Mechanism of lactate-induced relaxation of isolated rat mesenteric resistance arteries

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1. The effects of the sodium salt of the weak acid lactate on tension and intracellular pH (pH_i) were studied in rat mesenteric small arteries mounted on a wire myograph. Sodium lactate was substituted iso-osmotically for sodium chloride.

2. At a concentration of 50 mM, both L- and D-stereoisomers of lactate markedly relaxed arteries preconstricted with noradrenaline (NA) within 10 min. The concentration–response relationship for L-lactate showed that the NA contracture was relaxed by 50% at approximately 26 mM. L-Lactate did not, however, relax arteries preconstricted with high-K^+ (45 mM) solution.

3. L-Lactate did not alter extracellular pH (pH_o) but caused a small but significant decrease in pH_i, measured using the pH-sensitive fluorochrome, 2′,7′-bis(carboxyethyl)-5-(6′)-carboxyfluorescein (BCECF). Relaxation to L-lactate was unaffected when this change in pH_i was offset by the simultaneous addition of NH_4Cl to the solution.

4. Sodium pyruvate (50 mM) caused a significant intracellular acidosis but did not relax arteries preconstricted with NA.

5. L-Lactate-induced relaxations were unaffected by removal of the endothelium or when the synthesis of nitric oxide (NO) was inhibited by 10^{-4} M N^{ω}-nitro-L-arginine methyl ester (L-NAME).

6. The potassium channel blockers glibenclamide (10 μM), 4-aminopyridine (3 mM) and tetraethylammonium chloride (10 mM) did not affect L-lactate-induced relaxation in arteries preconstricted with NA. Inhibition of guanylate cyclase with Methylene Blue, or cyclooxgenase with indomethacin, also did not affect relaxation to L-lactate.

7. The R_p stereoisomer of adenosine-3′,5′-cyclic monophosphothioate (R_p-cAMPS), an analogue of cAMP which inhibits competitively stimulation of protein kinase A, reduced significantly L-lactate-induced relaxation at a concentration of 25 μM. R_p-cAMPS also significantly reduced forskolin-induced relaxation of the NA contracture.

8. It is concluded that L-lactate-induced relaxation in this vascular bed is pH_i-, endothelium-, and nitric oxide-independent. It is not mediated by inhibition of voltage-gated Ca^{2+} channels, opening of K^+ channels, prostacylin or cyclic GMP. cAMP may however play a role in L-lactate-induced relaxation.

Lactic acidosis is commonly associated with circulatory failure, lactate being synthesized as a result of anaerobic respiration. The situation is further compounded by reduced lactate disposal, as the organs responsible (kidneys, liver, heart) are themselves poorly perfused (Tashkin, Goldstein & Simmons, 1972). Lactic acid induces relaxation of vascular smooth muscle (Hilton & Eicholtz, 1925; Kurtz & Leake 1926; Mohme-Lundholm, 1957; Omar, Figueroa, Tejani & Wolin, 1993*a*; Omar, Mohazzab, Mortelliti & Wolin, 1993*b*), so further reducing peripheral vascular resistance and contributing to circulatory failure.

There is some evidence that weak acids cause vascular relaxation by decreasing intracellular pH (pH_i) (Austin & Wray, 1993, 1994). However, we have reported that intracellular acidosis may occur without vascular relaxation (Carr, Graves & Poston, 1993), and acute acidosis is often concurrent with a transient rise in tension (Aalkjaer &

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Cragoe, 1988; Aalkjaer & Mulvany, 1988; Aalkjaer & Hughes, 1991; Matthews, Graves & Poston, 1992; Jensen, Hughes, Boonen & Aalkjaer, 1993). The interplay between intracellular acidosis and tension in smooth muscle is multifactorial; acidification may reduce Ca^{2+} influx through voltage-gated channels (Klöckner & Isenberg, 1994), inhibit K^+ channel opening (Kume, Takagi, Satake, Tokuno & Tomita, 1990), cause intracellular Ca^{2+} release (Battle, Peces, LaPointe, Ye & Daugirdas, 1993), and lead to complex and tissue-specific effects on the sensitivity of the contractile apparatus to $[Ca^{2+}]_i$ (Crichton, Templeton & Smith, 1994). In order to examine the mechanisms underlying relaxation induced by the Na^+ salt of L-lactate we have simultaneously measured tension development and pH_i in rat mesenteric resistance arteries. By 'clamping' pH_i with NH_4Cl, it was possible to investigate the relationship between relaxation and pH_i. In view of an earlier report from our laboratory (Carr *et al.* 1993) describing the endothelium dependence of hypercapnia-induced relaxation, we have in addition, determined whether the endothelium or nitric oxide plays a role in the response. The mechanism of lactate-induced relaxation was also investigated using a variety of pharmacological inhibitors of possible contributory pathways.

