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THE INFLUENCE OF LACTIC ACID ON ADENOSINE RELEASE FROM SKELETAL MUSCLE IN ANAESTHETIZED DOGS

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SUMMARY

1. In anaesthetized and artificially ventilated dogs, a gracilis muscle was vascularly isolated and perfused at a constant flow rate of $11 \cdot 9 \pm 2 \cdot 2$ ml min^{-1} 100 g^{-1} (mean ± S.E.M., $n = 16$; equivalent to $170 \cdot 2 \pm 21 \cdot 3$ % of its resting free flow).

2. Stimulation (3 Hz) of the obturator nerve produced twitch contractions of the gracilis muscle, reduced venous pH from $7 \cdot 366 \pm 0 \cdot 027$ to $7 \cdot 250 \pm 0 \cdot 031$ ($n = 5$), increased oxygen consumption from $0 \cdot 62 \pm 0 \cdot 24$ to $2 \cdot 76 \pm 0 \cdot 46$ ml min^{-1} 100 g^{-1} ($n = 5$) and increased adenosine release from $-0 \cdot 40 \pm 0 \cdot 14$ (net uptake) to $1 \cdot 36 \pm 0 \cdot 50$ nmol min^{-1} 100 g^{-1} ($n = 8$).

3. Infusion of lactic acid (4·2 mM) into the artery reduced venous pH to $7 \cdot 281 \pm 0 \cdot 026$ ($n = 5$) and increased adenosine release to $0 \cdot 96 \pm 0 \cdot 40$ nmol min^{-1} 100 g^{-1} ($n = 8$), but did not significantly alter oxygen consumption ($0 \cdot 80 \pm 0 \cdot 19$ ml min^{-1} 100 g^{-1}; $n = 5$). Stimulation (3 Hz) in the presence of lactic acid infusion produced no further significant changes in venous pH or adenosine release, but increased oxygen consumption to $2 \cdot 53 \pm 0 \cdot 37$ ml min^{-1} 100 g^{-1} ($n = 5$).

4. Infusion of a range of lactic acid concentrations ($\geqslant 1 \cdot 83$ mM) produced dose-dependent increases in adenosine release. The maximum lactic acid concentration tested (5·95 mM) reduced venous pH to $7 \cdot 249 \pm 0 \cdot 023$ ($n = 5$) and increased adenosine release to $2 \cdot 64 \pm 1 \cdot 26$ nmol min^{-1} 100 g^{-1} ($n = 6$).

5. A strong correlation existed between the adenosine release and the venous pH ($r = -0 \cdot 92$); points obtained during muscle stimulation and/or lactic acid infusion fell on a single correlation line.

6. The vasoactivity of adenosine administered by close-arterial injection was unaltered by infusion of either lactic acid (7·2 mM) or saline.

7. These results suggest that the release of adenosine from skeletal muscle can be induced by a decrease in pH (probably at an intracellular site), and that this mechanism may contribute to the release of adenosine during muscle contractions.

INTRODUCTION

It is now well established that adenosine is released from skeletal muscle during contractions. The adenosine content of venous blood and muscle has long been known to increase during flow-restricted contractions (Berne, Rubio, Dobson &

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Curnish, 1971; Bockman, Berne & Rubio, 1976; Belloni, Phair & Sparks, 1979), whilst recent technological advances have allowed detection of the more moderate increases in venous adenosine which accompany contractions during high constant flow perfusion (Ballard, Cotterrell & Karim, 1987) or free flow perfusion (Fuchs, Gorman & Sparks, 1981; Karim, Ballard & Cotterrell, 1988). The released adenosine has been shown to make an important contribution to the steady-state vasodilatation accompanying contractions (Kille & Klabunde, 1984; Ballard *et al.* 1987; Goonewardene & Karim, 1989), but little is known about the mechanism of the adenosine release.

It was suggested that adenosine release was caused by oxygen insufficiency (Phair & Sparks, 1979) because the amount of adenosine released during muscle contractions was found to be greater when the blood flow was restricted. However, a systematic study of the relationship between flow rate and adenosine release showed that the release was not correlated with either the venous oxygen tension or the ratio of oxygen supply to free-flow oxygen consumption (Ballard, Cotterrell & Karim, 1989). Thus the increased adenosine release at low blood flow was likely to be caused by some other factor related to the flow restriction, rather than the oxygen lack. Both the adenosine release and the venous pH returned to control values over a time course of about 10 min during the recovery from muscle contractions, whereas the venous oxygen tension had returned to control within the first minute after stimulation was stopped (Ballard *et al.* 1987), indicating a possible involvement of acidity in the adenosine release.

The present investigation was undertaken to determine whether acidity played a role in the release of adenosine from contracting muscles. Adenosine release from an isolated gracilis muscle was determined by HPLC at rest and during contractions, under control conditions and in the presence of a lactic acid infusion. Since lactic acid rapidly permeates cells (Mason & Thomas, 1988), both intracellular and extracellular pH were depressed by this intervention. Different rates of lactic acid infusion were employed to determine whether the adenosine release was dose-dependent; a range of adenosine concentrations were administered by close-arterial injection in the presence or absence of lactic acid infusion to determine whether the vasoactivity of adenosine varied with pH.

METHODS

Sixteen mongrel dogs weighing 9–27 kg were anaesthetized by injection of sodium pentobarbitone (Sagatal; RMB Animal Health Ltd) into a cephalic vein; anaesthesia was subsequently maintained by infusions of sodium pentobarbitone (12 mg ml^{-1}) as required. The neck was opened in the mid-line and the trachea cannulated for positive pressure ventilation at a stroke rate of 18 min^{-1} and an initial stroke volume of 17 ml kg^{-1} body weight.

