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METABOLIC DEPRESSION DURING ENVIRONMENTAL STRESS: THE ROLE OF EXTRACELLULAR *VERSUS* INTRACELLULAR pH IN *SIPUNCULUS NUDUS*

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Summary

Environmental stresses such as hypoxia or hypercapnia are known to cause acid–base disturbances and in several organisms they lead to metabolic depression. The present study was undertaken to quantify the influence of these changes in acid–base parameters on metabolic rate. We determined the rate of oxygen consumption in a non-perfused preparation of the body wall musculature of the marine worm *Sipunculus nudus* at various levels of extra- and intracellular pH (pHe and pHi, respectively), P_{CO_2} and $[HCO_3^-]$. The acid–base status of the tissue was modified and clamped by long-term exposure to media set to specific values of extracellular pH, P_{CO_2} and $[HCO_3^-]$. At a pHe of 7.90, which is equivalent to the normoxic normocapnic *in vivo* extracellular pH, and an ambient P_{CO_2} of 0.03 kPa (control conditions), pHi was 7.26±0.02 (mean ± S.D., N=5). A reduction of extracellular pH from 7.90 to 7.20 resulted in a significant decrease of pHi to 7.17±0.05 at 0.03 kPa P_{CO_2} (normocapnia) and to 7.20±0.02 at 1.01 kPa P_{CO_2} (hypercapnia). At the same time, the rate of oxygen consumption of the tissue was significantly depressed by 18.7±4.7 % and 17.7±3.0 %, respectively. A significant depression of oxygen consumption by 13.7±4.7 % also occurred under hypercapnia at pHe 7.55 when pHi was elevated above control values (7.32±0.01). No significant changes in oxygen consumption were observed when pHe was either drastically elevated to 8.70 under normocapnia (pHi 7.36±0.05) or maintained at 7.90 during hypercapnia (pHi 7.37±0.03). ATP and phospho-L-arginine concentrations, as well as the Gibbs free energy change of ATP hydrolysis ($dG/d\xi_{ATP}$), were maintained at high levels during all treatments, indicating an equilibrium between energy supply and demand. We conclude that the depression of aerobic energy turnover in isolated body wall musculature of *S. nudus* is induced by low extracellular pH. A model is proposed which could explain a reduced ATP cost of pHi regulation during extracellular acidosis, thus contributing to metabolic depression.

Key words: intracellular pH, extracellular pH, hypercapnia, energetics, metabolic depression, sipunculid, *Sipunculus nudus*.

Introduction

The acid–base status of an animal is affected by a range of functional and environmental stresses, the pattern of change depending upon the effects of these stressors on the mode and rate of metabolism, respiration and the mechanisms of H^+-equivalent ion exchange. In a feedback reaction, acid–base parameters, especially pH, are seen to affect metabolic processes and are also considered to be relevant to the overall rate of energy turnover. An acid shift of intracellular pH was early on postulated to be a prime factor depressing metabolic rate during anaerobiosis (Busa and Nuccitelli, 1984). Metabolic depression occurring under environmental hypercapnia (Barnhart and McMahon, 1988; Hand and Gnaiger, 1988; Rees and Hand, 1990) is also attributed to a concomitant drop in intracellular pH. Interest has generally focused on intracellular pH since the activity of enzymes as well as many other cellular processes are highly pH-dependent. However, the changes in intra- and extracellular acid–base parameters during environmental hypercapnia are complex, and in an earlier study we demonstrated that, in *Sipunculus nudus*, the aerobic metabolic rate remained depressed at a time when the intracellular acidosis was fully compensated (H. O. Pörtner, A. Reipschläger and N. Heisler, in preparation). A key role of pHi in metabolic depression has also been questioned by Brooks and Storey (1989).