METHODS

Preparation of arteries

Male Wistar rats (weight 250–300 g) were killed by cervical dislocation. The mesentery was removed and resistance arteries (approximate i.d. 300 μM) were dissected free from the surrounding tissue and mounted as isometric preparations on either a Mulvany-Halpern myograph (J. P. Trading, Århus, Denmark; for the measurement of tension alone) or a computer-controlled myograph (Cambustion Ltd, Cambridge, UK; for the simultaneous determination of tension and pH_i). The arteries were bathed in physiological saline solution (PSS), containing (mM): NaCl, 119; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 0·289; $NaHCO_3$, 25; KH_2PO_4, 1·18; EDTA, 0·026; glucose, 5·5; and gassed with 5% CO_2–95% O_2. The arteries were set to a circumference 90% of that obtained when the vessels were stretched to a transmural pressure of 13·4 kPa (Mulvany & Halpern, 1977). Prior to experiments, arteries were subjected to a routine 'run-up' procedure consisting of one contraction to K^+ substituted PSS (equimolar substitution of KCl for NaCl), one to 5 μM noradrenaline (NA), and three to 5 μM NA and KCl substituted PSS.

pH_i measurement

Following mounting on the myograph, arteries were incubated for 1 h in PSS containing 5 μM 2′,7′-bis(carboxyethyl)-5-(6′)-carboxy-fluorescein acetoxy methyl ester (BCECF-AM, Calbiochem-Novabiochem). The myograph was mounted on the stage of a Nikon TMD inverted microscope with an epifluorescence attachment. BCECF was alternatively excited with light of wavelength 490 and 430 nm at 64 Hz and emitted light from the excited dye was monitored at 550 nm using a barrier filter and a photomultiplier tube, the output of which was digitized, stored, and displayed on a microcomputer using a spectrophotometer system (Cairn Ltd, Sittingbourne, UK). The 490:430 nm ratio was then used to calculate the pH_i, following calibration (Thomas, Buchsbaum, Zimniak & Racker, 1979) in each artery using a 140 mM potassium–10 μM nigericin medium in which this ratio was recorded at a minimum of six pH levels (pH adjusted with 0·1 M potassium hydroxide). The linear regression coefficient of the pH_i *vs.* the 490:430 nm ratio was greater than 0·95 for all arteries.

Assessment of control and weak-acid-mediated relaxation

Preliminary experiments showed that weak-acid-induced relaxation of the NA contracture was most consistent when the response to NA was approximately half-maximal; conversely, the effect of weak acids on maximal NA responses tended to vary between different arteries. For this reason, an initial NA concentration–response analysis was performed for each artery and a concentration sufficient to produce approximately 50% of the maximum contractile response was selected. Following this procedure, we assessed the intrinsic time-dependent change (usually a decline) of the NA response at this concentration by contracting the artery with NA for 13 min. At the end of the 3rd minute of this period, the NA-containing solution was replaced with an identical NA-containing solution which was present over the remaining 10 min of NA exposure. The decline of the NA response during this 10 min period is referred to in the text as the 'time control' relaxation. In order to then assess the effect of the weak acid on the NA response, NA was reapplied, and after 3 min the solution was replaced with one containing the identical concentration of NA, as well as 50 mM sodium lactate or sodium pyruvate (substituted for NaCl), for a further 10 min. Responses to the L- and D-stereoisomers of lactate were compared, and the effects of a number of pharmacological agents on the response to L-lactate were investigated. In experiments where high-K^+ (45 mM) solution was used instead of NA to constrict the artery, appropriate time controls were carried out to assess the intrinsic time-dependent alteration in the response to high K^+ between the 3rd and 13th minutes of the contracture; this was usually a slight rise. This high K^+ (45 mM, substituted for Na^+) was also the concentration which produced an approximately half-maximal response.

Removal of endothelium or inhibition of nitric oxide (NO) synthase

De-endothelialization was achieved by passing a human hair through the lumen of the mounted vessel several times. The presence of an intact endothelium was assessed by the demonstration of greater than 80% relaxation to 10^{-6} M acetylcholine (ACh) in vessels preconstricted with 1 μM NA. Successful de-endothelization was demonstrated by less than 10% ACh-induced relaxation. In other experiments, NO synthase was inhibited by incubating the vessel with 10^{-4} M N^{ω}-nitro-L-arginine methyl ester (L-NAME, Sigma), for 20 min. All subsequent solutions also contained L-NAME.

Chemicals and drugs

R_p-cAMPS and BCECF were obtained from Calbiochem-Novabiochem. Methylene Blue was obtained from the pharmacy at St Thomas' Hospital. All other chemicals were obtained from Sigma.

Statistical analysis

In each artery, the change in tension during the 10 min exposure to the weak acid was compared with that measured during the final 10 min of the time-control contracture. Data were compared using Student's paired t test, and a value of $P < 0·05$ was considered to indicate a significant effect. The term 'n.s.' in the text indicates the lack of a significant difference between two sets of data.

