Extracellular carbonic anhydrase activity facilitates lactic acid transport in rat skeletal muscle fibres

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1. In skeletal muscle an extracellular sarcolemmal carbonic anhydrase (CA) has been demonstrated. We speculate that this CA accelerates the interstitial CO_2/HCO_3^- buffer system so that H^+ ions can be rapidly delivered or buffered in the interstitial fluid. Because > 80 % of the lactate which crosses the sarcolemmal membrane is transported by the H^+–lactate cotransporter, we examined the contributions of extracellular and intracellular CA to lactic acid transport, using ion-selective microelectrodes for measurements of intracellular pH (pH_i) and fibre surface pH (pH_s) in rat extensor digitorum longus (EDL) and soleus fibres.

2. Muscle fibres were exposed to 20 mM sodium lactate in the absence and presence of the CA inhibitors benzolamide (BZ), acetazolamide (AZ), chlorzolamide (CZ) and ethoxzolamide (EZ). The initial slopes (dpH_s/dt, dpH_i/dt) and the amplitudes (ΔpH_s, ΔpH_i) of pH changes were quantified. From dpH_i/dt, ΔpH_i and the total buffer factor (BF_{tot}) the lactate fluxes (mM min^{-1}) and intracellular lactate concentrations ($[lactate]_i$) were estimated.

3. BF_{tot} was obtained as the sum of the non-HCO_3^- buffer factor ($BF_{non\text{-}HCO_3}$) and the HCO_3^- buffer factor (BF_{HCO_3}). $BF_{non\text{-}HCO_3}$ was 35 ± 4 mM ΔpH^{-1} for the EDL ($n = 14$) and 86 ± 16 mM ΔpH^{-1} for the soleus ($n = 14$).

4. In soleus, 10 mM cinnamate inhibited lactate influx by 44 % and efflux by 30 %; in EDL, it inhibited lactate influx by 37 % and efflux by 20 %. Cinnamate decreased $[lactate]_i$, in soleus by 36 % and in EDL by 45 %. In soleus, 1 mM DIDS reduced lactate influx by 18 % and efflux by 16 %. In EDL, DIDS lowered the influx by 27 % but had almost no effect on efflux. DIDS reduced $[lactate]_i$ by 20 % in soleus and by 26 % in EDL.

5. BZ (0.01 mM) and AZ (0.1 mM), which inhibit only the extracellular sarcolemmal CA, led to a significant increase in dpH_s/dt and ΔpH_s by about 40 %–150 % in soleus and EDL. BZ and AZ inhibited the influx and efflux of lactate by 25 %–50 % and reduced $[lactate]_i$ by about 40 %. The membrane-permeable CA inhibitors CZ (0.5 mM) and EZ (0.1 mM), which inhibit the extracellular as well as the intracellular CAs, exerted no greater effects than the poorly permeable inhibitors BZ and AZ did.

6. In soleus, 10 mM cinnamate inhibited the lactate influx by 47 %. Addition of 0.01 mM BZ led to a further inhibition by only 10 %. BZ alone reduced the influx by 37 %.

7. BZ (0.01 mM) had no influence on the K_m value of the lactate transport, but led to a decrease in maximal transport rate (V_{max}). In EDL, BZ reduced V_{max} by 50 % and in soleus by about 25 %.

8. We conclude that the extracellular sarcolemmal CA plays an important role in lactic acid transport, while internal CA has no effect, a difference most likely attributable to the high internal *vs.* low extracellular $BF_{non\text{-}HCO_3}$. The fact that the effects of cinnamate and BZ are not additive indicates that the two inhibitors act at distinct sites on the same transport pathway for lactic acid.

Several studies have provided histochemical (Ridderstråle, 1979; Lönnerholm, 1980; Riley *et al.* 1982; Dermietzel *et al.* 1985; Decker *et al.* 1996), biochemical (Wetzel & Gros, 1990, 1998) and functional evidence (Zborowska-Sluis *et al.* 1974; Effros & Weissman, 1979; Geers *et al.* 1985; De Hemptinne *et al.* 1987) for a membrane-bound, sarcolemmal carbonic anhydrase (CA) in skeletal muscle. Waheed *et al.* (1992) have demonstrated that in rat skeletal muscle the sarcolemmal CA is a 39 kDa, glycosylated, phosphatidylinositol-glycan anchored CA IV.

The present study investigates the functional role of this CA in lactic acid transport across the sarcolemmal membrane of skeletal muscle. Because of the very low concentration of non-bicarbonate buffers in the interstitial space, the CO_2/HCO_3^- buffer system is the most important pH buffer in the interstitial space. The CO_2 hydration/dehydration reaction in the interstitium is accelerated by the membrane-bound sarcolemmal CA so that H^+ ions needed for transport processes into the cell can very rapidly be delivered and, on the other hand, H^+ ions that are transported out of the muscle cell can very rapidly be buffered in the interstitial fluid. At lactate concentrations < 10 mM more than 80% of the lactate moving across the sarcolemmal membrane is transported by the H^+–lactate cotransporter at a ratio of 1:1 (Mason & Thomas, 1988; Roth & Brooks, 1990*a*,*b*; Juel, 1997). Therefore, we recorded intracellular pH (pH_i) and the pH directly on the surface (pH_s) of rat extensor digitorum longus (EDL) and soleus muscle fibres with microelectrodes to examine the roles of the sarcolemmal and intracellular CAs in lactic acid transport. The results obtained suggest that sarcolemmal CA, but not the cytosolic isoform, has a major influence on lactate transport.

METHODS

Muscle fibre preparation

Female Wistar rats (body mass 200 ± 30 g, mean ± S.D.) were killed by an overdose of diethylether. The soleus muscle and the EDL were dissected out within the next 2 min and were kept in oxygenated, 28 mM HCO_3^-–5% CO_2-buffered Ringer solution at room temperature. From these muscles the fibre bundles were prepared in a Petri dish with spring scissors under a Wild M8 microscope (Leica). The fibre bundles consisted of 15–30 muscle fibres and only the superficial ones were used in the experiments. To perform the experiment, each fibre bundle was transferred into a chamber with a volume of 800 ± 50 µl. The chamber was perfused at a rate of 2 ml min^{-1}, and a change of the perfusion solution in the chamber was 90% complete within ~50 s. All experiments were performed at room temperature.

All experiments were carried out in accordance with the guidelines laid down by the Bezirksregierung Hannover.

Microelectrodes

The membrane potential (E_m) electrodes were pulled from borosilicate glass tubing with a filament (KBF-112080, ZAK Products, Marktheidenfeld, Germany) and filled with a solution containing 1.5 M KCl and 1.5 M potassium acetate (pH adjusted to 6.6–6.7 with HCl; resistance 35–65 MΩ). The H^+-sensitive electrodes, one for measuring the intracellular pH (pH_i) and one for measuring the pH value on the surface of the cell (pH_s), were drawn from thin-walled tubing (GC 150T-7.5, Clark Electromedical Instruments, Pangbourne, UK). After silanization with *N*-*N*-dimethyl-trimethyl-silylamine (Fluka) vapour, the micropipettes were backfilled with 100 mM NaCl, 200 mM Hepes and 100 mM NaOH. A short column of the H^+ sensor Hydrogen Ionophore Cocktail A (Fluka) was sucked into the tip (resistance 20–35 MΩ). The resistance of the much coarser pH_s electrode was 1–2 GΩ, and the average slope of both electrodes was about 61 mV ΔpH^{-1}. The reference electrode was constructed from glass tubing (GC200-15) and filled with 3 M KCl and 3% agar.

Intracellular non-HCO_3^- buffer capacity

In order to determine the non-HCO_3^- buffer factor ($BF_{non\text{-}HCO_3}$), the fibre bundles of soleus and EDL were alternately exposed to 30 mM HCO_3^-–5% CO_2- (P_{CO_2} in the chamber 37 ± 1 mmHg, 22°C, $n = 14$) or to 60 mM HCO_3^-–10% CO_2- (P_{CO_2} in the chamber 70 ± 3 mmHg, 22°C, $n = 22$) buffered Ringer solution. The pH_i value was measured under steady-state conditions for both CO_2 concentrations. $BF_{non\text{-}HCO_3}$ was determined in Ringer solution with Na^+ as well as in Ringer solution without Na^+ in order to examine the influence of sodium-dependent acid extrusion (Aicken & Thomas, 1977*a*). Possible effects on $BF_{non\text{-}HCO_3}$ caused by a Cl^-–HCO_3^- exchanger were tested by the addition of 1 mM DIDS. Finally, the effects of the extracellular sarcolemmal CA on $BF_{non\text{-}HCO_3}$ were investigated using 10^{-5} M benzolamide (BZ). The values of $BF_{non\text{-}HCO_3}$ were not significantly different under the various conditions examined (Table 1). Therefore, they were combined to yield a mean value of 35 ± 4 mM ΔpH^{-1} for EDL ($n = 14$) and of 86 ± 16 mM ΔpH^{-1} for soleus ($n = 14$). This value of $BF_{non\text{-}HCO_3}$ for the EDL is much lower than the value reported by Grossie *et al.* (1988) (100 mM ΔpH^{-1}). Therefore, $BF_{non\text{-}HCO_3}$ was also measured under the conditions of Grossie *et al.* (1988), using 25 mM Hepes–100% O_2 (= 0% CO_2) and 24 mM HCO_3^-–5% CO_2. This resulted in a value of $BF_{non\text{-}HCO_3}$ of 83 ± 4 mM ΔpH^{-1} ($n = 3$) for EDL and 107 ± 6 mM ΔpH^{-1} ($n = 3$) for soleus. Because the range of pH_i values covered by the experiments with a lactic acid load (see below) was more comparable to the range of pH_i values occurring between 5 and 10% CO_2 than to that between 0 and 5% CO_2, we chose a $BF_{non\text{-}HCO_3}$ of 35 mM ΔpH^{-1} for EDL and a $BF_{non\text{-}HCO_3}$ of 86 mM ΔpH^{-1} for soleus in the calculations done for the present experiments.