The aim of the present study was to examine the specific influences of each individual acid–base parameter, i.e. intra- and extracellular pH, P_{CO_2} and $[HCO_3^-]$, on the aerobic metabolic rate of isolated body wall musculature of *Sipunculus nudus*. The use of isolated body wall musculature which, compared with most vertebrate muscle preparations, has the advantage of being

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viable as a non-perfused preparation, allowed us to define the extracellular acid–base status and thereby control and clamp intracellular steady-state acid–base parameters. Oxygen consumption rates of the tissue were determined under a variety of physiological conditions, including normocapnic extra- and intracellular acidosis and alkalosis and uncompensated as well as compensated hypercapnic acidosis. We were able to demonstrate that extracellular rather than intracellular pH is the parameter inducing metabolic depression in isolated body wall musculature of *S. nudus*.

Materials and methods

Animals

Large specimens (20–40 g) of *Sipunculus nudus* were dug up from sandy sediments of the intertidal zone in Locquémeau, Brittany, France, in February 1994. The animals were kept in aquaria with aerated artificial sea water and a bottom layer of sand (15–25 cm) at 11–15 °C for periods of up to several weeks.

Experimental procedure

For the preparation of isolated body wall musculature, individuals were killed by 'decapitating' them behind the base of the introvert retractor muscles. The animals were opened dorsally and all inner organs including the ventral nerve cord were removed. The body wall musculature was cut longitudinally to obtain two identical halves. One half was used for one experiment. The tissue was punctured at the edges with a fine needle and fixed with thread onto a plastic frame. Each tissue preparation was first subjected to 12 h of normocapnic control incubation at pH 7.90. The incubations were carried out in filtered 34‰ artificial sea water (Wiegand, Germany) with 0.1 g l^{-1} streptomycin, 10^5 i.u. l^{-1} penicillin and 0.01 mol l^{-1} TEA/HCl at a temperature of 15±0.5 °C. For each incubation, tissues from 1–3 animals were placed together in a volume of 2 l, equilibrated and bubbled continuously with 40 % air and 60 % nitrogen supplied by a gas-mixing pump (2M303/a-F, Wösthoff, Germany). A hypoxic gas mixture was used because normoxic P_{O_2} levels can be damaging to this sediment-dwelling animal. After 12 h of incubation, the control rate of oxygen consumption was determined at pHe 7.90. Subsequently, each tissue was subjected to a second incubation period of 24 h under normocapnic (0.03 kPa P_{CO_2}) or hypercapnic (1.01 kPa P_{CO_2}) conditions in media of different pH values. Normocapnic incubations (40 % air/60 % nitrogen) were carried out at pH 7.20, 7.90 or 8.70, and hypercapnic incubations (40 % air/59 % nitrogen/1 % CO_2) at pH 7.20, 7.55 or 7.90. Prior to the incubation of the tissue, hypercapnic solutions were equilibrated with the gas mixture for several hours. pH was then readjusted by addition of the appropriate amounts of solid $NaHCO_3$ calculated from the Henderson–Hasselbalch equation using a value of pK‴ determined according to Heisler (1986). pH was checked at the beginning and at the end of the experiment and confirmed to be within ±0.03 units of the initial value. After 24 h of incubation in the different media, the rate of oxygen consumption of the tissue was determined for a second time. At the end of the experiments, the tissue was freeze-clamped and stored under liquid nitrogen for further analyses. Water samples were taken and stored under liquid nitrogen for later analysis of the total content of CO_2.

Analyses

Oxygen consumption rates were determined by closed-system respirometry in a 93 ml respiration chamber equipped with a polarographic oxygen sensor (Eschweiler, Germany). The chambers were filled with solutions identical to those in which the previous incubations had been carried out. Oxygen consumption was recorded for 1–2 h. The determination of oxygen consumption rates in chambers without tissue showed that bacterial growth was completely inhibited by the added antibiotics.

Intracellular acid–base parameters were determined according to Pörtner *et al.* (1990). Total CO_2 (C_{CO_2}) was measured using a gas chromatograph (Hach Carle, USA) and apparent HCO_3^- concentrations were calculated as $[HCO_3^-] = C_{CO_2} - \alpha_{CO_2} \times P_{CO_2}$ using values of pK‴ and of the solubility coefficient α_{CO_2} determined according to Heisler (1986).