The right gracilis muscle was vascularly isolated as previously described (Ballard *et al.* 1987). The branch of the obturator nerve supplying the gracilis muscle was exposed for about 2 cm of its length. The proximal tendon of the muscle was fixed securely to a post on the operating table; the distal tendon was cut and attached to an isometric force transducer (Grass, model FT10) to record developed tension. The muscle was wrapped in plastic film to prevent dehydration.

Heparin sodium (500 IU kg^{-1}; Sigma Chemical Co.) was given 30 min after completion of the dissection; supplementary doses of 50 IU kg^{-1} were given every 30 min thereafter. The perfusion circuits (Fig. 1) were filled with an artificial plasma solution containing (g l^{-1}): NaCl, 6·92; KCl, 0·354; $NaHCO_3$, 2·10; $MgSO_4.7H_2O$, 0·317; KH_2PO_4, 0·162; $CaCl_2.2H_2O$, 0·191; glucose, 2·0; BSA,

1·0; and dextrans (60000–90000 molecular weight), 59, before connection to the animal. The muscle was perfused under free flow conditions through the right femoral artery with blood from the opposite femoral artery for 30–60 min following connection of the perfusion circuits to allow pressures and flows to stabilize. Blood flow was then measured by timed collections of the venous

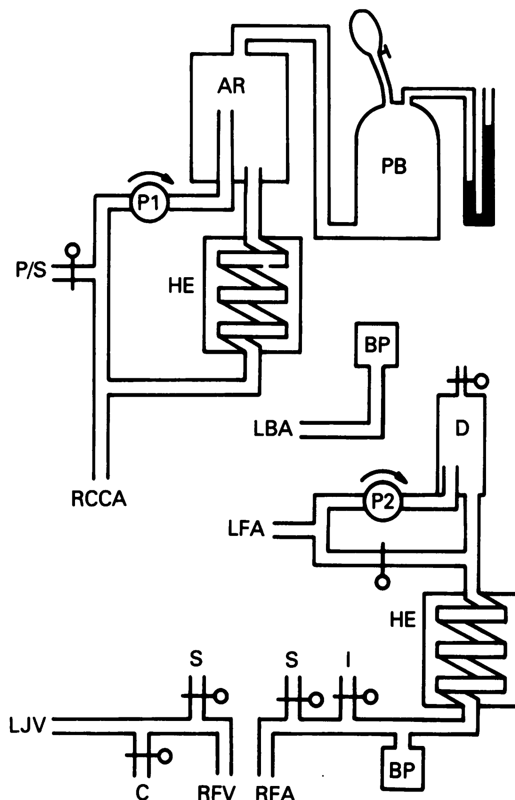

Fig. 1. Schematic diagram of the perfusion circuits. Blood pressure was held constant by connection of the right common carotid artery (RCCA) via a reservoir (AR) to a pressure bottle (PB). Blood in the reservoir was kept at 37 °C by circulation through a heat exchanger (HE) using a peristaltic pump (P1). Blood pressure was measured by a transducer (BP) connected to the left brachial artery (LBA). The gracilis muscle was perfused through an isolated section of the right femoral artery (RFA) with blood taken from the left femoral artery (LFA), either at free flow through the bypass tube or at constant flow using the peristaltic pump (P2). Pressure pulsations during pump perfusion were suppressed with a damping chamber (D). Venous blood was collected from the right femoral vein (RFV) and returned to the animal through the left jugular vein (LJV). C, port for collection of venous blood during flow measurements. S, sampling port; I, infusions of drugs; P/S, port for addition of artificial plasma/saline.

outflow from the muscle, which was temporarily diverted to a measuring cylinder. The peristaltic pump (Cole Parmer, model 7521-25) was set to deliver approximately double the free-flow rate, and constant flow perfusion was started. The obturator nerve was crushed, and the distal part was placed over bipolar stimulating electrodes.

Systemic arterial blood pressure was measured in the left brachial artery with a Gould P23XL blood pressure transducer, and held constant at $104·8 \pm 6·5$ mmHg (mean ± S.E.M., $n = 16$) by connection of either the right brachial artery or the right carotid artery to a pressure bottle (Fig. 1). A mixture of artificial plasma and saline (up to 1·5 l per experiment) was added to the arterial

reservoir whenever necessary to maintain the blood volume there. Arterial perfusion pressure was recorded from the perfusion circuit using a similar P23XL transducer. Signals were recorded via Lectromed coupling units (model 4960) on two Lectromed MX216 recorders.

Rectal temperature was recorded by a thermistor probe and telethermometer (Yellow Springs Instruments), and maintained between 35 and 38 °C by heating coils under the table. Systemic arterial blood gases and pH were determined frequently using a Corning blood gas analyser (model 278). P_{O_2} was kept at 129·0 ± 6·9 mmHg by supplementing the inspired air with oxygen; P_{CO_2} was kept at 37·9 ± 1·1 by adjustments to the stroke volume, and pH was kept at 7·425 ± 0·018 by infusions of 1 M-$NaHCO_3$.