An error in $BF_{non\text{-}HCO_3}$ of 10% (see variations in $BF_{non\text{-}HCO_3}$ in Table 1) would affect lactate fluxes and intracellular lactate concentrations by 7% in soleus and by 5% in EDL.

Parameters derived from pH recordings

The initial slope (dpH_s/dt; $\Delta pH\ min^{-1}$) and the maximal amplitude (ΔpH_s) of the pH_s signal were separately determined for the phases of lactic acid uptake and of lactic acid release. The initial slopes of the pH_i signal (dpH_i/dt) were evaluated and used to calculate the rates of lactic acid uptake and of lactic acid release (mM min^{-1}). Additionally, the maximal pH_i amplitude (ΔpH_i), which reflects the maximal concentration of lactic acid achieved in the muscle cell, was determined. Using these parameters, directly derived from the pH recordings, the following quantities were calculated from eqns (1)–(4):

$$\text{Flux of lactate} = BF_{tot} \times dpH_i/dt, \quad (1)$$

where flux is defined as the change in intracellular concentration over time and BF_{tot} is the total buffer factor (mM ΔpH^{-1}; see below). By inserting dpH_i/dt derived from the phase of lactic acid uptake the rate of lactate influx is obtained, and by inserting dpH_i/dt derived from the phase of lactic acid release the rate of lactate efflux is obtained.

$$[\text{Lactate}]_i = BF_{tot} \times \Delta pH_i, \quad (2)$$

where $[\text{lactate}]_i$ (mM) is the intracellular lactate concentration at equilibrium and ΔpH_i is the change in pH_i due to lactic acid loading after steady state has been established.

Table 1. Values of the non-HCO_3^- buffer factor $BF_{non\text{-}HCO_3}$ in rat EDL and soleus

	+ Na^+	− Na^+	− Na^+ + 10^{-5} M BZ	− Na^+ + 1 mM DIDS
EDL	38 (1)	36 ± 4 (7)	32 ± 6 (4)	35 (2)
Soleus	74 ± 17 (5)	92 ± 13 (6)	94 ± 13 (6)	—

Experiments were done in Ringer solution with Na^+ (+ Na^+), without Na^+ (− Na^+), without Na^+ but with the carbonic anhydrase inhibitor benzolamide, BZ (− Na^+ + 10^{-5} M BZ), and without Na^+ but with DIDS (− Na^+ + 1 mM DIDS). Na^+ was replaced by choline. $BF_{non\text{-}HCO_3}$ values (mm ΔpH^{-1}) are means ± S.D. Numbers in parentheses give numbers of experiments.

$$BF_{tot} = BF_{HCO_3} + BF_{non\text{-}HCO_3}, \quad (3)$$

where BF_{tot} is the sum of the HCO_3^- buffer factor BF_{HCO_3} (mM ΔpH^{-1}), and the non-bicarbonate buffer factor $BF_{non\text{-}HCO_3}$. $BF_{non\text{-}HCO_3}$ has been determined as described above, and BF_{HCO_3} has been derived using the equation:

$$BF_{HCO_3} = \Delta[HCO_3^-]_i \times \Delta pH_i^{-1}, \quad (4)$$

where $\Delta[HCO_3^-]_i$ is the difference in the intracellular HCO_3^- concentration before and during lactate exposure under steady-state conditions. The intracellular HCO_3^- concentration ($[HCO_3^-]_i$) was estimated using the Henderson-Hasselbalch equation.

Solutions

Solutions for the experiments with lactic acid. Standard Ringer solution without lactate (mM): 100 NaCl, 3.7 KCl, 1.2 $MgSO_4$, 1.3 $CaCl_2$, 28 $NaHCO_3$, 20 methanesulfonic acid. Ringer solution with lactate (mM): 100 NaCl, 3.7 KCl, 1.2 $MgSO_4$, 1.3 $CaCl_2$, 28 $NaHCO_3$, 20 sodium lactate. In the experiments without Na^+, NaCl was replaced by choline chloride, $NaHCO_3$ by choline bicarbonate and sodium lactate by lactic acid; the pH was adjusted to 7.0 by choline bicarbonate. In the experiments with 10 mM cinnamate and with 10 mM $CaCl_2$, NaCl was reduced from 100 to 90 and 91 mM, respectively. In the experiments with 1 mM DIDS in 15 mM Hepes–100 % O_2 the NaCl concentration was 110 mM and HCO_3^- was replaced by Hepes.

Solutions for the determination of $BF_{non\text{-}HCO_3}$. Solution A (mM): 120 NaCl, 3.7 KCl, 1.2 $MgSO_4$, 1.3 $CaCl_2$, 30 $NaHCO_3$/5 % CO_2; solution B (mM): 90 NaCl, 3.7 KCl, 1.2 $MgSO_4$, 1.3 $CaCl_2$, 60 $NaHCO_3$–10 % CO_2. In the case of the experiments without Na^+, Na^+ was replaced by choline in solutions A and B. Solution C (mM): 125 choline chloride, 3.7 KCl, 1.2 $MgSO_4$, 1.3 $CaCl_2$, 25 Hepes–100 % O_2; solution D (mM): 120 choline chloride, 3.7 KCl, 1.2 $MgSO_4$, 1.3 $CaCl_2$, 30 choline bicarbonate–5 % CO_2.

Cinnamate (α-cyano-4-hydroxycinnamic acid), DIDS (4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid), acetazolamide and ethoxzolamide were purchased from Sigma. Chlorzolamide and benzolamide were kind gifts from Lederle Laboratories (Pearl River, NY, USA).

RESULTS

pH changes associated with lactate uptake and release

Figure 1 shows the pH_s and pH_i transients during exposure of a superficial EDL fibre to lactate. In the control solution, the values of pH_s and pH_i were 7.39 and 7.21, respectively. After switching to 20 mM lactate, pH_s showed an alkaline shift with a peak value of 7.43 and pH_i an acidic shift with its nadir at 7.14. The uptake of lactate and protons by the H^+–lactate cotransporter as well as by diffusion of undissociated lactic acid consumed H^+ ions and caused an alkalinization on the surface of the fibre and induced an intracellular acidification. In this phase the pH_i transient reached a plateau at pH = 7.14, indicating the steady-state distribution of lactate, where $[lactate]_i$ was 5.4 mM. By switching from lactate-containing Ringer solution back to standard Ringer solution, lactic acid was released from the fibre. The release of lactate and H^+ ions caused pH_i to return to its control value and produced a pH_s shift in the acidic direction. The resting potential was not influenced by the exposure to or washout of lactate.

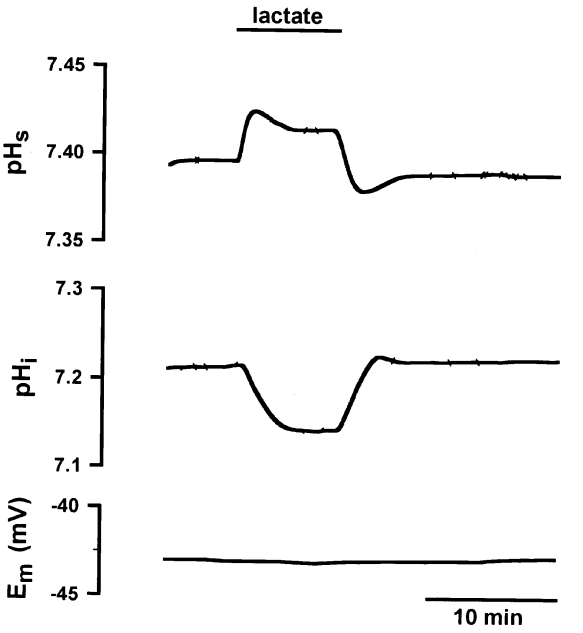

Figure 1. Changes in pH_s, pH_i and E_m in a single EDL fibre evoked by 20 mM lactate in the superfusing solution

The uptake of lactic acid led to an alkalinization on the surface of the muscle fibre and to an intracellular acidification. The release of lactic acid caused a pH_s transient in the acidic direction and caused pH_i to return to its control value. The transport of lactate had no effect on E_m.

