^{-1}}$ lactate. Similarly, Farrell *et al.* (1988*b*) reported that, in the absence of exogenous fuel, cardiac performance declined. This did not happen in the present study; $0.5\,\mathrm{mmol\,l^{-1}}$ IAA had no effect on cardiac performance. Hearts were able to achieve and maintain a high power output, even though lactate utilization was limited by substrate availability (series I and III, Fig. 3B). Clearly, the hearts in the present study had enough endogenous fuel to meet energy demands. Further evidence to support this is seen in Fig. 3B; the hearts in series IV and V were able to maintain performance even when lactate utilization was reduced by lactate transport blockers. The reasons for the discrepancy between the current study and the previous ones are not clear, but may be related to the level of endogenous triglycerides and/or tonic adrenergic stimulation to the preparation. Triglyceride levels in salmonid hearts are at least as high as glycogen levels ($20\,\mu\mathrm{mol\,g^{-1}}$ wet mass; Patton *et al.* 1975) and can serve as a significant source of fuel (Patton *et al.* 1975). Although the regulation of triglyceride metabolism in teleost cardiac metabolism is not well understood, in mammalian cardiac muscle, lipolysis is stimulated by both adrenaline and increased work (Crass *et al.* 1969, 1975; Rovetto, 1981). Tonic adrenergic stimulation has been shown to be important in maintaining performance of the *in situ* perfused heart (Graham and Farrell, 1989), though the underlying mechanism is not known. In the present study, the combination of tonic adrenergic stimulation and the increased workload may have been important in promoting triglyceride utilization when lactate availability was reduced, thus maintaining performance. Tonic adrenergic stimulation was not provided to the perfused hearts in either the study of Driedzic and Hart (1984) or that of Farrell *et al.* (1988*b*). Furthermore, the hearts in the former study were performing at sub-physiological levels. Some of the reduction in cardiac performance observed by Driedzic and Hart (1984) in

the presence of 0.5 mmol l^{-1} IAA and Farrell *et al.* (1988*b*) in the absence of exogenous fuel could be explained by a wash-out of adrenaline from the perfused heart, thus potentially limiting the capacity for lipolysis.

When faced with exogenous lactate limitation, the perfused heart maintained its performance, presumably by mobilizing endogenous triglycerides. However, when exogenous lactate was plentiful (e.g. series II), the heart preferentially oxidized lactate. Assuming that the heart is working at 15 % efficiency (Farrell, 1985), in series II lactate oxidation can account for as much as 89–110 % of the ATP requirement at the low workload. Lactate oxidation can also account for almost 100 % of the energy requirement at the high workload, assuming the efficiency of the heart increased with performance to 25 % (Farrell, 1985). A *caveat* to this interpretation lies in the assumption that all the $^{14}CO_2$ produced is a result of complete oxidation of lactate. Driedzic *et al.* (1985) suggest that, in the perfused sea raven and ocean pout hearts, a portion of lactate utilized is channelled from acetyl CoA possibly into lipid, rather than directed into the citric acid cycle. However, the hearts in their study were performing at sub-physiological levels and lacked adrenergic stimulation. While the situation is not known in teleost hearts, in mammalian hearts, adrenaline and increased workload not only promote lipolysis (see above), but also inhibit lipid synthesis (Crass *et al.* 1969). Therefore, if fatty acid synthesis occurred in the trout heart in the present study, it was probably minimal. Nonetheless, these estimates clearly demonstrate that lactate can be a significant fuel to the working heart.

It has been shown that, *in vitro*, trout hearts prefer lactate to glucose or fatty acids as an exogenous fuel (Lanctin *et al.* 1980). The present results extend this observation, suggesting that exogenous lactate, when plentiful, is preferred over endogenous fuel, and 10 mmol l^{-1} lactate can supply nearly all the heart's energy needs. This is an important observation since, during near-maximal sustained swimming and after burst swimming, a portion (about 10 %) of the lactate produced in the white muscle is released into the blood, raising blood lactate levels to 10–20 mmol l^{-1} (Milligan and Wood, 1986). The present observations indicate that this lactate can serve as an important fuel for maintaining the high levels of cardiac performance required during recovery from exhaustive exercise.

The effect of lactate uptake blockade on lactate utilization

Pyruvate, at levels 10 times those of lactate, did not appear to affect lactate utilization. This is inconsistent with the known competitive effect of pyruvate on lactate uptake and metabolism in a number of preparations (e.g. red blood cell, Halestrap, 1976; guinea pig cardiac muscle, Poole *et al.* 1989). This apparent lack of competition between pyruvate and lactate may be an artefact induced by the design of the present experiments. Prior to the addition of pyruvate, there was a control period during which lactate utilization was not challenged. By the end of this control period, a number of oxidative intermediates were no doubt labelled and the continued production of $^{14}CO_2$ during the pyruvate challenge may represent oxidation of this 'residual' label. Pyruvate may, in fact, have inhibited

lactate uptake and utilization. However, under the current experimental conditions, we were unable to demonstrate it.