Analytical procedure. Plasma adenosine concentrations were determined by HPLC using methods similar to those described previously (Ballard, Cotterrell & Karim, 1986). Duplicate plasma samples (0·5 ml) were deproteinized with acetone and, after the addition of 10^{-9} mol methyl adenosine as an internal standard, extracted with chloroform and evaporated under nitrogen for 30 min at 0·5 °C to remove residual organic solvent. Samples were chromatographed on a 15 cm column containing 3 μm ODS packing (Supelco) with a gradient of methanol and 10 mM-KH_2PO_4, pH 5·5, using a Bio Rad series 700 HPLC system. The absorbance of separated peaks was monitored simultaneously at 254 nm for peak area quantification and 280 nm to allow identification of peaks by the absorbance ratio at the two wavelengths.

Experimental procedure. Control blood samples were collected from the artery and vein for adenosine and blood gas analysis. The distal end of the obturator nerve was stimulated at 10 V, 3 Hz for 10 min, and blood samples were again collected. Stimulation was then stopped, and the pressure was allowed to return to its control level. Lactic acid (15 mM solution in saline) was infused at 1·1 ml min^{-1} so as to produce a final plasma concentration of about 4 mM. Control samples were collected 5–8 min later when steady-state conditions for both intracellular and extracellular lactate concentration were expected to have been achieved (Mason & Thomas, 1988). Stimulation for 10 min was repeated, and blood samples were again collected. Stimulation and infusion were then stopped, and pressure allowed to return to its control value. Systemic arterial pH was checked, and corrected with bicarbonate infusion if necessary.

The relationship between rate of lactic acid infusion and adenosine release was then investigated. The infusion rate was increased in a stepwise fashion using rates of 0·15, 0·42, 1·1 and 2·2 ml min^{-1}. In each case, perfusion pressure was allowed to reach a new stable value (3–8 min) before arterial and venous blood samples were collected for adenosine and pH analysis, and the infusion rate was increased to the next value. After the last samples had been collected, the infusion was stopped and perfusion pressure was allowed to return to control. Systemic arterial pH was checked and corrected with bicarbonate infusion if necessary.

Finally, the effect of lactic acid on the vasoactivity of adenosine was tested. Adenosine doses (10^{-4}–10^{-2} M) were given as duplicate 1 ml bolus injections, firstly in the absence of infusion, then in the presence of a 0·84 ml min^{-1} infusion of 15 or 30 mM-lactic acid, and finally in the presence of a 0·84 ml min^{-1} saline infusion. Each adenosine dose–response curve was preceded by sufficient 1 ml saline injections to obtain a reproducible control response (three to six injections) and followed by the withdrawal of arterial and venous blood samples for pH and blood gas analysis.

When all experiments had been completed, the animal was killed by intravenous infusion of saturated potassium chloride and the gracilis muscle was removed and weighed.

RESULTS

The average resting flow to the gracilis muscle was 7·6 ± 2·0 ml min^{-1} 100 g^{-1} ($n = 16$) and the average muscle weight was 52·8 ± 5·3 g ($n = 16$). Muscles were perfused at a constant flow rate of 11·9 ± 2·2 ml min^{-1} 100 g^{-1} (170·2 ± 21·3 % of their resting free flow).

Results are expressed as means ± S.E.M.; n is the number of tests stated at the beginning of each series of results unless otherwise indicated. Student's t test was used to test the significance of results, except for adenosine concentrations which were not normally distributed, and were therefore compared using the non-parametric Wilcoxon signed ranks test.

Effect of lactic acid infusion on the response to obturator nerve stimulation

Stimulation was performed twice in each of eight dogs, once under control conditions and once in the presence of lactic acid. The average plasma concentration of lactic acid achieved during infusion was 4·21 ± 0·62 mM.

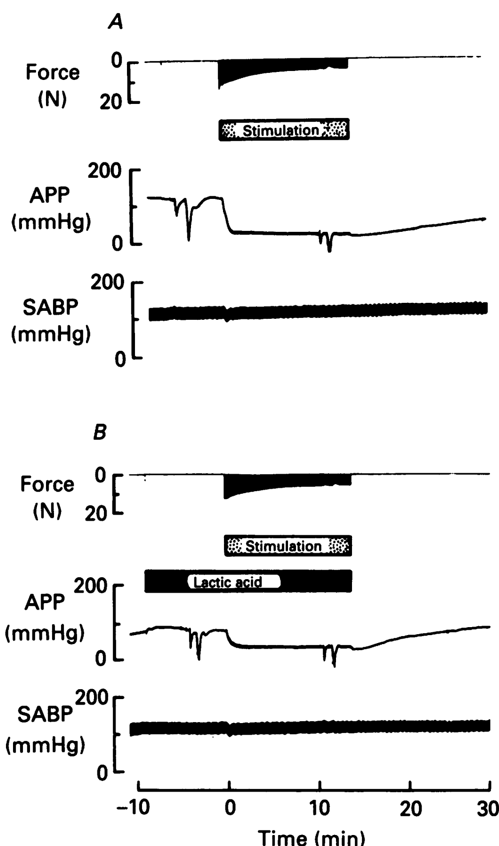

Fig. 2. Example of traces showing the response to muscle stimulation at 3 Hz in the absence (*A*) or presence (*B*) of lactic acid infusion. Stimulation (shown by the stippled bars) resulted in twitch contractions. Lactic acid infusion at 1·1 ml min^{-1} (shown by the filled bar) produced an arterial plasma concentration of 4·5 mM. The perfusion rate for this muscle was 4·9 ml min^{-1}, and the muscle weight was 33 g. APP, femoral arterial perfusion pressure; SABP, systemic arterial blood pressure.