Transport processes affecting pH_s and pH_i transients

Na^+–H^+ exchanger

Under a lactic acid load, H^+ extrusion by the Na^+–H^+ exchanger is about one-sixth of the H^+ transport capacity of the H^+–lactate cotransporter in muscle (Juel, 1995). In order to test whether the Na^+–H^+ exchanger affects the observed changes in pH_s and pH_i, Na^+ ions were replaced by choline. The results are shown in Fig. 2. In both muscles, the absence of Na^+ ions hardly affected either ΔpH_s (Fig. 2*A*) or lactate fluxes (Fig. 2*B*). Only values of $[lactate]_i$ were slightly decreased in soleus and EDL (Fig. 2*C*). In contrast, any pH_i regulatory effect by the Na^+–H^+ exchanger would be expected to lead to a decrease in ΔpH_i and $[lactate]_i$, respectively, and accordingly the absence of Na^+ ions to an increase of ΔpH_i and $[lactate]_i$. From these data, we conclude that pH_i regulation by the Na^+–H^+ exchanger does not affect the transients of pH_s and pH_i to a noticeable extent.

H^+–lactate cotransporter

The influence of 10 mM cinnamate, an inhibitor of the H^+–lactate cotransporter, on pH_s and pH_i transients was investigated. Figure 3 shows the transients of pH_s and pH_i and the resting potential in the absence and presence of 10 mM cinnamate for a soleus fibre. Cinnamate led to a reduction of the amplitude of the pH_s transient during lactate uptake as well as lactate release. It can be seen from the pH_i transient that the intracellular acidification was slowed down and reduced by cinnamate. The membrane potential was unaffected. The quantitative results for soleus and EDL are shown in Fig. 2. Cinnamate reduced ΔpH_s by about 50 % in both muscles (Fig. 2*A*). In soleus, the rate of lactate influx was slowed down from 5.5 to 3.1 mM min^{-1} (by 44 %) and the rate of lactate efflux from 5.3 to 3.7 mM min^{-1}, i.e. by 30 % (Fig. 2*B*). In EDL, lactate influx was reduced from 3.5 to 2.2 mM min^{-1} and lactate efflux from 2.9 to 2.3 mM min^{-1}. Cinnamate caused a significant decrease in $[lactate]_i$ from 10.7 to 6.8 mM in soleus and from 6.0 to 3.3 mM in EDL (Fig. 2*C*).

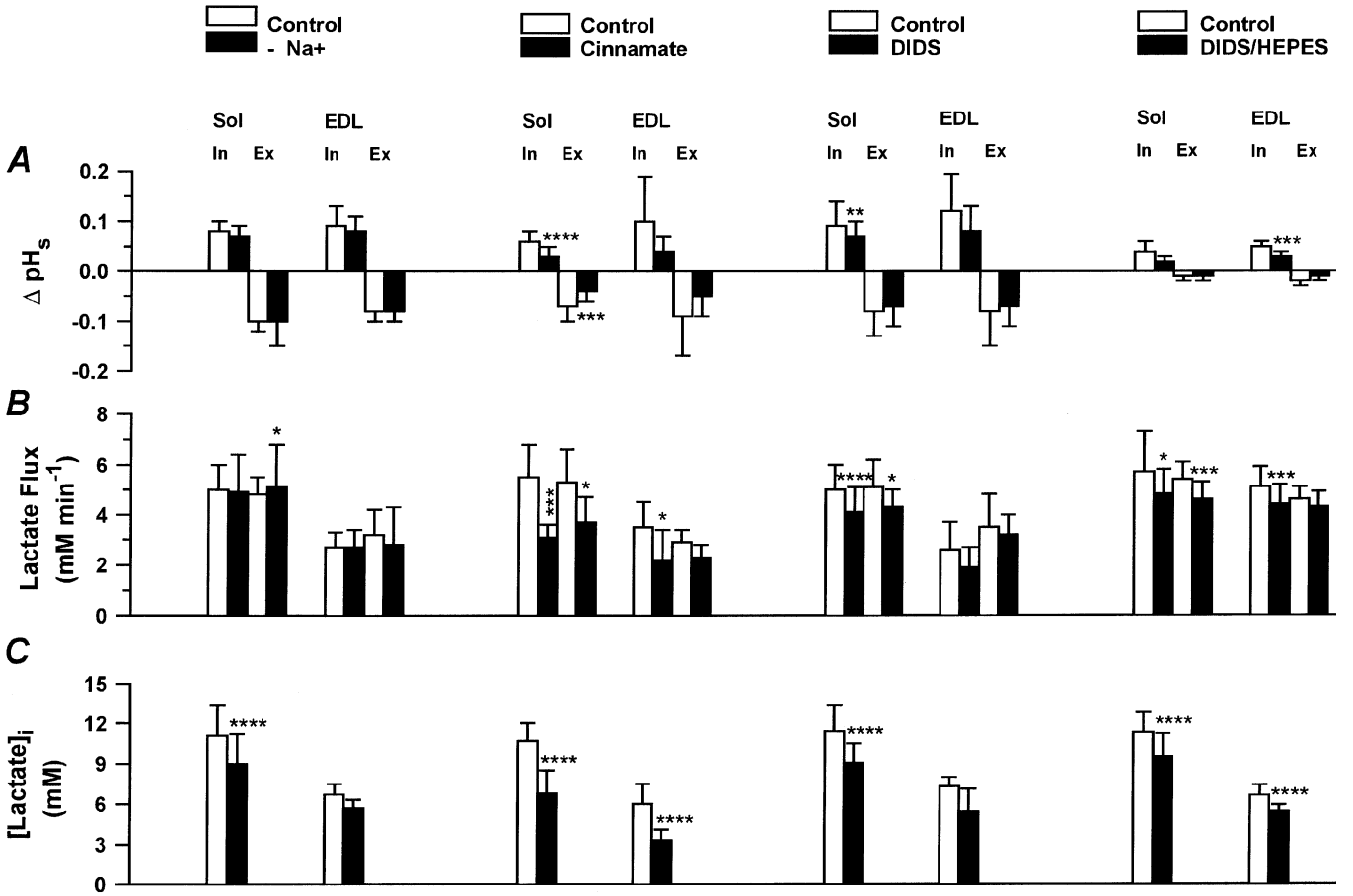

Figure 2. Changes in pH_s, lactate flux and $[lactate]_i$ in soleus (Sol) and EDL fibres in the absence and presence of extracellular sodium, cinnamate and DIDS

□, control data; ■, data without Na^+ in superfusing solution ($n = 4$ Sol, $n = 3$ EDL, left column), with 10 mM cinnamate ($n = 7$ Sol/EDL, second column from the left), with 1 mM DIDS in 28 mM HCO_3^-–5 % CO_2-buffered superfusing solution ($n = 10$ Sol, $n = 3$ EDL, third column from the left) or with 1 mM DIDS in 15 mM Hepes-buffered superfusing solution ($n = 7$ Sol, $n = 8$ EDL, right column). Values are means ± S.D. 'In' indicates lactate influx, 'Ex' lactate efflux, asterisks show levels of significance (Student's paired *t* test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$.

The effects of 1 mM DIDS in 28 mM HCO_3^-–5% CO_2-buffered superfusion solution on the pH_s and pH_i transients are qualitatively similar to those of cinnamate although quantitatively less pronounced. As seen in Fig. 2, during lactate uptake DIDS reduced ΔpH_s by between 20 and 33% in soleus and EDL. During lactate release, values of ΔpH_s were not significantly affected by DIDS. In soleus, DIDS significantly slowed down lactate fluxes decreasing the influx by 18% and the efflux by 16%, and reduced $[lactate]_i$ by 20%. In EDL, all effects of DIDS were qualitatively similar to what was seen in soleus, but they were almost all not statistically significant (see Fig. 2*A*–*C*).

Experiments in 15 mM Hepes–100% O_2-buffered Ringer solution were performed in order to answer the question of whether DIDS exerted its effects on the H^+–lactate cotransporter or on the Cl^-–HCO_3^- exchanger acting as a lactate–HCO_3^- exchanger in skeletal muscle. Figure 4 shows an experiment in the EDL, and Fig. 2 (*A*–*C*, right column) summarizes the results obtained for soleus and EDL. The effects of 1 mM DIDS in Hepes buffer are quantitatively similar to the effects observed in the presence of CO_2/HCO_3^- (see Fig. 2*A*–*C*). This finding validates the assumption that the effects of DIDS observed on lactate transport are caused by inhibition of the H^+–lactate cotransporter rather than of the Cl^-–HCO_3^- exchanger acting as a lactate–HCO_3^- exchanger.