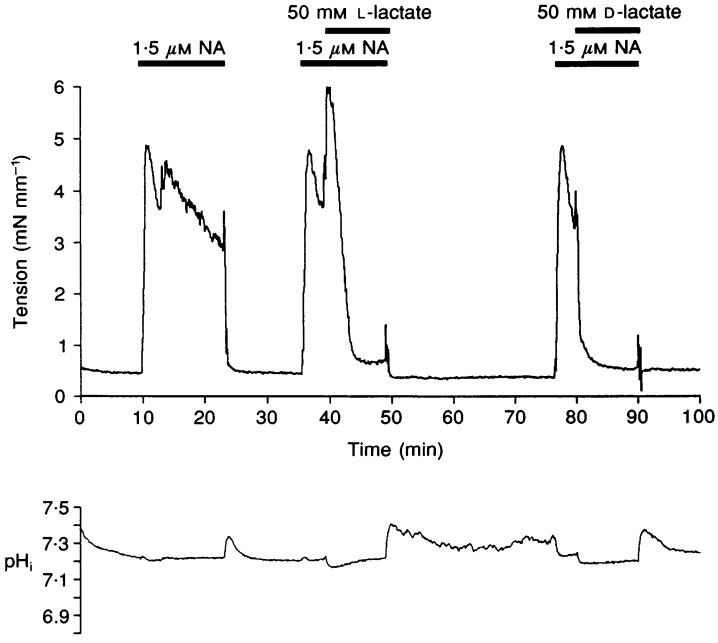

Figure 1. Relaxation of a rat mesenteric resistance artery contracted with noradrenaline by the L- and D-stereoisomers of lactate

Experimental traces showing tension (in mN mm^{-1}) elicited by 1·5 μM NA alone (time control) and in the presence of the L- and D-stereoisomers of lactate (added 3 min after NA), as indicated by the bars. The lower trace shows pH_i, measured simultaneously. In this and other figures, NA-containing solution was reapplied during the time control 3 min after the NA response was initiated, in order to mimic the solution change which occurred when lactate was applied during subsequent NA contractures.

RESULTS

Relaxation induced by lactate

The pH of the bathing media was unaffected by the addition of the sodium salts of L- or D-lactate. When added during a NA contracture, both stereoisomers caused a progressive and significant relaxation (Fig. 1). Measurement of tension at the end of the 10 min exposure revealed that 50 mM L-lactate induced a relaxation of 89·4 ± 1·5% compared with 17·7 ± 2·8% in time controls ($n = 12$, $P = < 0{\cdot}001$). D-Lactate caused a similar relaxation of the NA contracture (relaxation to D-lactate 90·6 ± 5·9%, compared with 13·2 ± 2·2% in time controls ($P < 0{\cdot}01$, $n = 3$), which was not significantly different in magnitude to the relaxation induced by L-lactate in the same arteries (93·0 ± 1·7%, $n = 3$, $P < 0{\cdot}01$; Fig. 1). However, D-lactate induced a more rapid relaxation than L-lactate. The time taken for the tension to decay by 50% was 7·0 ± 0·6 min in L-lactate and 3·7 ± 0·2 min in D-lactate ($n = 3$, $P < 0{\cdot}05$).

The concentration–response relationship for the relaxation of the NA contracture resulting from a 10 min exposure to L-lactate is illustrated in Fig. 2. There was an approximately linear relationship between the L-lactate concentration and relaxation. NA-induced tension was significantly reduced by 6 mM L-lactate, and was inhibited by 50% by approximately 26 mM L-lactate.

A concentration of 50 mM L-lactate did not, however, cause relaxation if arteries were preconstricted with high K^+ (45 mM), as shown in Fig. 3. Tension in L-lactate increased by 11·4 ± 2·4% over 10 min, while tension increased by 8·5 ± 1·5%; in time controls ($n = 3$, n.s.).

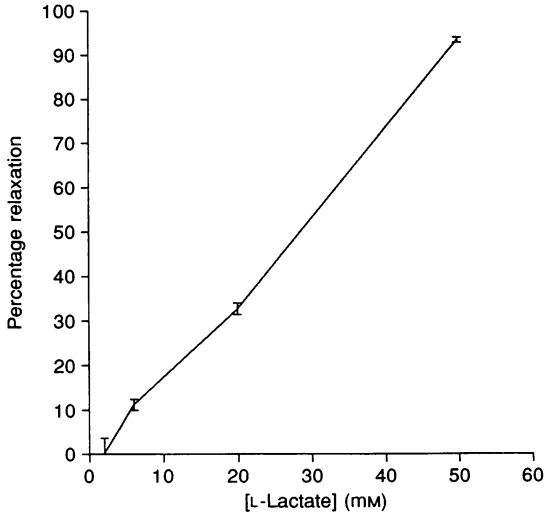

Figure 2. The concentration–response relationship for L-lactate-mediated relaxation of the NA contracture

Arteries were stimulated with the concentration of NA producing a half-maximal response and then after 3 min L-lactate was added. The degree of relaxation was assessed after a further 10 min. NA and L-lactate were then washed out of the bath and the artery was equilibrated with PSS for 10 min prior to reapplication of NA and then lactate. Each artery was exposed to 2, 6, 20 and 50 mM L-lactate. Time controls were also carried out in each artery and percentage relaxation to L-lactate was calculated taking into account the relaxation in its presence, over and above the intrinsic decay in the NA contracture measured in the time controls.

Effects of pH_i 'clamp' and sodium pyruvate

NA induced a small decrease in pH_i, from 7·30 ± 0·06 to 7·26 ± 0·06 ($n = 21$, $P < 0·02$). The addition of 50 mM L-lactate subsequently caused a further slight fall in pH_i to 7·23 ± 0·06 ($n = 21$) which was highly significant when data from all arteries were analysed ($P < 0·001$). The lactate-induced decrease in pH_i tended to be transient, as shown in Fig. 1.