Haemodynamic responses

Typical haemodynamic and force recordings are shown in Fig. 2. In the absence of infusion, stimulation caused the arterial perfusion pressure to decrease 50·0 ± 6·5 % from 137·6 ± 9·6 mmHg to a new stable value of 67·3 ± 7·5 mmHg over 3·04 ± 0·67 min. Pressure took 16·90 ± 3·32 min to return to a stable post-control value of 127·3 ± 12·2 mmHg after stimulation was stopped.

Infusion of lactic acid caused an immediate increase in perfusion pressure, followed by a decrease to a new stable value which was higher than control in five tests, lower

than control in one test, and unchanged in two tests. The mean perfusion pressure during lactic acid infusion was thus not significantly different from control (121·9 ± 10·0 mmHg before and 129·6 ± 10·7 mmHg during lactic acid infusion). Stimulation caused the perfusion pressure to decrease 44·1 ± 6·3 % to a new stable value of 71·4 ± 8·2 mmHg over 3·60 ± 0·88 min. When stimulation and infusion were stopped, pressure returned to 122·6 ± 9·0 mmHg over 20·51 ± 4·18 min.

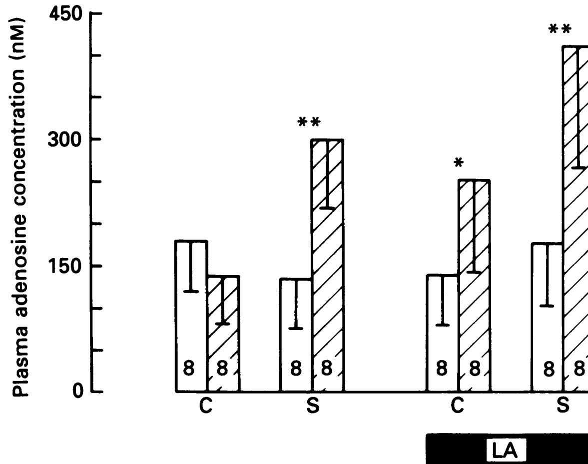

Fig. 3. Arterial (□) and venous (▨) plasma adenosine concentrations under control conditions (C) and during muscle stimulation (S) in the absence or presence (LA) of lactic acid infusion. Values are the means and S.E.M. of the number of tests shown on the bars. Asterisks denote a significant difference between arterial and venous concentrations in the Wilcoxon signed ranks test (*, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

Force of contraction

Acid infusion did not significantly alter the contractile force. In the absence of infusion, contractile force reached a peak value of 9·04 ± 0·95 N 100 g^{-1} in the first 2 min of stimulation, and decreased to a new stable value of 5·26 ± 0·61 N 100 g^{-1}; during lactic acid infusion, the peak tension was 7·55 ± 0·92 N 100 g^{-1} and the sustained tension was 5·25 ± 0·62 N 100 g^{-1}.

Blood gases and pH

Arterial and venous blood gases and pH were measured at rest and after 10 min stimulation in five animals. Lactic acid infusion reduced arterial pH from 7·398 ± 0·027 to 7·268 ± 0·024 ($P < 0{\cdot}01$) and increased arterial P_{CO_2} from 38·2 ± 2·8 to 43·0 ± 1·7 mmHg ($P < 0{\cdot}05$). Arterial P_{O_2} was unchanged by acid infusion (132·6 ± 9·5 and 134·6 ± 9·8 mmHg in the absence of infusion and in the presence of lactic acid respectively).

Stimulation had no effect on arterial blood gases or pH. Muscle contractions reduced venous pH from 7·366 ± 0·027 to 7·250 ± 0·031 in the absence of infusion, but

did not significantly alter it in the presence of lactic acid infusion (7·281 ± 0·026 at rest and 7·220 ± 0·030 during contractions). Stimulation in the absence of infusion increased venous P_{CO_2} from 43·0 ± 3·0 to 69·9 ± 6·8 mmHg ($P < 0·01$) and decreased venous P_{O_2} from 59·0 ± 5·7 to 23·1 ± 1·7 mmHg ($P < 0·001$). Changes in venous P_{CO_2} and P_{O_2} upon stimulation in the presence of acid infusion were not significantly different from those under control conditions.

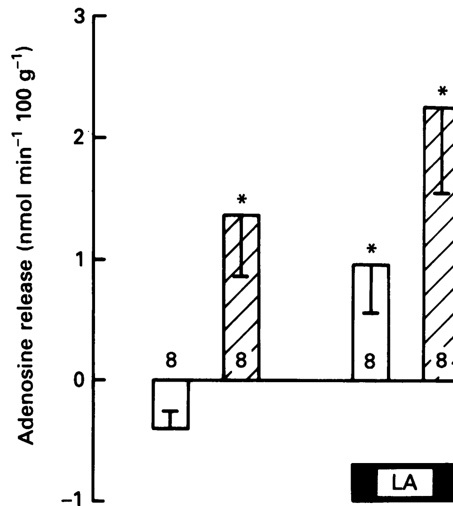

Fig. 4. Influence of lactic acid infusion (LA) and/or muscle stimulation on the adenosine release from isolated gracilis muscle. Values are the means and S.E.M. of the number of tests shown on the bars. Asterisks indicate where the adenosine release is significantly greater than control (*, $P < 0·01$, Student's t test). □, rest; ▨, stimulation.