Samples of the body wall musculature were ground under liquid nitrogen and extracted in ice-cold perchloric acid as described by Beis and Newsholme (1975). The extracts were pH-neutralized with 5 mol l^{-1} KOH and solid $K_2CO_3/KHCO_3$ (1:6 w/w). ATP was analyzed according to Bergmeyer (1984), phospho-L-arginine and L-arginine according to Grieshaber *et al.* (1978) and inorganic phosphate was assayed according to Pörtner (1990). Levels of free ADP and AMP were calculated from the equilibria of the arginine kinase and myokinase reactions. The Gibbs free energy change of ATP hydrolysis ($dG/d\xi_{ATP}$) was calculated on the basis of the determined metabolite concentrations and pHi values as outlined by Pörtner *et al.* (1993, 1996*a*). For these calculations, a constant free cellular Mg^{2+} level of 1 mmol l^{-1} was assumed.

Statistics

For each treatment, the oxygen consumption rates under control and experimental conditions were compared using Student's *t*-test for paired values. Oxygen consumption rates are expressed as a percentage of the respective control value at pHe 7.90 and 0.03 kPa P_{CO_2} in order to facilitate comparison between different treatments. Intracellular pH values, intracellular bicarbonate concentrations and tissue metabolite levels were analyzed using one-way analysis of variance (ANOVA). When a significant difference between treatments was indicated by the ANOVA, each treatment was compared with the control treatment (pHe 7.90, 0.03 kPa P_{CO_2}) using Student–Newman–Keuls *post-hoc* test. In all cases, $P<0.05$ was accepted to indicate a significant difference.

All values are presented as means ± S.D., $N=5$.

Results

Modification of intracellular acid–base parameters

Intracellular pH and apparent HCO_3^- concentration could

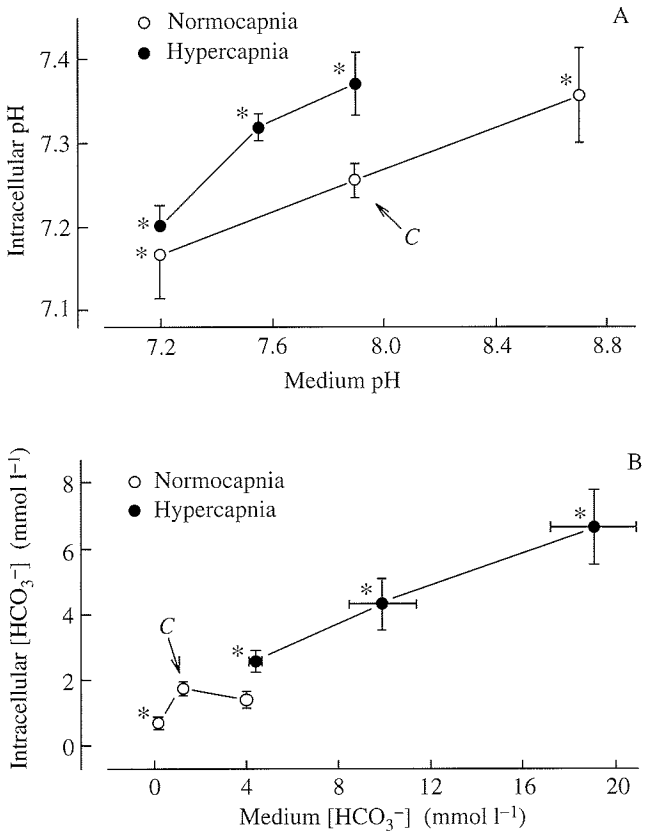

Fig. 1. (A) Intracellular pH values (pHi) of *Sipunculus nudus* isolated body wall musculature after long-term exposure of the tissue to media of different pH. pHi increases with medium pH (pHe). Normo- and hypercapnia give rise to different pHi/pHe relationships. (B) Intracellular HCO_3^- concentrations of isolated body wall musculature exposed to media containing different extracellular HCO_3^- concentrations. *C*, control; values are means ± S.D., *N*=5, * indicates a significant difference from the respective control value.