In order to determine whether this change in pH_i played a role in L-lactate-mediated relaxation, NH_4Cl was used to offset the acidification, as shown in Fig. 4. The pH_i change induced by L-lactate was noted and after washout of L-lactate, various concentrations of NH_4Cl were added to the bath until a concentration which produced an alkalinization equal to the L-lactate-induced acidification was found. The arteries were again pre-constricted with NA for 3 min, and L-lactate was added, together with the appropriate concentration of NH_4Cl. Using NH_4Cl the maximum relaxation to L-lactate was not affected (89·6 ± 6·7% without NH_4Cl *vs.* 91·7 ± 5·1% with NH_4Cl, $n = 3$, n.s.). pH_i in these experiments was 7·12 ± 0·10 prior to NA addition, 7·08 ± 0·13 after the addition of NA, and 7·14 ± 0·11 in the presence of NH_4Cl, NA and lactate.

We also explored the effects of the Na^+ salt of a second weak acid, pyruvate. In three arteries preconstricted with NA, 50 mM pyruvate caused a significant decrease in pH_i (7·31 ± 0·03 to 7·23 ± 0·03, $n = 3$, $P < 0·001$). Pyruvate did not, however, cause relaxation. In arteries from four rats, pyruvate had little effect on the NA contracture over 10 min, as illustrated in Fig. 5, while in one rat pyruvate enhanced the NA contracture (relaxation of time control 27·5 ± 7·9%; relaxation in pyruvate 7·5 ± 24·2%, $n = 5$, n.s.). In these arteries, 50 mM L-lactate caused a relaxation of 89·5 ± 6·5% ($P < 0·02$, $n = 5$, compared with pyruvate).

Effects of potassium channel blockers

As shown above, L-lactate produced a relaxation of the NA contracture, but not that elicited by high K^+. By analogy with similar results observed for K^+ channel openers such as minoxidil (Meisheri, Cipkus & Taylor, 1988), it seemed possible that L-lactate might be acting to open K^+ channels and causing relaxation through hyperpolarization. We therefore, examined whether the L-lactate-induced relaxation was inhibited by drugs known to block three major classes of K^+ channels. These included 10 mM tetraethylammonium (TEA), a blocker of large-conductance Ca^{2+}-activated K^+ channels, 3 mM 4-aminopyridine (4-AP), a blocker of the delayed rectifier K^+ channel, and glibenclamide (10 μM) a blocker of the ATP-sensitive K^+ channel.

None of these agents had any effect upon 50 mM L-lactate-induced relaxation. The lactate-mediated relaxation was 91·6 ± 2·3% ($n = 3$) in the presence of TEA, 90·2 ± 1·8% ($n = 3$) in 4-AP, and 88·6 ± 2·0 ($n = 3$) in glibenclamide; none of these differed significantly from the relaxations observed in these arteries in the absence of the agent. These data suggested that the opening of K^+ channels was not necessary for the L-lactate-induced relaxation.

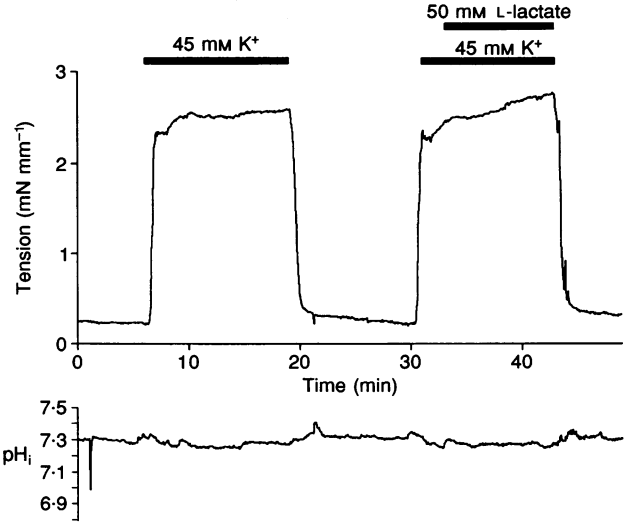

Figure 3. Effect of L-lactate on the contraction elicited by high-K^+ solution

Arteries were preconstricted with a concentration of K^+ sufficient to give an approximately half-maximal contracture (45 mM) and then exposed to 50 mM L-lactate after 3 min. L-Lactate did not significantly affect the response to high K^+.

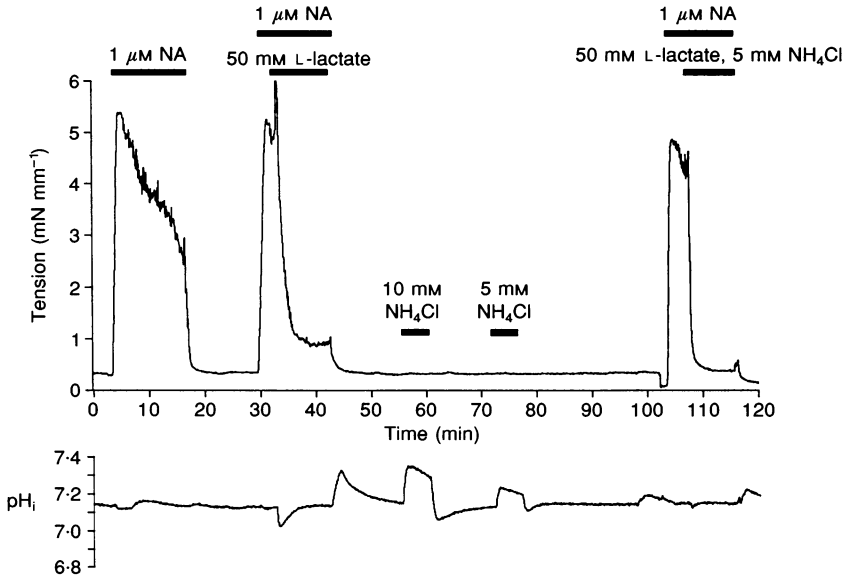

Figure 4. The effect on relaxation of offsetting the intracellular acidosis induced by L-lactate by the addition of ammonium chloride