The oxygen content of the blood was calculated from the haematocrit, P_{O_2} and pH. The rate of oxygen supply to the muscle was not altered by lactic acid infusion (3·71 ± 0·54 and 3·68 ± 0·52 ml min^{-1} 100 g^{-1}). The oxygen consumption by the muscle increased during contractions from 0·62 ± 0·24 to 2·76 ± 0·46 ml min^{-1} 100 g^{-1} in the absence of infusion, and from 0·80 ± 0·19 to 2·53 ± 0·37 ml min^{-1} 100 g^{-1} during lactic acid infusion. The ratio of oxygen supply:consumption (an index of tissue oxygenation) during muscle contractions was 1·39 ± 0·11 or 1·49 ± 0·20 in the absence or presence respectively of lactic acid infusion (no significant difference).

Plasma adenosine concentrations

Mean arterial and venous plasma adenosine concentrations are shown in Fig. 3. The arterial adenosine concentration was not altered by stimulation or lactic acid infusion (average value 156·5 ± 30·1 nM, $n = 32$). There was no significant difference between the arterial and venous adenosine concentration under control conditions. However, the venous adenosine concentration was increased to a value significantly greater than arterial by either lactic acid infusion ($P < 0·05$) or obturator nerve stimulation ($P < 0·01$) or both ($P < 0·01$).

Adenosine release was calculated from the veno-arterial concentration difference,

the flow rate and the muscle weight, and is shown in Fig. 4. Under control conditions there was a small net uptake of adenosine by the muscle (negative value for release), whereas lactic acid infusion or stimulation led to an adenosine release which was significantly greater than the control value ($P < 0.01$). However, the adenosine release during stimulation in the presence of lactic acid was not significantly greater

TABLE 1. Effect of lactic acid infusion on arterial and venous pH

Infusion rate (ml min^{-1})	Arterial pH	P	Venous pH	P
0	7·398 ± 0·027	—	7·366 ± 0·027	—
0·15	7·327 ± 0·023	n.s.	7·306 ± 0·029	n.s.
0·42	7·320 ± 0·018	< 0·05	7·301 ± 0·026	n.s.
1·1	7·278 ± 0·026	< 0·05	7·271 ± 0·025	< 0·05
2·2	7·280 ± 0·023	< 0·01	7·249 ± 0·023	< 0·05

Values are the means ± S.E.M. of five tests. Student's t test was used to determine whether pH values differed significantly from control.

than that at rest during acid infusion, although it was greater than that in the non-infusion control ($P < 0.01$).

Effect of different rates of lactic acid infusion

Six tests were performed in six dogs. Lactic acid infusion produced arterial plasma concentrations of 0·73 ± 0·19 mM at 0·15 ml min^{-1}, 1·83 ± 0·43 mM at 0·42 ml min^{-1}, 3·86 ± 0·77 mM at 1·1 ml min^{-1} and 5·95 ± 0·99 mM at 2·2 ml min^{-1}.

Haemodynamic responses

Arterial perfusion pressure was 132·0 ± 17·3 mmHg under control conditions; it did not change significantly during lactic acid infusion (135·9 ± 18·7, 141·9 ± 17·1, 143·9 ± 17·8 and 144·3 ± 18·7 mmHg at infusion rates of 0·15, 0·42, 1·1 and 2·2 ml min^{-1} respectively).

pH

Arterial and venous pH was determined at each infusion rate in five of the animals. Values are shown in Table 1.

Plasma adenosine concentrations

The arterial plasma adenosine concentration was not altered by lactic acid infusion at any of the rates employed (average value 94·0 ± 12·8 nM, $n = 30$). Venous adenosine concentrations are shown in Fig. 5. Arterial and venous adenosine concentrations were not significantly different under control conditions; infusion of lactic acid at rates of 0·42 ml min^{-1} or above produced a significant elevation of venous adenosine concentration ($P < 0.05$). The adenosine release from the muscle was also significantly greater than control at these infusion rates (Fig. 6).

The adenosine release during lactic acid infusion and/or obturator nerve stimulation was plotted against the venous pH (Fig. 7). A strong correlation existed between the two parameters ($r = -0.92$); points obtained during muscle stimulation fell on the same correlation line as those obtained during acid infusion.

Effect of lactic acid on the vasoactivity of adenosine

Eight tests were performed in eight dogs. The average plasma concentration of lactic acid achieved during infusion was 7·16 ± 0·76 mM.

Haemodynamic responses

In the absence of infusion, the arterial perfusion pressure was 132·2 ± 12·0 mmHg. Lactic acid infusion reduced perfusion pressure from 134·5 ± 9·9 to

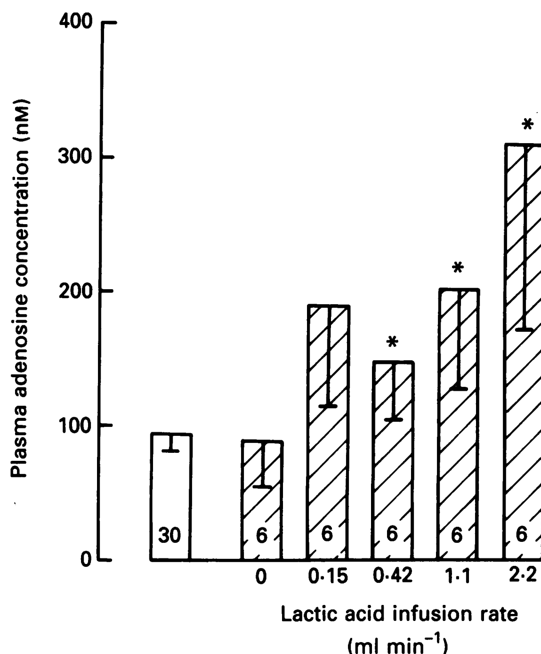

Fig. 5. The effect of different rates of lactic acid infusion on plasma adenosine concentrations. Values are the means and S.E.M. of the number of tests shown on each bar. The mean arterial concentration (□) includes the pooled data from all tests, since the arterial adenosine concentration did not change. Venous concentrations (▨) were compared to their own arterial concentrations in the Wilcoxon signed ranks test. *, significant difference ($P < 0·05$).