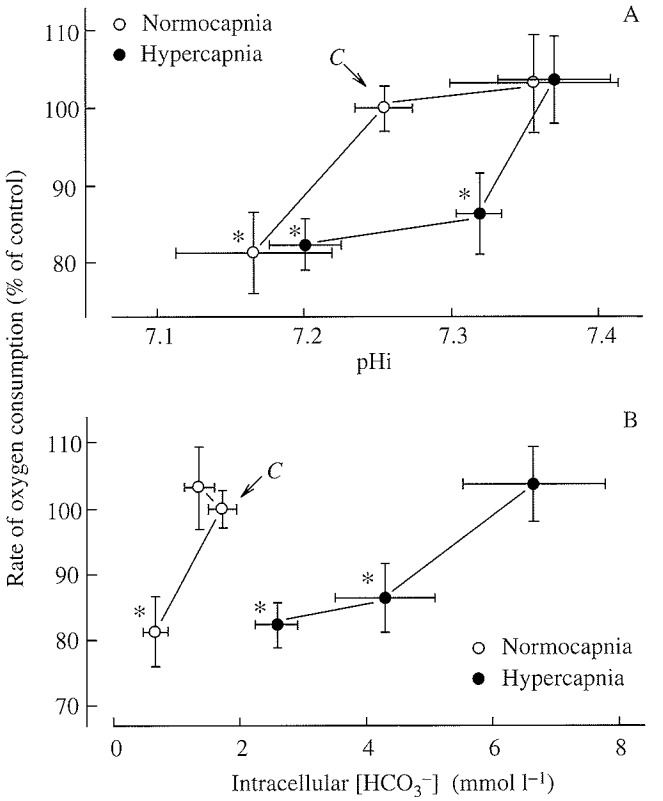

Fig. 2. (A) Oxygen consumption rates of *Sipunculus nudus* isolated body wall musculature at different values of intracellular pH (pHi) during normocapnia and hypercapnia. (B) Intracellular HCO_3^- concentrations and oxygen consumption rates of isolated body wall musculature under the same conditions. *C*, control; *oxygen consumption rate significantly different from the control value; values are means ± S.D., *N*=5.

be set to specific values by choosing the appropriate 'extracellular' parameters for the incubation medium. The changes in tissue acid–base parameters with those of the medium are shown in Fig. 1A,B. pHi increased with medium pH (Fig. 1A). At a low pHe of 7.20, pHi was 7.17±0.05 during normocapnia and 7.20±0.02 during hypercapnia. Increasing pHe led to a larger increase in pHi under hypercapnia than under normocapnia. During hypercapnia, pHi was elevated to 7.37±0.03 at pHe 7.90, while during normocapnia pHe had to be set to 8.70 to reach a comparable pHi value of 7.36±0.05. This difference is obviously due to the differing extra- and intracellular HCO_3^- concentrations under hypercapnia and normocapnia (Fig. 1B). Extracellular HCO_3^- concentrations under hypercapnia had to be severalfold higher than under normocapnia to reach the same extracellular pH. At a given medium pH, the dependence of intracellular on extracellular HCO_3^- concentrations (Fig. 1B) led to higher intracellular HCO_3^- concentrations and pH values under hypercapnia.

Dependence of rates of oxygen consumption on acid–base status

Fig. 2A shows the rate of oxygen consumption as a function of pHi. At low pHi (≤7.20), oxygen consumption was significantly depressed to about 80 % of control values under both hypercapnia and normocapnia. High pHi values between 7.35 and 7.40 were associated with a slight, but insignificant, elevation of oxygen consumption rates above control values, regardless of hypercapnic or normocapnic conditions. The normocapnic control tissue (pHi=7.26±0.02) exhibited a higher metabolic rate than the hypercapnic tissue at pHi 7.32±0.01, which showed a depression similar to that of the tissues at low pHi.

Fig. 2B depicts the relationships between oxygen consumption rates and intracellular HCO_3^- concentrations. The HCO_3^- levels of the tissues differed considerably between normo- and hypercapnia, and an overall correlation between intracellular HCO_3^- concentrations and oxygen consumption rates was not observed. However, within each treatment, falling intracellular HCO_3^- concentrations were associated with a reduced rate of oxygen consumption of the tissue.