Addition of 50 mM L-lactate during a NA contracture produced a typical relaxation and acidosis, the extent of which was estimated using the displacement of the 490:430 BCECF signal. The effect of 5 and 10 mM NH_4Cl on the 490:430 ratio was subsequently examined, and it was observed that 5 mM NH_4Cl caused an intracellular alkalosis equal in magnitude to the L-lactate-induced acidosis. The addition of L-lactate in the presence of 5 mM NH_4Cl completely relaxed a subsequent NA contracture, without a significant change in pH_i.

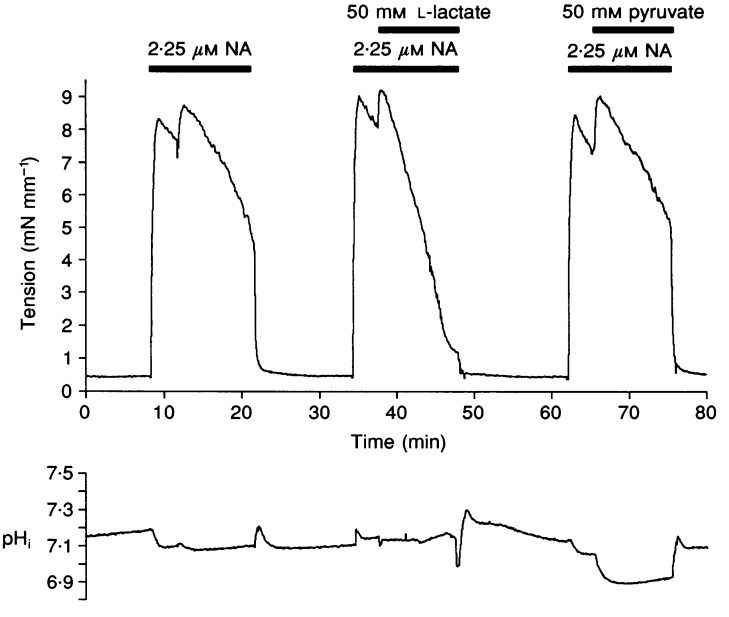

Figure 5. A comparison of the effects of 50 mM pyruvate and lactate on NA-stimulated contracture

Addition of L-lactate, but not pyruvate, caused relaxation. Addition of pyruvate, but not L-lactate, caused acidosis in this artery.

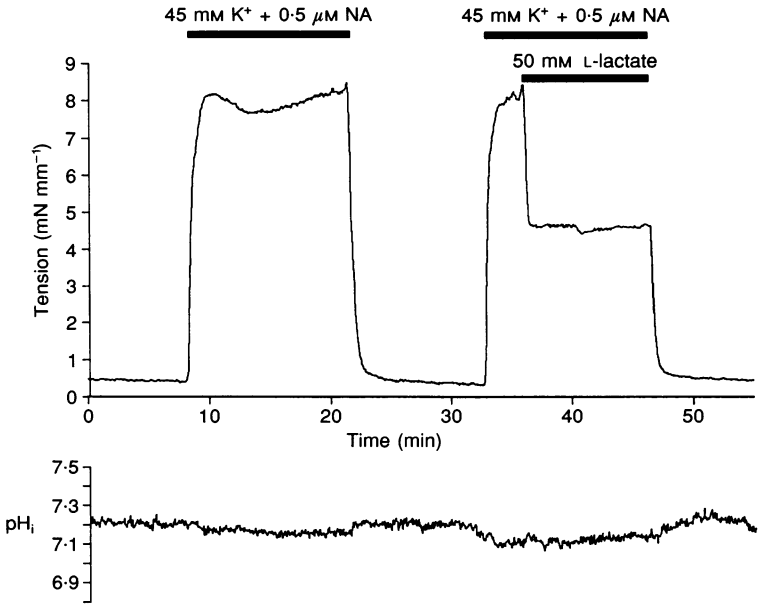

Figure 6. The effect of 50 mM L-lactate on the contracture induced by simultaneous addition of 45 mM K^+ and 0·5 μM NA

Simultaneous exposure of the artery to these concentrations of NA and high-K^+ solution elicited a sustained, near-maximal contracture which was then relaxed by approximately 50% by 50 mM L-lactate.