124·0 ± 7·6 mmHg ($P < 0·05$, paired t test), but saline infusion did not alter it significantly (145·9 ± 17·0 mmHg before and 142·2 ± 16·6 mmHg during infusion). The responses to close-arterial injection of adenosine or the saline vehicle are shown in Fig. 8; the per cent change in perfusion pressure in response to adenosine injection did not differ significantly from control in the presence of either lactic acid or saline infusion.

pH

Arterial pH was 7·375 ± 0·033 under control conditions. Lactic acid infusion reduced it to 7·281 ± 0·046 ($P < 0·05$, paired t test), but saline infusion produced no significant change (7·382 ± 0·027). Venous pH was 7·278 ± 0·048 under control

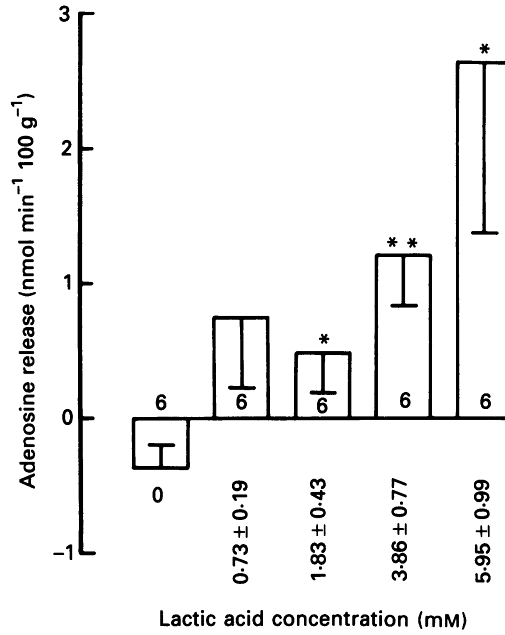

Fig. 6. Adenosine release at different rates of lactic acid infusion. Values are the means and S.E.M. of the number of tests shown on the bars. Asterisks indicate where the adenosine release is significantly greater than control in Student's t test (*, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

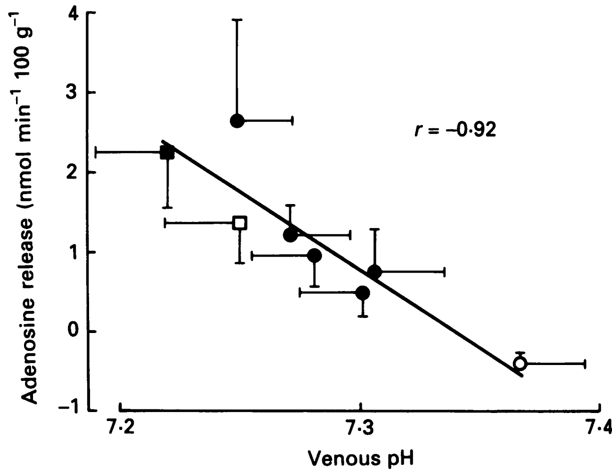

Fig. 7. Relationship between venous pH and adenosine release. Error bars indicate the S.E.M. for measurements of pH and adenosine release. Points obtained during lactic acid infusion and/or muscle stimulation fell on a single correlation line, with a regression coefficient of −0·92. ○, control; ●, lactic acid infusion; □, muscle stimulation; ■, lactic acid infusion and muscle stimulation.

conditions, 7·223±0·065 during lactic acid infusion and 7·268±0·047 during saline infusion.

DISCUSSION

The main findings of this study were that lactic acid infusion into an isolated gracilis muscle resulted in a dose-dependent release of adenosine from the muscle (Fig. 6), and that the adenosine release caused by nerve stimulation and/or lactic acid infusion was strongly correlated with the venous pH (Fig. 7). These data support a role for pH in the control of adenosine release from skeletal muscle.

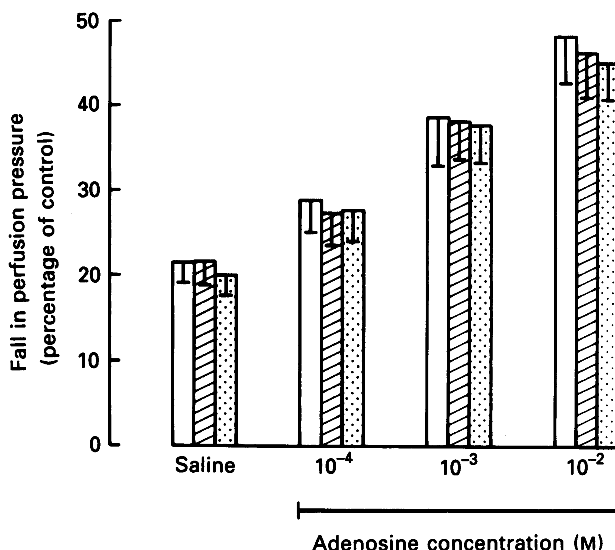

Fig. 8. Comparison between the decrease in femoral arterial perfusion pressure produced by close-arterial injection of adenosine in the absence of infusion (□) or in the presence of an infusion of either saline (⚄) or 7·2 mM-lactic acid (▨). Values are the means and S.E.M. of eight tests. Adenosine, at the concentrations shown, or the saline vehicle was delivered as a 1 ml bolus. Neither saline nor lactate infusion significantly altered the response to any given dose of adenosine.