Finally, the relationship between pHe and the oxygen

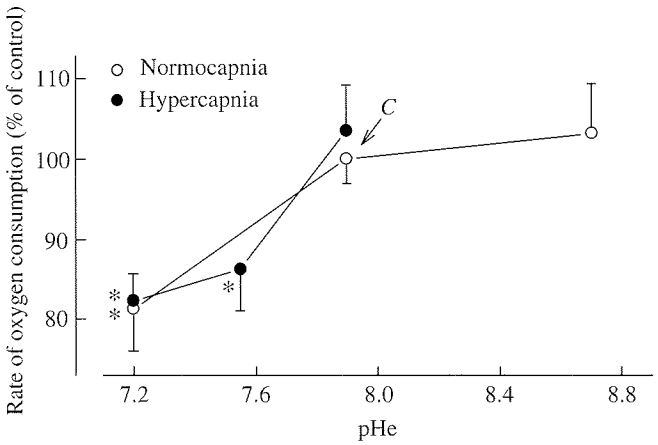

Fig. 3. Oxygen consumption rates of *Sipunculus nudus* isolated body wall musculature as a function of extracellular pH (pHe). *C*, control; *oxygen consumption rate significantly different from the control value; values are means ± S.D., *N*=5. This plot gives a congruent picture for normo- and hypercapnic data: the oxygen consumption rate of the tissue is significantly depressed below a threshold value of pHe.

consumption rates of the tissue preparations (Fig. 3) shows that the depression of oxygen consumption depends upon low values of pHe. Below a threshold value of pHe, the oxygen consumption rate is linearly reduced with falling pHe. It should be noted that this is the only congruent relationship (including all data points) that can be established between oxygen consumption rates and one of the six intra- and extracellular acid–base parameters.

Concentrations of high-energy phosphates and Gibbs free energy change of ATP hydrolysis

Table 1 shows the concentrations of ATP, phospho-L-arginine, L-arginine, inorganic phosphate, free ADP and free AMP in the body wall musculature under the different treatments. We also calculated the ratio of phospho-L-arginine to L-arginine plus phospho-L-arginine concentrations, and the free energy change of ATP hydrolysis ($dG/d\xi_{ATP}$). None of the treatments led to significant changes in the concentrations of the high-energy phosphates (ATP and phospho-L-arginine), and both the free energy change of ATP hydrolysis and the other parameters remained unaffected.

Discussion

In the present study, we examined the effects of intra- and extracellular pH, P_{CO_2} and $[HCO_3^-]$ on the aerobic metabolic rate of *S. nudus* at the tissue level. The use of isolated body wall musculature allows us to determine whether cellular mechanisms are involved in the depression of aerobic metabolic rate that occurs *in vivo* under environmental hypercapnia in *S. nudus* (H. O. Pörtner, A. Reipschläger and N. Heisler, in preparation) and to identify the acid–base parameter(s) eliciting metabolic depression.

Isolated tissue preparations of the body wall musculature were subjected to hypercapnic conditions at pHe 7.20, 7.55 or 7.90. The first two values were chosen according to *in vivo* data for *S. nudus* exposed to environmental hypercapnia. A pHe of 7.20 represents the *in vivo* pHe found during acute uncompensated hypercapnia, and pHe 7.55 represents the steady-state pHe value reached after compensation of the hypercapnic acidosis (H. O. Pörtner, A. Reipschläger and N. Heisler, in preparation). At both pHe values, we measured a significant depression of oxygen consumption rate in isolated body wall musculature during hypercapnia (Fig. 3). At pHe 7.20, the average rate of consumption was depressed by 17.7 % and at pHe 7.55 it was depressed by 13.7 %. *In vivo* measurements of oxygen consumption rate during environmental hypercapnia of 1.01 kPa P_{CO_2} demonstrated a comparable depression of 22 % (H. O. Pörtner, A. Reipschläger and N. Heisler, in preparation). It can therefore be concluded that at least part of this response is evoked at the cellular level and not by systemic mechanisms.