Isobutyl carbonyl lactyl anhydride (iBCLA; 20 $\mu mol\,l^{-1}$), a synthetic anhydride of lactic acid, virtually abolished lactate oxidation at the high workload (Fig. 3B), even though lactate was present at a concentration 500 times that of iBCLA. Furthermore, even though lactate oxidation was reduced, cardiac performance was not affected by iBCLA. The observed depression of lactate oxidation in the absence of an effect on cardiac performance suggests that the iBCLA effect was lactate-specific. Two hypotheses can be put forward to explain the inhibitory effect of iBCLA on lactate utilization: (1) iBCLA was directly inhibiting lactate metabolism; (2) iBCLA was inhibiting lactate transport into the cell.

A number of observations tend to rule out the hypothesis that iBCLA was directly affecting lactate metabolism. First, 20 $\mu mol\,l^{-1}$ iBCLA does not affect the activity of lactate dehydrogenase in crude homogenates of trout hearts (C. L. Milligan, unpublished observations). It is possible that iBCLA was acting further along the oxidative pathway (e.g. tricarboxylic acid cycle, electron transport system): however, the observation that cardiac performance was unchanged suggests that mitochondrial activity was not affected. Furthermore, iBCLA does not appear to enter the cell (Johnson *et al.* 1980). The latter observation lends support to the alternative hypothesis: iBCLA is inhibiting lactate uptake. This hypothesis is consistent with the known actions of iBCLA in a number of other preparations. In Ehrlich ascites tumour cells and human erythrocytes, iBCLA specifically inhibits lactate transport by acylating the carrier (Johnson *et al.* 1980). Furthermore, in sarcolemmal vesicles prepared from trout cardiac muscle, iBCLA (8 $\mu mol\,l^{-1}$) significantly inhibited lactate uptake, typically reducing the rate of lactate uptake 10-fold (G. Tibbits, personal communication). These observations, taken together, suggest that the inhibition of lactate oxidation by 20 $\mu mol\,l^{-1}$ iBCLA is due to an inhibition of uptake, thus limiting substrate availability. Clearly, verification of this hypothesis and delineation of the uptake mechanism requires further experimental work with either sarcolemmal vesicles or isolated cardiac myocytes from trout heart.

Like iBCLA, 100 $\mu mol\,l^{-1}$ SITS inhibited lactate oxidation without affecting cardiac performance. By the same reasoning, a direct effect of SITS on lactate metabolism can be ruled out. (1) 100 $\mu mol\,l^{-1}$ SITS does not affect lactate dehydrogenase activity in crude homogenates of trout heart (C. L. Milligan, unpublished results). (2) SITS probably does not affect metabolism further along the oxidative pathway, since performance was not affected. (3) SITS does not appear to enter cells (Halestrap, 1976). If lactate entry into cardiac myocytes is carrier-mediated, which is suggested by the results of series IV (20 $\mu mol\,l^{-1}$ iBCLA), it would appear that SITS is reducing lactate oxidation by limiting substrate at the level of uptake. Whether SITS acts directly or indirectly (through coupled transporters) on inward lactate transport into the trout heart is not known.

In the *in situ* perfused trout heart, 2.5 $mmol\,l^{-1}$ α-CIN proved to be toxic, as

indicated by the rapid deterioration of cardiac performance. The reason for this toxicity is not immediately clear. Numerous other studies have reported that at low concentrations (1–100 μmol l^{-1}), α-CIN is a specific inhibitor of mitochondrial pyruvate transport (e.g. Halestrap and Denton, 1975) and at higher concentrations (1–10 mmol l^{-1}), α-CIN inhibits pyruvate and lactate transport across the cell membrane (Halestrap and Denton, 1975; Halestrap, 1976; Hemptine *et al.* 1983; Koch *et al.* 1981; Mann *et al.* 1985). In none of these studies was α-CIN reported to have a toxic effect. In fact, 10 mmol l^{-1} α-CIN was shown to block lactate uptake by a perfused rabbit heart without any apparent ill effect (Mann *et al.* 1985). The inhibitory effect of α-CIN on mitochondrial pyruvate transport may present the perfused trout heart with a fuel limitation. However, since triglycerides were most probably fuelling the heart, and mitochondrial transport and β-oxidation of fatty acids are not dependent upon mitochondrial pyruvate transport, it is unlikely that the deterioration of the heart was due to a fuel limitation. Whatever the reason for the α-CIN toxicity, it is clearly not an appropriate compound to use for the study of either lactate or pyruvate transport in trout cardiac muscle.

In conclusion, the present study has confirmed the previous observation that the rate of lactate oxidation by fish hearts is limited by lactate availability. However, cardiac performance in iodoacetic-acid-blocked hearts is not limited by exogenous fuel availability, suggesting that endogenous triglycerides and/or protein are an adequate fuel for the heart. Furthermore, exogenous lactate, when plentiful, is preferred over endogenous fuel. Finally, evidence is presented suggesting that in the working trout heart lactate uptake is carrier-mediated.

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