Obturator nerve stimulation reduced venous pH from 7·36 to 7·25 and increased adenosine release from −0·40 (net uptake) to 1·36 nmol min^{-1} 100 g^{-1}. Infusion of 4·2 mM-lactic acid produced similar values for both venous pH (7·28) and adenosine release (0·96 nmol min^{-1} 100 g^{-1}). Stimulation at the same time as lactic acid infusion produced a venous pH of 7·22 and an adenosine release of 2·25 nmol min^{-1} 100 g^{-1}; neither of these values differed significantly from those at rest during acid infusion if evaluated using Student's *t* test, but adenosine release did increase upon stimulation in seven of the eight tests, and the small decrease in pH was significant ($P < 0.05$) in a paired *t* test. In other words, when muscle contractions produced little or no decrease in pH, then there was also little or no increase in adenosine release. Lactic acid infusion did not alter the force production by the muscle or the

oxygenation of the muscle (ratio of oxygen supply to oxygen consumption). This suggests that the pH change during muscle contractions may be the factor which normally brings about the adenosine release.

Lactic acid infusion produced dose-dependent increases in the adenosine release from the muscle. Points obtained during lactic acid infusion and during muscle stimulation fell on the same correlation line (Fig. 7), even though the pH change during muscle contractions is mainly caused by an increased release of carbon dioxide. Furthermore, infusion of sufficient hydrochloric acid to reduce arterial pH to 7·23 caused a small increase in venous adenosine concentration to 218 ± 67 nM ($n = 10$; Ballard, 1990). Thus it is likely that the adenosine release resulted from the pH change *per se* rather than from some specific effect of lactic acid.

It has previously been reported that flow restriction during muscle contractions increased the adenosine content of the muscle (Berne *et al.* 1971; Dobson, Rubio & Berne, 1971; Belloni *et al.* 1979; Phair & Sparks, 1979) or the adenosine release into the venous effluent (Berne *et al.* 1971; Dobson *et al.* 1971; Bockman, Berne & Rubio, 1975). The flow restriction was thought to have caused hypoxia, which in turn led to the adenosine release. However, a later study (Ballard *et al.* 1989) concluded that hypoxia could not have caused the adenosine release, since an increase in the blood flow rate could not abolish it, and no relationship could be found between the amount of adenosine released and indices of muscle oxygenation. A re-examination of the results of that study indicated that the amounts of adenosine released at rest and during contractions at different flow rates did correlate well with the venous pH ($r = -0{\cdot}94$). It is likely, therefore, that the large amounts of adenosine which are released during flow-restricted contractions result from the large decrease in pH which occurs. Thus severe hypoxia can stimulate adenosine release because it can cause lactic acid release and contribute to the decrease in pH, but flow restriction can also stimulate adenosine release independently of any hypoxia, since the failure to remove carbon dioxide from the tissue would also depress pH.

A reduction in pH to 6·8 by CO_2 has previously been reported to cause an increase in adenosine release from Langendorf-perfused guinea-pig hearts (Mustafa & Mansour, 1984), whereas both cardiac function and adenosine release were depressed when a similar pH change was induced by HCl infusion (Bardenheuer, Whelton & Sparks, 1987). Similarly, the adenosine released from cultured vascular smooth muscle is reported to be increased by raising the CO_2 but reduced by lowering the pH (Belloni, Bruttig, Rubio & Berne, 1986). CO_2 would have depressed intracellular pH more rapidly than HCl, since it can easily diffuse through the cell membrane. One possible explanation for these observations is that the increase in adenosine release observed during CO_2 treatment was caused by a reduction in intracellular pH, whereas HCl treatment could not depress intracellular pH within the time frame of the test, and so failed to stimulate adenosine release. Lactic acid can cross muscle cell membranes rapidly, both by diffusion (Roos, 1975; Mason, Mainwood & Thoden, 1986) and via a carrier for L-lactate and proton equivalents (Mason & Thomas, 1988), and was likely to have depressed intracellular pH by around 0·1 units in the present experiments (Mason & Thomas, 1988). HCl would enter the cell more slowly, and was found in skeletal muscle, also, to be less effective than lactic acid in causing adenosine release, even though it produced a larger fall in pH (Ballard, 1990). The present

results are therefore consistent with the suggestion that it is an intracellular pH reduction which stimulates adenosine release. However, the mechanism by which such a reduction in intracellular pH might stimulate adenosine release remains unclear.

The change in perfusion pressure in response to lactic acid infusion was quite variable; pressure increased in some muscles but decreased in others. This probably occurred because the infusion rates for lactic acid (up to 2·2 ml min^{-1}) were high in relation to the perfusion rate (range 3·3–16·8 ml min^{-1}) for some of the muscles. The overall change in perfusion pressure was the resultant sum of an increase in pressure due to the increased flow rate, a decrease in pressure due to vasodilatation caused by the decrease in pH and any myogenic effects resulting from the flow change. Since pilot experiments indicated that a lower infusion rate coupled with a higher lactic acid concentration caused some red cell damage, and the release of intracellular constituents could have greatly influenced the observed adenosine concentrations, it seemed preferable to sacrifice observations on the vasodilator properties of lactic acid (which are already well documented, e.g. McDowall, 1928), in order to obtain an accurate determination of the effects of lactic acid on plasma adenosine concentrations.