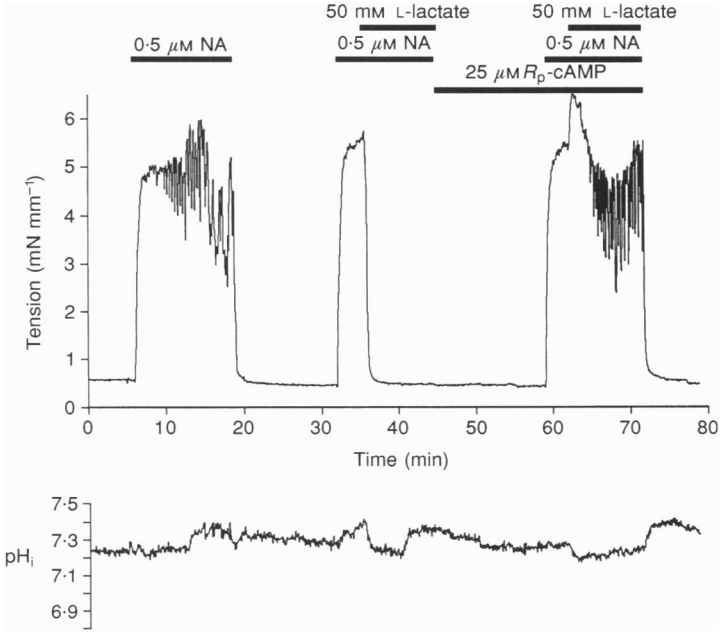

Figure 7. The effect of 25 μM R_p-cAMPS on L-lactate-induced relaxation

Complete relaxation of the contracture induced by 0·5 mM NA was observed in this artery upon exposure to 50 mM L-lactate. Application of 25 μM R_p-cAMPS 15 min prior to a subsequent exposure to NA and 50 mM L-lactate then markedly suppressed the L-lactate-induced relaxation.

Figure 6 illustrates further evidence that hyperpolarization was not required for relaxation. L-Lactate caused a marked relaxation of the contracture induced by the combination of NA and high-K^+ (45 mM) solution. In four experiments, this contracture was relaxed by 44·0 ± 8·4% after 10 min in the presence of L-lactate, whereas the corresponding time controls carried out in the absence of L-lactate increased by 21·0 ± 6·0% ($P < 0·002$). These results, taken together with the lack of effect of L-lactate on the contracture elicited by high K^+ alone, demonstrated that L-lactate was able to relax the NA contracture even when arteries were depolarized by high K^+.

Role of endogenous vasodilators

In order to assess the possible contribution of endothelium-derived vasodilators to L-lactate-induced relaxation, the effect of L-lactate was measured in endothelium-denuded arteries. The lactate-mediated relaxation of the NA contracture persisted in the absence of a functional endothelium. A concentration of 50 mM L-lactate produced an 83·8 ± 6·0% relaxation in nine de-endothelialized arteries, compared with the time control relaxation of 15·4 ± 3·4% ($P \leqslant 0·03$). Furthermore, blockade of NO synthase with 10^{-4} M L-NAME (applied 20 min prior to NA) did not significantly affect the L-lactate-mediated relaxation in arteries with an intact endothelium; 50 mM L-lactate relaxed the arteries by 91·2 ± 1·7% in the absence of L-NAME, and by 93·5 ± 2·1% in its presence ($n = 4$, n.s.). Similarly, indomethacin (10 μM), a blocker of cyclo-oxygenase which should prevent the elaboration of vasodilating prostanoids, had no effect upon L-lactate-induced relaxation (85·1 ± 6·3%, $n = 3$) when added 10 min prior to NA.

It has been reported that the guanylate cyclase inhibitor, Methylene Blue, inhibited lactate-induced relaxation of human placental arteries (Omar *et al.* 1993*a*). In our preparation, however, incubation of arteries in PSS containing 10 or 50 μM Methylene Blue for 20 min prior to NA addition had no effect upon 50 mM lactate-induced relaxation (92·7 ± 2·6%, $n = 3$).

Effect of R_p-cAMPS

In order to evaluate the possible role of cyclic AMP in the response to L-lactate, the effect of the membrane-permeable biologically inactive stereoisomer R_p-cAMPS, which competes with cAMP for its binding site on protein kinase A, was examined. Pre-incubation of the arteries with 25 μM R_p-cAMPS for 25 min prior to NA addition led to a significant reduction in L-lactate-induced relaxation (Fig. 7). Relaxation amounted to 89·9 ± 4·1% in lactate alone, but was reduced to 15·9 ± 12·6% in R_p-cAMPS ($n = 5$, $P < 0·003$). R_p-cAMPS did not itself cause tension development, and had no significant effect on the amplitude of the NA contracture.

In order to provide direct evidence that R_p-cAMPS was acting by inhibiting the cAMP system, we evaluated the effect of R_p-cAMPS on the response to forskolin, an agent which increases cAMP production by directly stimulating adenylate cyclase (Seamon & Daly, 1981). Preliminary experiments revealed that forskolin relaxed the NA contracture, but that the response to forskolin was not reproducible during multiple exposures. The effect of R_p-cAMPS was thus determined using a protocol in which arteries were exposed to forskolin only once. One group of arteries was exposed to NA for 18 min, with 0·04 μM forskolin present during the final 15 min. In this case, relaxation amounted to 88·5 ± 5·4% ($n = 5$) after 15 min. The second group was exposed to 25 μM R_p-cAMPS for 20 min, and subsequently treated with NA and then forskolin. In these arteries, relaxation was significantly reduced to 49·0 ± 6·0% ($n = 6$, $P < 0·01$). Taking into consideration the underlying decay of the NA contraction (by 18·7 ± 2·9%, $n = 5$) revealed by the time-control experiments, R_p-cAMPS reduced the response to forskolin by approximately 50%. These data show that this concentration of R_p-cAMPS was capable of attenuating vasorelaxation associated with stimulation of the cAMP second messenger system.