Cl^-–HCO_3^- exchanger

In resting muscle as well as in muscle under a lactate load (Aickin & Thomas, 1977*a*; Juel, 1995), the Cl^-–HCO_3^- exchanger contributes to the total H^+ transport capacity by 20%. In order to estimate the influence of this exchanger on the recordings of pH_i, we compared control data for lactate fluxes and $[lactate]_i$ derived from experiments performed in HCO_3^--free, Hepes-buffered solutions (Fig. 2*B* and *C*, right column) with control data for fluxes and $[lactate]_i$ in the presence of HCO_3^-, obtained from the series with and without Na^+, cinnamate and DIDS in HCO_3^-/CO_2-buffered solutions (Fig. 2*B* and *C*, first to third columns). In soleus, data for lactate influx and efflux in HCO_3^--free, Hepes-buffered solutions (Fig. 2*B*, right column) did not differ from fluxes obtained in HCO_3^-/CO_2-buffered solutions (Fig. 2*B*), but in EDL lactate fluxes under HCO_3^--free conditions (Fig. 2*B*, right column, EDL) appeared to be greater by about 30% compared to fluxes in the presence of HCO_3^-/CO_2 (Fig. 2*B*, first to third columns, data for EDL). Control data for $[lactate]_i$ in soleus and EDL under HCO_3^--free conditions (Fig. 2*C*, right column) were not different from control data for $[lactate]_i$ in the presence of HCO_3^-/CO_2 (Fig. 2*C*). From these results, we conclude that the Cl^-–HCO_3^- exchanger does not affect the changes in pH_i and their time course in soleus, but seems to affect the time course of pH_i in the EDL to a minor extent.

Effects of CA inhibitors on pH_s and pH_i transients

Figures 5 and 6 show the pH_s and pH_i transients in the absence and presence of 0.01 mM BZ in EDL and soleus, respectively. BZ qualitatively caused the same effects in EDL and soleus. On the surface of the muscle fibre, BZ

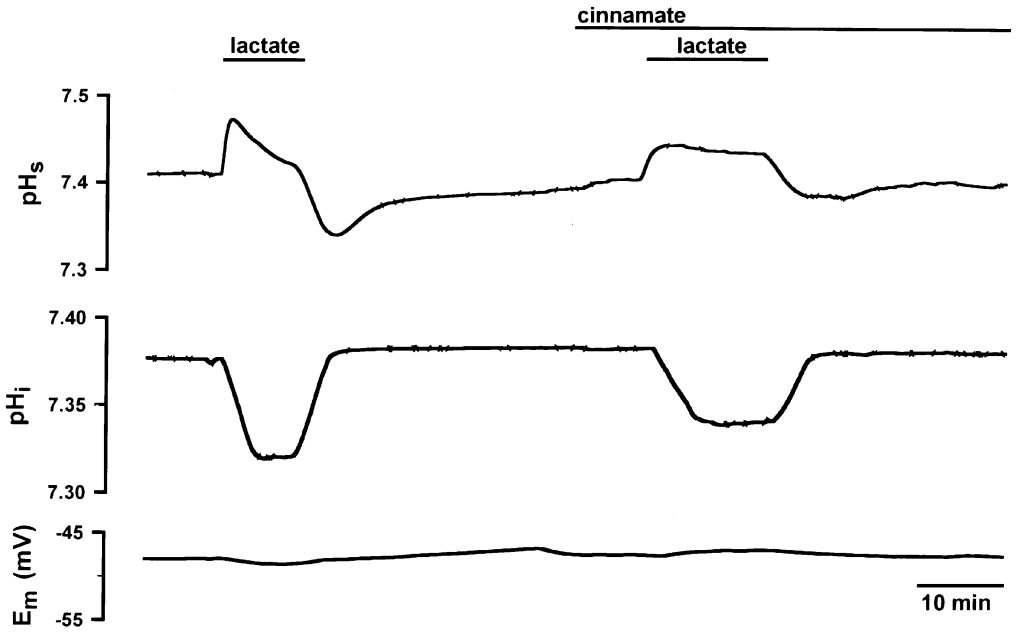

Figure 3. Changes in pH_s, pH_i and E_m in a single soleus fibre in the absence and presence of 10 mM cinnamate

Cinnamate led to a slow-down of dpH/dt and to a reduction in the amplitude in the pH_s transient as well as in the pH_i transient. The resting potential was not affected either by lactate or by cinnamate.

markedly increased the amplitude and the initial slope of the pH_s signal. This effect was much greater during lactate uptake than during lactate release. Intracellularly, BZ led to a slow-down of dpH_i/dt and to a reduction in ΔpH_i. The resting potential was not influenced by the inhibitor.

Figure 7 shows the results of different specific CA inhibitors, of BZ (0.01 mM) and acetazolamide (AZ, 0.1 mM), both hydrophilic and therefore poorly membrane-permeable inhibitors, and of chlorzolamide (CZ, 0.5 mM) and ethoxzolamide (EZ, 0.1 mM), two lipophilic and membrane-permeable CA inhibitors (Maren *et al.* 1983). Because EZ cannot be dissolved at a higher concentration than 0.1 mM and because of the presence of the sulfonamide-insensitive intracellular CA III in soleus, it was neccessary to use 0.5 mM CZ instead of 0.1 mM EZ in soleus fibres. During lactate influx all four CA inhibitors increased the values of ΔpH_s by about 100 % in soleus as well as in EDL (Fig. 7*A*, influx). This holds also for dpH_s/dt (Fig. 7*B*, influx). During lactate efflux the inhibitors also led to an increase in ΔpH_s and dpH_s/dt (Fig. 7*A* and *B*, efflux), but on average these effects were less pronounced than those during lactate influx. Figure 7*C* shows the changes in lactate fluxes, which in most cases were highly significant. In soleus, BZ and AZ reduced lactate influx by 36 and 50 %, respectively, and CZ by 36 %. In EDL, both hydrophilic inhibitors decreased the rate of lactate influx by 33 %, and the lipophilic EZ decreased the rate of lactate influx by 42 %. During lactate efflux BZ and AZ reduced lactate fluxes of soleus by 23 % and 50 %, respectively; CZ reduced lactate efflux by 36 %. In EDL, lactate efflux was slowed 24 % by BZ, 33 % by AZ (not significant), and 17 % by EZ (not significant). $[Lactate]_i$ was significantly decreased by the CA inhibitors by between 20 and 45 % (Fig. 7*D*), except for AZ and EZ in EDL where the decrease was not significant.

Effects of BZ on pH_s and lactate flux under inhibition of the H^+–lactate cotransporter

Does the CA inhibitor BZ still influence the lactate transport rate after the H^+–lactate cotransporter has been inhibited by cinnamate or by DIDS? Figure 8 (first two columns) shows the results with cinnamate and BZ in soleus fibres. During lactate influx 10 mM cinnamate reduced ΔpH_s from 0.05 to 0.03. Addition of 0.01 mM BZ did not exert an effect on ΔpH_s in the presence of cinnamate. Both inhibitors did not influence the amplitude of pH_s during lactate efflux. Lactate influx

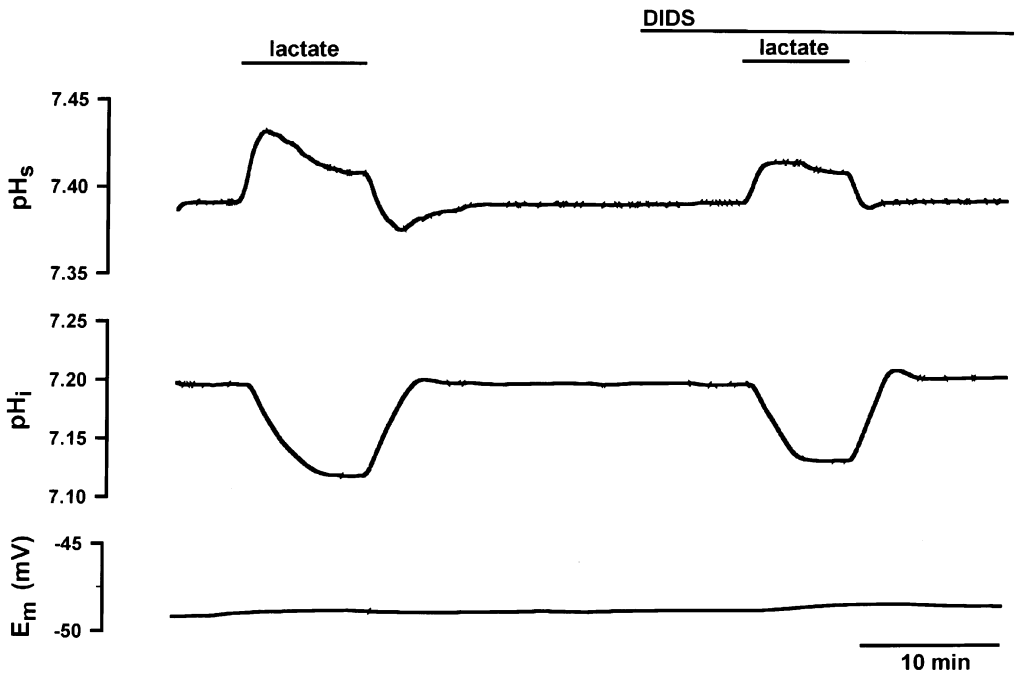

Figure 4. Changes in pH_s, pH_i and E_m in a single EDL fibre in the absence and presence of 1 mM DIDS in HCO_3^--free, Hepes-buffered Ringer solution

The muscle bundle was superfused with 15 mM Hepes–100 % O_2-buffered Ringer solution in the absence of HCO_3^-. Under these conditions DIDS led to a slow-down of dpH/dt and to a reduction of the amplitude (ΔpH) of both pH transients: during lactate uptake DIDS reduced dpH_s/dt from 0.09 to 0.05 $\Delta pH\ min^{-1}$ and ΔpH_s from 0.049 to 0.024; during lactate release dpH_s/dt was reduced from 0.04 to 0.03 $\Delta pH\ min^{-1}$ and ΔpH_s from 0.026 to 0.005 by DIDS. DIDS decreased dpH_i/dt from 0.082 to 0.077 $\Delta pH\ min^{-1}$ during lactate uptake and from 0.065 to 0.046 $\Delta pH\ min^{-1}$ during lactate release. In the absence of DIDS pH_i changed from 7.20 to 7.10 due to the uptake of lactic acid, but in the presence of DIDS pH_i changed only from 7.20 to 7.12. The resting potential was constant during the experiment.