Infusion of lactic acid did not alter the vasoactivity of adenosine in these experiments. A reduction in pH of $\geqslant 0{\cdot}2$ units has previously been reported to enhance adenosine-induced vasodilatation in the coronary circulation (Mustafa & Ghai, 1981; Downing, Lee & Weinstein, 1982). However, the decrease in venous pH was limited to about 0·1 units in the present experiments, in order to be comparable with the change in pH which occurred during muscle contractions. Thus enhancement of adenosine-induced vasodilatation in the skeletal muscle circulation may result from larger reductions in pH, but were unlikely to have occurred in the present experiments.

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REFERENCES

BALLARD, H. J. (1990). The role of pH in the release of adenosine from skeletal muscle in anaesthetized dogs. *Journal of Physiology* **429**, 78*P*.

BALLARD, H. J., COTTERRELL, D. & KARIM, F. (1986). Analysis of submicromolar concentrations of adenosine in plasma using reversed phase high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis* **4**, 207–219.

BALLARD, H. J., COTTERRELL, D. & KARIM, F. (1987). Appearance of adenosine in venous blood from the contracting gracilis muscle and its role in vasodilatation in the dog. *Journal of Physiology* **387**, 401–403.

BALLARD, H. J., COTTERRELL, D. & KARIM, F. (1989). The influence of blood flow rate on adenosine release from contracting dog skeletal muscle. *Quarterly Journal of Experimental Physiology* **74**, 97–107.

BARDENHEUER, H., WHELTON, B. & SPARKS, H. V. (1987). Adenosine release by the isolated guinea pig heart in response to isoproterenol, acetylcholine, and acidosis: the minimal role of vascular endothelium. *Circulation Research* **61**, 594–600.

BELLONI, F. L., BRUTTIG, S. P., RUBIO, R. & BERNE, R. M. (1986). Uptake and release of adenosine by cultured rat aortic smooth muscle. *Microvascular Research* **32**, 200–210.

BELLONI, F. L., PHAIR, R. D. & SPARKS, H. V. (1979). Role of adenosine in prolonged vasodilation following flow-restricted exercise of canine skeletal muscle. *Circulation Research* **44**, 759–766.

BERNE, R. M., RUBIO, R., DOBSON, J. G. & CURNISH, R. R. (1971). Adenosine and adenine nucleotides as possible mediators of cardiac and skeletal muscle blood flow regulation. *Circulation Research* **28–29**, suppl. 1, 115–119.

BOCKMAN, E. L., BERNE, R. M. & RUBIO, R. (1975). Release of adenosine and lack of release of ATP from contracting skeletal muscle. *Pflügers Archiv* **355**, 229–241.

BOCKMAN, E. L., BERNE, R. M. & RUBIO, R. (1976). Adenosine and active hyperemia in dog skeletal muscle. *American Journal of Physiology* **230**, 1531–1537.

DOBSON, J. G., RUBIO, R. & BERNE, R. M. (1971). Role of adenine nucleotides, adenosine and inorganic phosphate in the regulation of skeletal muscle blood flow. *Circulation Research* **29**, 375–384.

DOWNING, S. E., LEE, J. C. & WEINSTEIN, E. M. (1982). Coronary dilator actions of adenosine and CO_2 in experimental diabetes. *American Journal of Physiology* **243**, H252–258.

FUCHS, B. D., GORMAN, M. W. & SPARKS, H. V. (1981). Adenosine release from skeletal muscle during free flow exercise. *Physiologist* **24**, 76.

GOONEWARDENE, I. P. & KARIM, F. (1989). Adenosine deaminase attenuates exercise vasodilatation. *Proceedings of the International Union of Physiological Sciences* **17**, 64.

KARIM, F., BALLARD, H. J. & COTTERRELL, D. (1988). Changes in adenosine release and blood flow in the contracting dog gracilis muscle. *Pflügers Archiv* **412**, 106–112.

KILLE, J. M. & KLABUNDE, R. E. (1984). Adenosine as a mediator of postcontraction hyperemia in dog gracilis muscle. *American Journal of Physiology* **246**, H274–282.

McDOWALL, R. J. S. (1928). The influence of acid base equilibrium on the activities of blood vessels. *Journal of Physiology* **65**, 25–31.

MASON, M. J., MAINWOOD, G. W. & THODEN, J. S. (1986). The influence of extracellular buffer concentration and propionate on lactate efflux from frog muscle. *Pflügers Archiv* **406**, 472–479.

MASON, M. J. & THOMAS, R. C. (1988). A microelectrode study of the mechanisms of L-lactate entry into and release from frog sartorius muscle. *Journal of Physiology* **400**, 459–479.

MUSTAFA, S. J. & GHAI, G. (1981). Effect of adenosine on the relaxation of coronary arteries at varying pH values. *Basic Research in Cardiology* **76**, 380–386.

MUSTAFA, S. J. & MANSOUR, M. M. (1984). Effect of perfusate pH on coronary flow and adenosine release in isolated rabbit heart. *Proceedings of the Society for Experimental Biology and Medicine* **176**, 22–26.

PHAIR, R. D. & SPARKS, H. V. (1979). Adenosine content of skeletal muscle during active hyperemia and ischemic contraction. *American Journal of Physiology* **237**, H1–9.

ROOS, A. (1975). Intracellular pH and distribution of weak acids across cell membranes. A study of D- and L-lactate and of DMO in rat diaphragm. *Journal of Physiology* **249**, 1–25.