DISCUSSION

Lactate caused relaxation in small mesenteric resistance arteries of the rat, in agreement with previous publications that have described lactate-induced vasodilatation in arteries from other vascular beds. Thus, lactate has been found to induce peripheral vasodilatation in amphibians (Hilton & Eicholtz, 1925; Kurtz & Leake, 1926) and relaxation of human chorionic plate placental arteries (Omar *et al.* 1993*a*), bovine coronary arteries (Mohme-Lundholm, 1957) and calf pulmonary arteries (Omar *et al.* 1993*b*).

The concentration of plasma lactic acid varies widely. In resting humans the arterial lactate concentration is approximately 0·6 mM, but can vary extensively due to mental stress, muscle tone, exercise or state of health e.g. following a brief period of intense leg exercise skeletal muscle lactate increased to more than 30 mM and femoral venous lactate increased to 12 mM (Bangsboo, Johansen, Graham & Saltin, 1993). In addition, venous plasma lactate concentrations as high as 26 mM have been measured in hospital inpatients suffering from a number of unrelated conditions (Huckabee, 1961). In this study we chose to focus on responses to 50 mM lactate, since this concentration gave a prompt and marked relaxation. The lactate-induced relaxation appeared, however, to be approximately linearly related to the lactate concentration, suggesting that this effect may be relevant at lactate concentrations attained *in vivo*.

Dissociation between intracellular acidosis and relaxation of vascular smooth muscle was evident in this study, in agreement with previous publications (Aalkjaer & Cragoe, 1988; Aalkjaer & Mulvany, 1988; Aalkjaer & Hughes, 1991; Matthews *et al.* 1992; Nutting, Islam, Ye, Battle & Daugirdas, 1992; Jensen *et al.* 1993). This dissociation was indicated by three observations. Firstly, lactate caused marked relaxation with only a small fall in pH_i. Secondly, when this fall in pH was offset with NH_4Cl, relaxation to lactate was unaffected. Finally, the addition of the weak acid pyruvate caused a significant intracellular acidosis but did not induce any relaxation. Dissociation between weak-acid-induced acidosis and relaxation has also been suggested by Nutting *et al.* (1992), who demonstrated that acetate caused relaxation in the rat caudal artery, but that only a transient acidosis was elicited by acetate in cultured vascular smooth muscle cells. This was strengthened by their observation that the anion transport inhibitor 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS) inhibited acetate-induced relaxation in the intact artery, but also caused prolonged acidosis in the cultured cells, presumably as a result of inhibition of bicarbonate-dependent pH_i regulatory pathways (Aalkjaer & Hughes, 1991).

It is of interest that D-lactate caused a relaxation which was of similar magnitude to, but faster than that caused by L-lactate. In rat aortic vascular smooth muscle D-lactate gains entry but at a slower rate than L-lactate (Kutchai, Mann-Geddis & Martin, 1978). If this were to occur in the small mesenteric arteries, the faster rate of relaxation observed with D-lactate could be explained by intracellular accumulation of the D- but not L-stereoisomer, since the latter, but not the former, would be subsequently metabolized to pyruvate, which does not cause relaxation. Both the effectiveness of D-lactate, and the lack of effect of pyruvate, also suggested that metabolism of L-lactate to pyruvate by lactate dehydrogenase was not causing dilatation via the production of vasoactive intermediary metabolites generated in the Krebs cycle (Frohlich, 1965; Miller, Nolph, Joshua, Wiegman, Harris & Anderson, 1981). We did not, however, directly assess lactate uptake or metabolism, and our experiments do not rule out the possibility that either stereoisomer of lactate might have been exerting its action via an extracellular effect.

A number of possible mechanisms might contribute to the L-lactate-induced relaxation. For example, relaxation due to hyperosmolarity has previously been demonstrated (Miller *et al.* 1981). Although L-lactate was applied under isosmolar conditions in the present experiments, an imbalance between the influx of L-lactate and the efflux of Cl^-, which is likely to be more permeable, might lead to cell shrinkage as KCl and water leave the cells. This non-specific mechanism for relaxation appears, however, to be ruled out by the observation that the high-K^+ contracture was not relaxed by L-lactate. The possibility that reduction of the Cl^- concentration due to the substitution of sodium lactate for NaCl might contribute to relaxation of the NA contracture also seems unlikely, in that the substitution of sodium pyruvate for NaCl did not cause relaxation.

The lack of effect of L-lactate on the high-K^+ stimulated contracture also suggested that L-lactate was not acting by blocking voltage-gated Ca^{2+} channels. A recent analysis of the effects of pH_i on force development in rat mesenteric resistance arteries demonstrated that acidification with butyrate caused a relaxation of the high-K^+ contracture (Austin & Wray, 1994). This finding is consistent with the study of Klöckner & Isenberg (1994), which described a reduction of Ca^{2+} channel open probability caused by intracellular acidification. The relationship between pH_i and relaxation which was reported by Austin & Wray (1994), however, was such that the very small magnitude of the L-lactate induced acidification we measured should cause only a negligible effect on the K^+ contracture.