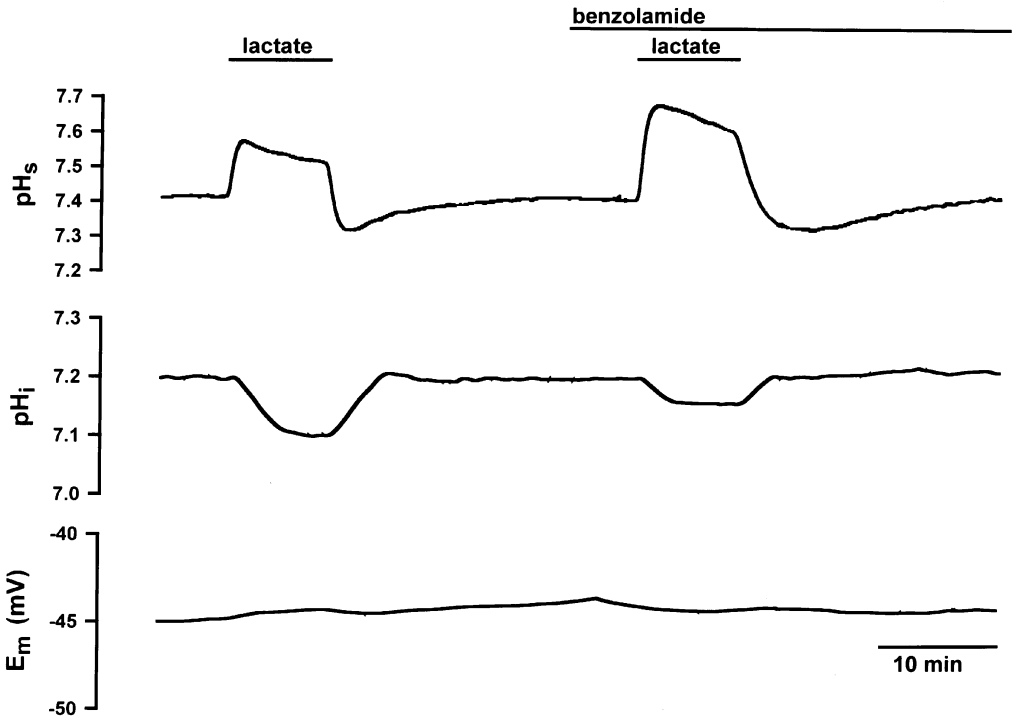

Figure 5. Changes in pH_s, pH_i and E_m in a single EDL fibre in the absence and presence of 0.01 mM BZ

BZ induced an enhanced extracellular alkalinization on the surface of the muscle fibre during lactate uptake and a greater acidic pH_s shift during lactate release as compared to control conditions. BZ slowed down dpH_i/dt and reduced ΔpH_i. The resting potential was not affected either by the lactate transport or by BZ.

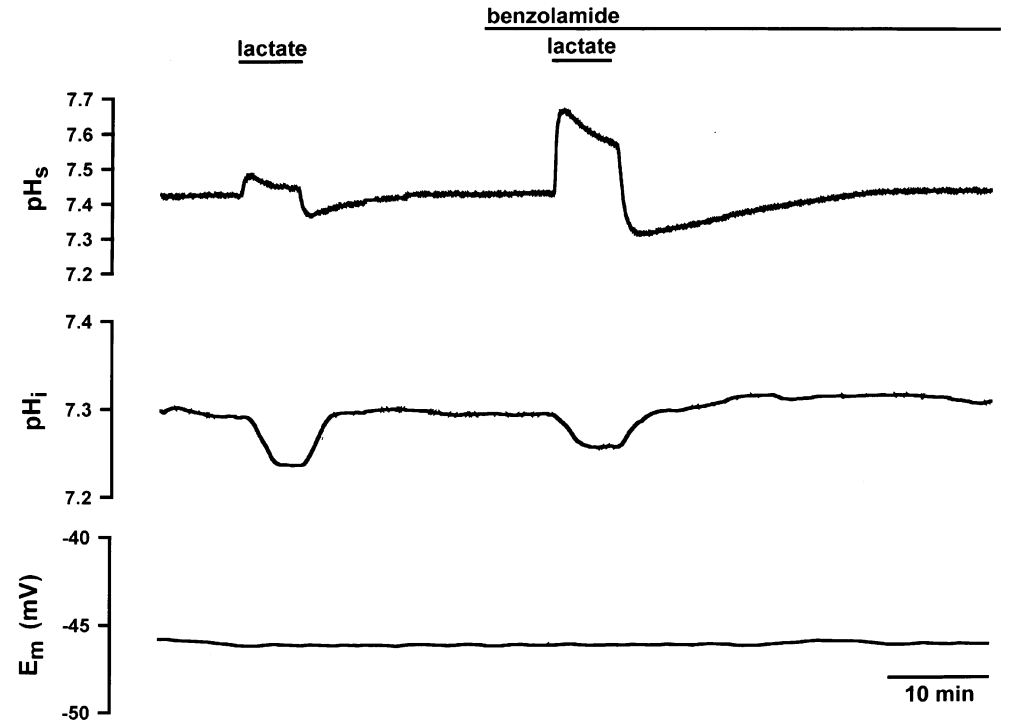

Figure 6. Changes in pH_s, pH_i and E_m in a single soleus fibre in the absence and presence of 0.01 mM BZ

In both EDL and soleus fibres, BZ led to a marked increase in dpH_s/dt and ΔpH_s and to a distinct decrease in dpH_i/dt and ΔpH_i. The resting potential was constant during the experiment.

was reduced from 6.0 to 3.2 mM min^{-1} (= 47 % inhibition) by cinnamate and further reduced to 2.6 mM min^{-1} by the addition of BZ (= further inhibition by 10 %). Lactate efflux was reduced from 5.9 to 4.1 mM min^{-1} (= 30 % inihibition) by cinnamate and further reduced to 3.0 mM min^{-1} by the addition of BZ (= further inhibition by 19 %).

Figure 8 (two columns on the right) also shows the effects of 1 mM DIDS and of 1 mM DIDS plus 0.01 mM BZ in soleus fibres. During lactate influx ΔpH_s decreased from 0.07 to 0.05 in the presence of DIDS and increased from 0.05 to 0.06 in the additional presence of BZ. Neither DIDS nor DIDS plus BZ affected ΔpH_s during lactate efflux. Lactate influx was reduced from 5.4 to 4.5 mM min^{-1} by DIDS (= 17 % inhibition) and from 4.5 to 2.8 mM min^{-1} by the further addition of BZ (= further inhibition by 31 %). Lactate efflux was reduced from 5.7 to 4.3 mM min^{-1} by DIDS (= 25 % inhibition) and was further reduced to 3.4 mM min^{-1} by addition of BZ (= further inhibition by 15 %).

Effects of BZ on Michaelis-Menten kinetics of lactate transport

The transport rates during lactate influx and lactate efflux were estimated in mM min^{-1} at various extracellular lactate concentrations between 10 and 80 mM. These fluxes represent total net fluxes. No correction for the contribution of non-ionic diffusion of lactic acid was attempted. Figure 9 shows lactate influx (*A*) and lactate efflux (*B*) plotted against the extracellular lactate concentration in the absence and presence of 0.01 mM BZ in EDL fibres. It is seen that the curves follow Michaelis-Menten kinetics. The maximal transport rates, V_{max}, and the Michaelis-Menten constants, K_m, are summarized in Table 2. In EDL, 0.01 mM BZ reduced the V_{max} of lactate influx from 6.0 ± 0.6 to 3.3 ± 0.4 mM min^{-1} and the V_{max} of lactate efflux from 7.8 ± 1.3 to 4.0 ± 0.7 mM min^{-1}.