In a recent study (Carr *et al.* 1993), we have shown that relaxation of rat mesenteric small arteries to elevation of the P_{CO_2} is associated with a fall in pH_i, but that relaxation does not occur when the endothelium has been removed or in the presence of a NO synthase inhibitor, despite an identical fall in pH_i. It was, therefore, concluded that hypercapnia caused relaxation through NO release. In the present study, removal of the endothelium or addition of the nitric oxide synthase inhibitor, L-NAME, did not prevent relaxation with L-lactate and a role for NO release can therefore be excluded. This is in agreement with previous findings that weak-acid-induced relaxation is not influenced by endothelial removal or suppression of NO generation in placental arteries (Omar *et al.* 1993*a*). Indomethacin, an inhibitor of cyclo-oxygenase, also did not effect the relaxation produced by L-lactate and it is unlikely, therefore, that vasodilating prostanoids play an important role.

There is evidence that cGMP may be involved in L-lactate-induced relaxation in the placental circulation as the soluble guanylate cyclase inhibitor Methylene Blue inhibits relaxation to L-lactate in human placental arteries (Omar *et al.* 1993*a*). These authors suggested that cGMP synthesis might result indirectly from the conversion of L-lactate to pyruvate which results in proton generation. The resultant reduction of NAD might lead to production of hydrogen peroxide and stimulation of cGMP. However in our experiments Methylene Blue did not affect L-lactate-induced relaxation and cGMP-induced relaxation is therefore unlikely to be involved.

Conversely, the inhibition of the response to L-lactate by R_p-cAMPS indicated involvement of cAMP in relaxation. R_p-cAMPS is a inactive competitor of cAMP at its binding site on protein kinase A. R_p-cAMPS is relatively resistant

to hydrolysis and so, if present in sufficiently high concentrations will block activation of protein kinase A (van Haastert, van Driel, Jastorff, Baraniak, Stec & De Witt, 1984; de Witt *et al.* 1984). The usefulness of R_p-cAMPS as an antagonist of the cAMP system was confirmed by the ability of this agent to attenuate the response to forskolin, which directly stimulates adenylate cyclase (Seamon & Daly, 1981). The effect of R_p-cAMPS on the L-lactate response was consistent with experiments carried out in cultured vascular smooth muscle from the rat caudal artery (Nutting, Islam & Daugirdas, 1991; Nutting *et al.* 1992) in which intracellular cAMP increased after exposure to the weak acids acetate, butyrate and propionate. All three weak acids increased cAMP levels independently of a functional endothelium and the presence of indomethacin (Daugirdas *et al.* 1988; Nutting *et al.* 1991). The time course of cAMP production by acetate, propionate and butyrate in single cells was comparable with the vasorelaxant effects of the weak acids in intact rat caudal artery. The increase in cAMP in single cells and relaxation of artery sections were blocked by 10^{-3} M DIDS, which at this concentration blocks the monocarboxylate transport pathway, suggesting that the response was mediated by weak-acid transport into the intracellular compartment. The present experiments provide direct evidence that this rise in cAMP does, in fact, contribute to weak-acid-induced relaxation in rat mesenteric arteries.

Cyclic AMP has been shown to cause vascular smooth muscle relaxation via a number of mechanisms, including stimulation of Ca^{2+} uptake by the sarcoplasmic reticulum (Tada & Katz, 1982). Rat mesenteric resistance arteries permeabilized using staphylococcal α-toxin and treated with ionomycin to prevent Ca^{2+} accumulation by the sarcoplasmic reticulum relax to cAMP (Nishimura & van Breeman, 1989), implying a direct effect of cAMP upon the contractile apparatus of the cells. There is also evidence that cAMP causes hyperpolarization via phosphorylation of a Ca^{2+}-dependent K^+ channel (Sadoshima, Akaike, Kanaide & Nakamura, 1988; Kume, Takai, Tokuno & Tomita, 1989). Since, however, lactate was able to relax a contracture induced by NA and high K^+ added in combination, this suggested that hyperpolarization was not an important factor in the present case. It is also of interest that forskolin has been shown to inhibit $^{45}Ca^{2+}$ influx stimulated by NA more effectively than that stimulated by high K^+ in rabbit aorta (Lukeman, 1987), an observation consistent with our finding that L-lactate relaxed the NA, but not the high-K^+-induced contracture.

In summary, the present observations indicate that L-lactate-induced relaxation is independent of changes in pH_i. It is not mediated by inhibition of voltage-gated Ca^{2+} channels, the opening of K^+ channels, cyclic GMP, and does not require a functional endothelium. The observation that the effect of L-lactate is blocked by R_p-cAMPS confirms and extends previous evidence (Nutting *et al.* 1991, 1992) that weak-acid-induced relaxation depends to an important degree on the stimulation of protein kinase A via a rise in cAMP.

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Acknowledgements

This work was supported by The British Heart Foundation. We are grateful to Dr Sergey Smirnov and Dr Christian Aalkjaer for their helpful suggestions concerning the manuscript and experiments therein.

Received 12 December 1994; accepted 21 August 1995.