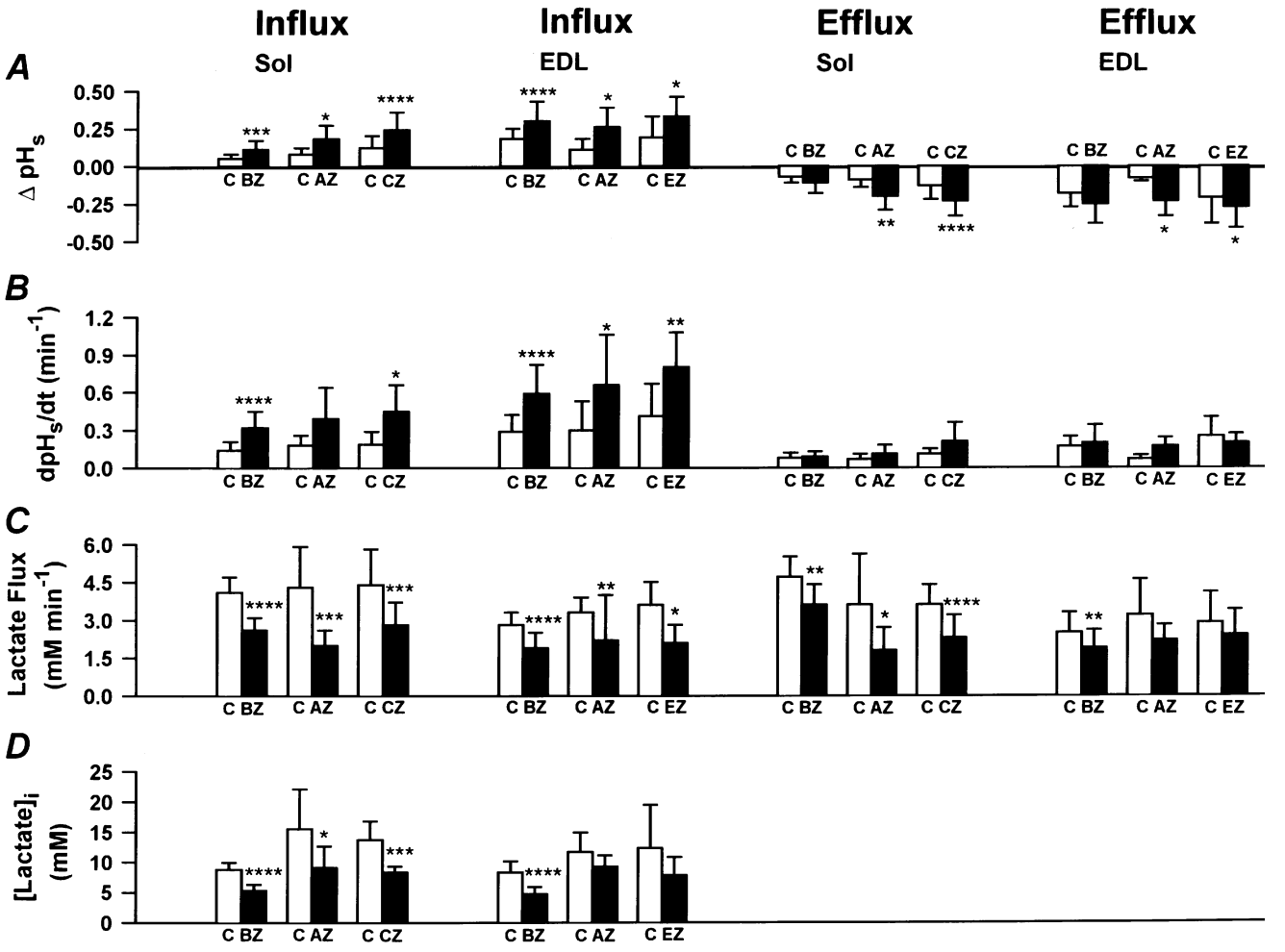

Figure 7. Effects of different CA inhibitors on ΔpH_s, dpH_s/dt, lactate flux and $[lactate]_i$ in soleus and EDL fibres

□, control data (C); ■, data with 0.01 mM BZ ($n = 8$), 0.1 mM AZ ($n = 6$), 0.5 mM CZ ($n = 7$) or 0.1 mM EZ ($n = 4$). Values are means ± S.D. Asterisks indicate levels of significance (Student's paired *t* test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$.

Table 2. Values of the maximal transport rate, V_{max}, and of the Michaelis constant, K_m, of lactic acid transport from fibre bundles of soleus and EDL in the absence and presence of 0.01 mM benzolamide

		Lactate – influx		Lactate – efflux	
		Control	BZ	Control	BZ
EDL	V_{max} (mM min^{-1})	6.0 ± 0.6	3.3 ± 0.4**	7.8 ± 1.3	4.0 ± 0.7*
	K_m (mM)	23 ± 6	20 ± 7	43 ± 16	21 ± 11
Soleus	V_{max} (mM min^{-1})	20 ± 7	15 ± 3	17 ± 2	14 ± 2
	K_m (mM)	56 ± 35	54 ± 23	42 ± 9	42 ± 11

Values of V_{max} and K_m are means ± S.E.M. and were derived from data sets as shown in Fig. 9. The number of fibre bundles studied at each of the lactate concentrations 10, 20, 40, 60 and 80 mM was 4 in the case of EDL and 3 in the case of soleus. Levels of significance of differences in parameters under control conditions and in the presence of BZ are indicated by asterisks and were derived from a statistical analysis of Lineweaver-Burke plots (Sachs, 1999, pp. 553–556): * $P < 0.05$, ** $P < 0.002$.

The K_m values were 23 ± 6 mM for lactate influx and 43 ± 16 mM for lactate efflux. These latter constants were not affected by BZ. In soleus, the maximal transport rates were also reduced by BZ, from 20 ± 7 to 15 ± 3 mM min^{-1} for lactate influx and from 17 ± 2 to 14 ± 2 mM min^{-1} for lactate efflux. In soleus, the K_m values again were not affected by BZ.

DISCUSSION

Critique of the method

Intrinsic (non-HCO_3^-) buffer capacity of the muscle

Table 3 summarizes published values of $BF_{non\text{-}HCO_3}$ as estimated in homogenates that were titrated with HCl/NaOH or with CO_2 showing a variation between 50

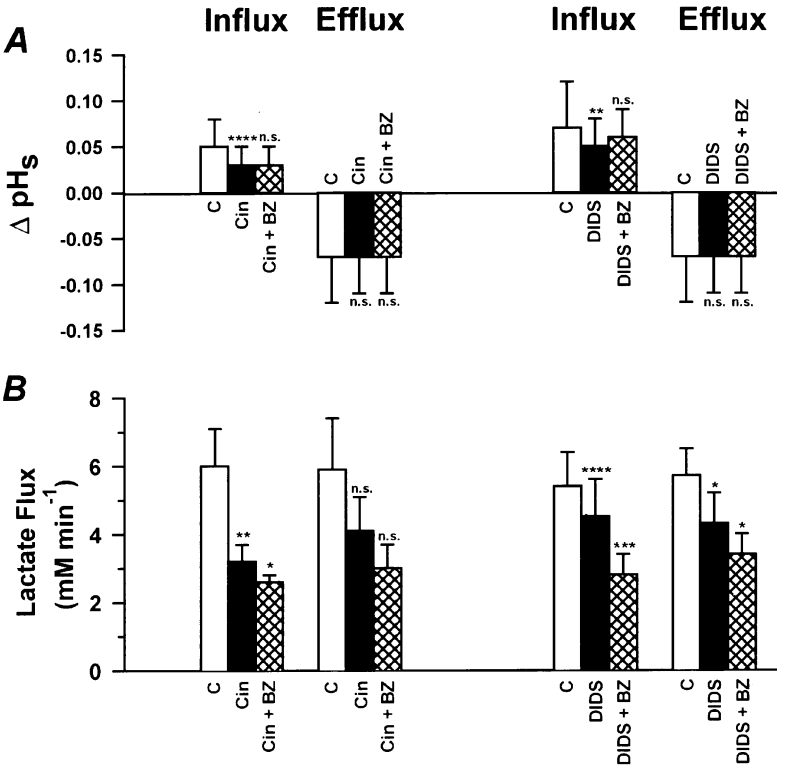

Figure 8. Changes in ΔpH_s and lactate flux in soleus fibres under inhibition of the H^+–lactate cotransporter by cinnamate or DIDS and under inhibition by cinnamate or DIDS plus inhibition of CA by BZ

□, control data (C); ■, data with 10 mM cinnamate (Cin, $n = 4$) or 1 mM DIDS ($n = 7$); ▩, data with 10 mM Cin plus 0.01 mM BZ or with 1 mM DIDS plus 0.01 mM BZ. Values are means ± S.D. Asterisks indicate levels of significance (Student's paired t test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$. n.s., not significant.

Table 3. Non-bicarbonate buffer factors, $BF_{non\text{-}HCO_3}$, of rat skeletal muscles

		$BF_{non\text{-}HCO_3}$ (mequiv H^+ (pH unit)$^{-1}$ (kg cell H_2O)$^{-1}$)	Material	Titrated with	Temp.
Eckel *et al.* (1959)	Skeletal muscle	50–73	Homogenate	HCl/NaOH	37 °C
Heisler & Pieper (1971)	Diaphragm	67	Homogenate	CO_2	37 °C
Lai *et al.* (1973)	Thigh	68	Homogenate	CO_2	37 °C
Bettice *et al.* (1976)	Thigh	60–78	Homogenate	CO_2 content	?
Adams *et al.* (1990)	M. gastrocnemius (white portion)	51	Homogenate	HCl/NaOH	37 °C
This paper	EDL	35 ± 4	Fibre bundle	5 %/10 % CO_2	22 °C
	Soleus	86 ± 16	Fibre bundle	5 %/10 % CO_2	22 °C

and 78 mequiv H^+ (pH unit)$^{-1}$ (kg cell water)$^{-1}$. It is worth mentioning that most homogenates examined in the studies cited consisted of a mixture of slow- and fast-twitch muscles. For the EDL, a fast-twitch muscle, we measured a $BF_{non\text{-}HCO_3}$ of about 35 mequiv H^+ (pH unit)$^{-1}$ (kg cell water)$^{-1}$ which is at the lower end of the buffer capacities described in the literature, while in the soleus, a slow-twitch muscle, we obtained a $BF_{non\text{-}HCO_3}$ of 86 mequiv H^+ (pH unit)$^{-1}$ (kg cell water)$^{-1}$ which is at the upper end of the values in Table 3. Such a large difference between intracellular non-HCO_3^- buffer capacities of fast- and slow-twitch muscles of the same species has not been described before. Data exist only for mouse and exhibit the same tendency: Westerblad & Allen (1992) estimated a value of $BF_{non\text{-}HCO_3}$ of 33.5 mequiv H^+ (pH unit)$^{-1}$ (kg cell water)$^{-1}$ in the fast-twitch flexor brevis, and Aickin & Thomas (1977*b*) reported a value of $BF_{non\text{-}HCO_3}$ of 45 mequiv H^+ (pH unit)$^{-1}$ (kg cell water)$^{-1}$ in the slow-twitch soleus.

Influence of the resting membrane potential

The superficial fibres of the muscle bundles from EDL and soleus usually displayed a resting potential between −45 and −60 mV. In contrast, the fibres of the same bundle which are directly beneath the superficial ones regularly exhibited a resting potential of −80 to −90 mV. The impalement of both intracellular microelectrodes, the membrane potential and the pH_i electrode, in one fibre usually led to a loss of potential of about 8 mV (see Aickin & Thomas, 1977*b*). However, this cannot explain the difference in membrane potential of superficial and deeper fibres. We speculate that the superficial fibres of a bundle might be mechanically irritated by the preparation or by the high rate of superfusion with

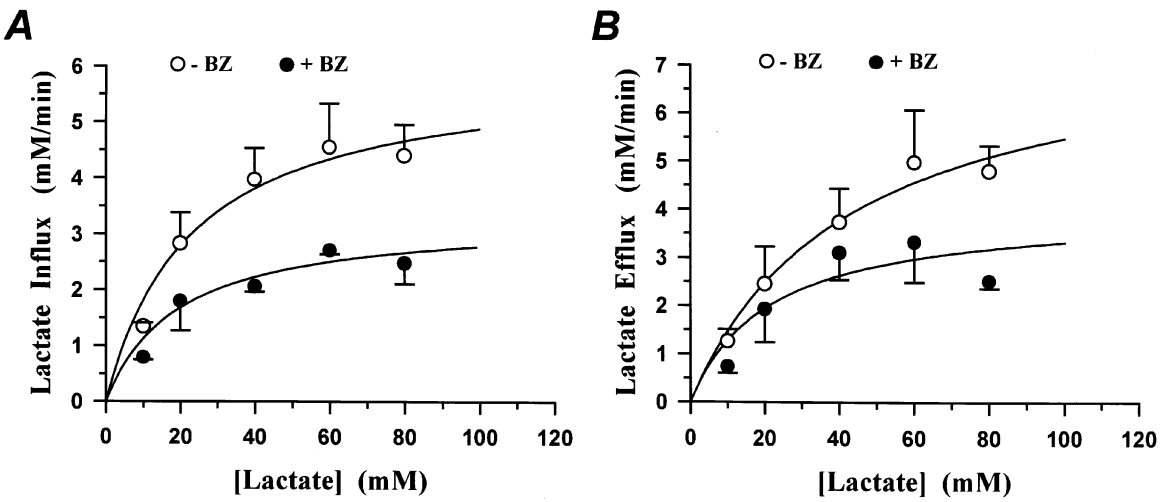

Figure 9. Michaelis-Menten kinetics of lactate fluxes in fibres of the rat EDL in the absence and presence of 0.01 mM BZ

A, lactate influx (mM min^{-1}) in the absence and presence of BZ. *B*, lactate efflux (mM min^{-1}) in the absence and presence of BZ. Abscissa gives extracellular concentration of lactate. Each data point represents the average of measurements on 4 fibre bundles. V_{max} values with and without BZ were significantly different for influx (*A*) and efflux (*B*) (see Table 2).

Ringer solution which is essential to achieve a fast rise and fall in extracellular lactate. Raising the extracellular $CaCl_2$ concentration from 1.3 to 10 mM led to a shift in the resting potential of superficial fibres from −45 to about −70 mV in soleus fibres. This has also been observed by Aickin & Thomas (1977*a*) in mouse soleus fibres. In order to test whether the more negative resting potential affects the transport rate of lactic acid, three fibres of EDL were exposed to 20 mM lactate and 0.01 mM BZ in the presence of 10 mM extracellular $CaCl_2$. The resting potential was -65 ± 2 mV. During lactate uptake BZ led to a reduction of lactate influx by 45% from 3.6 to 2.0 mM min^{-1} and to a reduction in $[lactate]_i$ by 45% from 4.4 to 2.4 mM. In EDL fibres with a resting potential of only -49 ± 4 mV at normal levels of extracellular Ca^{2+}, BZ decreased lactate influx by 32% from 2.8 to 1.9 mM min^{-1} and $[lactate]_i$ by 43% from 8.3 to 4.7 mM (Fig. 7*C* and *D*). It is concluded that the inhibitory effect of BZ on lactate transport does not depend on the resting potential in the range −45 to −65 mV.

Mechanisms of lactic acid permeation in muscle

Cinnamate is a competitive inhibitor of the H^+–lactate cotransporter in skeletal muscle (Roth & Brooks, 1993; Poole & Halestrap, 1993). Cinnamate has been used in several studies to investigate lactic acid transport. Watt *et al.* (1988) and Gladden *et al.* (1995) reported that cinnamate leads to a reduction in lactate transport by 30 and 70%, respectively, in a group of perfused muscles. Studies on soleus from mouse and rat (Vanheel *et al.* 1986; Madureira & Hasson-Voloch, 1988; Juel & Wibrand, 1989; McDermott & Bonen, 1994) demonstrated an inhibition of lactate transport by 4–5 mM cinnamate of between 22 and 55%. The greatest inhibitory effect of 69–90% by 10 mM cinnamate was observed in studies using sarcolemmal vesicles (Roth & Brooks, 1990*a*, 1993; McDermott & Bonen, 1993, 1994; Juel, 1997). In a single fibre of the rat soleus, 10 mM cinnamate in the present study reduced lactate influx by 44% and efflux by 30% (Fig. 2*B*). In a single fibre of the rat EDL, cinnamate inhibited the lactate influx by 37% and efflux by 20% (Fig. 2*B*). These values are closer to those estimated on whole soleus muscles than to those derived from sarcolemmal vesicles. This result may be ascribed to the accessibility of cinnamate to the H^+–lactate cotransporter molecule, which may be higher in sarcolemmal vesicles than in intact fibres of muscle bundles.

An inhibitory effect of DIDS on the transport of lactic acid so far has only been described for sarcolemmal vesicles. Roth & Brooks (1990*a*, 1993) reported an inhibition by 13% in hindlimb muscles from rat. Juel (1997) described an inhibition by 14% in vesicles from slow-twitch but not from fast-twitch rat muscles. The microelectrode technique described here allowed us to investigate separately the effects of DIDS on lactate influx and efflux. In soleus fibres, DIDS reduced lactate influx by 18% and efflux by 16%; in EDL fibres, DIDS inhibited the lactate influx by 27% and had almost no effect on lactate efflux (Fig. 2*B*). The experiments with DIDS in 15 mM Hepes-buffered solutions without CO_2 confirmed these results (see also Fig. 2*B*, right column). In contrast to Juel (1997), our findings demonstrate that DIDS inhibits the lactate transport not only in soleus, but also in EDL with stronger inhibition of lactate uptake than of lactate release.

CA and lactic acid transport in skeletal muscle

Membrane-impermeable extracellular CA inhibitors

At lactate concentrations < 10 mM, 80% of the lactate transport across the sarcolemmal membrane occurs by a cotransport of H^+ and lactate ions at a ratio of 1:1 (Roth & Brooks, 1990*a*,*b*; Juel, 1997), the remainder being transported by diffusion of undissociated lactic acid. However, each pathway requires H^+ and lactate ions in equimolar amounts. The CO_2/HCO_3^- system is the dominant extracellular buffer system capable of delivering H^+ ions for the uptake of lactic acid or of buffering H^+ ions released from the cell. The present results show that the CO_2 hydration/dehydration reaction must be accelerated by an extracellular CA in order to deliver or buffer rapidly enough the H^+ ions to be cotransported with lactate. The inhibition of this CA has two consequences: (1) during uptake of lactic acid the fast delivery of H^+ ions is impaired, which leads to a faster and greater alkalinization on the surface of the fibre and (2)during lactic acid release the fast buffering of H^+ ions is impaired, which leads to a faster and larger acidification on the surface of the muscle fibre. The retarded delivery of extracellular H^+ ions reduces the supply of lactic acid at a given extracellular concentration of lactate which reduces the rate of lactate influx. The decreased extracellular buffering of H^+ ions released from the muscle cell suppresses the rate of lactate efflux because an acid pH builds up on the cell surface, diminishing the transmembrane proton gradient, which has been shown to be important for lactic acid transport (Mainwood & Worsley-Brown, 1975; Watt *et al.* 1988; Juel & Wibrand, 1989; Roth & Brooks, 1990*b*). Also, this situation increases the 'trans-inhibitory' effect of H^+ ions on the H^+–lactate cotransporter molecule (Juel, 1991, 1996). In a manner analogous to its role in skeletal muscle, extracellular CA in brain tissue may facilitate transmembrane fluxes of lactic acid (Kaila & Chesler, 1998).

Membrane-permeable CA inhibitors

The membrane-permeable CA inhibitors CZ and EZ inhibit the extracellular sarcolemmal CA as well as the intracellular CAs: in EDL, the membrane-bound sarcoplasmic reticulum CA (SR-CA) is inhibited and in soleus the SR-CA and the cytoplasmic CA III are both inhibited. In order to investigate whether the intracellular CAs contribute to the transport of lactic acid, the effects of CZ and EZ were compared to those of BZ and AZ (see Fig. 7). This comparison very clearly shows that

both membrane-permeable CA inhibitors exert their inhibitory effects on the pH_s and pH_i signals to an extent quite similar to that of the membrane-impermeable CA inhibitors. We conclude that the intracellular CAs do not contribute to the kinetics of lactic acid transport but that the extracellular CA plays an essential role in the transport process of H^+ and lactate ions. This result is not surprising because non-HCO_3^- buffers exist at high concentrations in the intracellular but not in the extracellular interstitial space (see Table 3). The reduction of lactate influx and efflux rates seen here upon inhibition of muscle CA is in good agreement with the results of Scheuermann *et al.* (2000*a*,*b*), who found a delayed appearance of lactate in plasma during increasing ramp exercise after an acute infusion of AZ.

CA block under inhibition of the H^+–lactate cotransporter by cinnamate or DIDS

DIDS induced an inhibition of lactate influx by 17 %, and BZ led to a further inhibition by 31 % (Fig. 8*B*). In the case of cinnamate, lactate influx was reduced by 47 %, and BZ caused an additional inhibition by 10 % (Fig. 8*B*). BZ alone inhibited the lactate influx by 37 % (Fig. 7*C*). If cinnamate and BZ acted on two different pathways, their inhibitory effects should be additive: 47 % + 37 % = 84 %. However, as is seen in Fig. 8*B*, cinnamate and BZ together inhibited the influx of lactate only by 47 % + 10 % = 57 %. Therefore, it is concluded that cinnamate and BZ act on the same pathway. The implication that BZ is more effective when DIDS, an inhibitor that is less powerful than cinnamate, is present, is compatible with the idea that DIDS and BZ also act on the same pathway. Cinnamate/DIDS directly inhibit the H^+–lactate cotransporter while BZ indirectly inhibits this transporter by slowing down the delivery of extracellular H^+ ions.

Effects of CA inhibition on Michaelis-Menten kinetics

Several authors have determined the Michaelis constant of lactic acid transport in rat skeletal muscle. Madureira & Hasson-Voloch (1988) and McDermott & Bonen (1994) reported K_m values of 11 and 13 mM, respectively, in the soleus muscle and Watt *et al.* (1988) observed a K_m of 21 ± 4 mM in mixed, mainly white, muscles of the rat. Studies on sarcolemmal vesicles of white muscles reported K_m values of 40.1 ± 4.6 mM (Roth & Brooks, 1990*a*), 12.5 mM (McDermott & Bonen, 1994) and of 13–25 mM (Juel, 1991, 1997). Allen & Brooks (1994) found a K_m value of 46.2 ± 6.6 mM for the isolated H^+–lactate carrier which had been reconstituted in proteoliposomes. Table 2 shows that the K_m values determined in the present study for fibres from EDL and soleus range between 23 ± 6 and 56 ± 35 mM. The K_m values determined in the fibres agree very well with those described in the literature, which range from 11 to 46 mM. Juel (1991, 1997) investigated the affinity of the H^+–lactate carrier during lactate uptake and lactate release by sarcolemmal vesicles and found no difference in K_m values. Our results (Table 2) confirm this finding.

It may be noted that, while Fig. 9*A* allows us to correctly determine K_m values, Fig. 9*B* does not. In Fig. 9*B* the initial lactate efflux is plotted *versus* the extracellular lactate concentration at which the muscle cells were loaded. This kind of plot has also been used by other authors who have studied lactate efflux (Juel, 1991; Brown & Brooks, 1994). When we plot the effluxes of Fig. 9*B* *versus* the intracellular lactate concentrations calculated from ΔpH_i, the curve shifts to the left because $[lactate]_i$ is lower than $[lactate]_o$ and no longer exhibits clearly a transport maximum. Therefore, the 'true' K_m values for efflux may be different from the values in Table 2 but cannot be derived from the present data.

As seen in Table 2, BZ does not alter the K_m values. This is compatible with the view that BZ does not directly affect the H^+–lactate cotransporter. However, BZ decreases the maximal transport rate in EDL and soleus (Table 2). The slow-down of the delivery of H^+ ions by CA inhibition appears to limit the maximally possible transport rate of lactic acid uptake. Similarly, the impairment of the buffering of extracellular H^+ ions by CA inhibition, which leads to a build-up of $[H^+]$ on the cell surface and to an increase in the trans-inhibition of the H^+–lactate carrier, is responsible for the decrease in the maximal transport rate of lactic acid release.

Effects of cinnamate, DIDS and the CA inhibitors on ΔpH_i

ΔpH_i was reduced by 45 % by cinnamate, by 22–27 % by DIDS and by about 40 % by all four CA inhibitors. This means that all these inhibitors reduced $[lactate]_i$ under steady-state conditions. For example, in soleus, $[lactate]_i$ was reduced from 10.7 to 6.8 mM by cinnamate, from 11.4 to 9.1 mM by DIDS and from 8.8 to 5.3 mM by BZ. How can the reduction of $[lactate]_i$ be explained? If $[lactate]_i$ were not in equilibrium but determined by the balance between lactate utilization in the muscle cell and lactate influx, it would be conceivable that $[lactate]_i$ decreases when lactate influx is inhibited. From the resting O_2 consumption it can be estimated that the observed initial (= maximal) influx rates of lactate are > 50 times greater than the maximal rates of lactate oxidation under resting conditions. We conclude from this that under the present conditions lactate utilization does not markedly affect $[lactate]_i$. Thus, the latter is expected to represent the equilibrium distribution of lactate across the cell membrane. The equilibrium distribution of lactic acid across the sarcolemmal membrane is mainly determined by the distribution of H^+ (see e.g. Roos, 1975; Roth & Brooks, 1990*a*). Assuming $pH_s = 7.4$, $pH_i = 7.1$ and extracellular [lactate] = 20 mM, $[lactate]_i$ is calculated to be 10 mM if it were strictly distributed according to pH. On the other hand, a potential-dependent distribution of lactate will give a $[lactate]_i$ of 2.2 mM with a resting potential of −50 mV and an extracellular [lactate] of 20 mM. So, in the latter case $[lactate]_i$ will be much smaller. Roos (1975) showed that depolarization of the

membrane potential by elevation of the extracellular K^+ concentration leads to an increase in lactate uptake by one-tenth. This fraction is normally too small to significantly affect lactic acid distribution, which is dominated by the pH dependency. But if the H^+–lactate transporter is inhibited, e.g. by cinnamate, the permeability of lactic acid will be reduced whereas the potential-dependent permeation of lactate will be unaffected. Thus, the distribution of lactic acid will shift from being pH dependent to being more potential dependent. This shift will lead to a smaller $[lactate]_i$. We speculate that this shift is the reason why cinnamate, DIDS and the CA inhibitors reduce $[lactate]_i$ under steady-state conditions. In the case of the CA inhibitors, there is another, additional explanation. Figures 5 and 6 show that in the presence of BZ, pH_s is obviously more alkaline when the pH_i signal has achieved its plateau than in the absence of BZ. This elevated pH_s at a given pH_i will reduce the equilibrium concentration of lactic acid in the cell, i.e. $[lactate]_i$.

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