Table 1. *Intracellular pH values and the concentrations of ATP, phospho-L-arginine (PLA), L-arginine (L-Arg), inorganic phosphate (P_i), free ADP and free AMP in the body wall musculature of* Sipunculus nudus *after 24 h of incubation at different values of extracellular pH during normocapnia (0.03 kPa ambient P_{CO_2}) and hypercapnia (1.01 kPa ambient P_{CO_2})*

Condition	pHe	pHi	ATP (μmol g^{-1})	PLA (μmol g^{-1})	L-Arg (μmol g^{-1})	P_i (μmol g^{-1})	Free ADP (nmol g^{-1})	Free AMP (nmol g^{-1})	[PLA]/([PLA]+[L-Arg])	$dG/d\xi_{ATP}$ (kJ mol^{-1})
Normocapnia (control)	7.90	7.26±0.02	2.45±0.41	44.54±6.11	1.81±0.24	0.73±0.65	5.91±1.89	0.017±0.010	0.96±0.01	−66.31±4.72
Normocapnia	7.20	7.17±0.05*	2.77±0.59	45.63±4.30	2.04±0.28	0.77±0.46	5.88±1.39	0.014±0.004	0.96±0.01	−65.65±4.20
Normocapnia	8.70	7.36±0.05*	2.26±0.27	48.62±6.83	1.60±0.27	1.66±1.04	5.25±1.15	0.016±0.009	0.97±0.01	−63.73±2.79
Hypercapnia	7.20	7.20±0.02*	2.65±0.68	45.25±3.87	1.99±0.33	0.59±0.40	6.00±1.96	0.016±0.007	0.96±0.01	−65.59±2.76
Hypercapnia	7.55	7.32±0.01*	2.52±0.45	53.86±5.94	2.11±0.29	0.85±0.21	6.71±2.07	0.021±0.009	0.96±0.01	−63.83±1.00
Hypercapnia	7.90	7.37±0.03*	3.24±0.43	52.55±7.31	2.19±0.62	1.18±0.64	10.14±3.59	0.040±0.024	0.96±0.01	−63.73±2.98

The levels of free ADP and free AMP were calculated from the equilibrium of myokinase reaction.

Also given are the ratio of phospho-L-arginine to L-arginine plus phospho-L-arginine concentrations and the free energy change of ATP hydrolysis ($dG/d\xi_{ATP}$).

* indicates a significant difference from the respective control value (means ± S.D., *N*=5).

Apart from free AMP and ATP levels, which are given in nmol g^{-1} fresh mass, all other concentrations are in μmol g^{-1} fresh mass.

Our data show that the depression of aerobic metabolic rate during hypercapnia at pHe 7.20 and 7.55 cannot be attributed to a low pHi (Figs 1A, 2A). pHi values were low (7.20±0.02) at pHe 7.20, but reached a value of 7.32±0.01 at pHe 7.55 and were thus significantly elevated above the control value of 7.26±0.02. This result is in accordance with results obtained from intact *S. nudus*, where the oxygen consumption rate remained depressed when the intracellular acidosis was fully compensated (H. O. Pörtner, A. Reipschläger and N. Heisler, in preparation). As a corollary, pHi can be excluded as the regulatory signal which elicits metabolic depression during hypercapnia in *S. nudus*.

P_{CO_2} and $[HCO_3^-]$ can also be excluded as parameters responsible for metabolic down-regulation. If there were a direct effect of P_{CO_2} on energy turnover, it would be present in all tissues exposed to 1.01 kPa P_{CO_2}. However, the hypercapnic tissues incubated at a high pHe of 7.90 (pHi 7.37) did not show a reduction but instead a slight, although insignificant, elevation of their oxygen consumption rate (Fig. 3). Elevated intra- or extracellular HCO_3^- concentrations during hypercapnia also exert no depressing effect; Figs 1B, 2B demonstrate that the hypercapnic tissues showed no metabolic depression at high $[HCO_3^-]_e$ or $[HCO_3^-]_i$. Finally, as indicated by Fig. 3, it can be concluded that the parameter inducing a depression of energy turnover in the isolated body wall musculature of *S. nudus* is extracellular pH. A low extracellular pH is correlated with a depressed oxygen consumption rate during both hypercapnia and normocapnia.

To our knowledge, this is the first study presenting evidence for a role of extracellular pH in metabolic depression. Whether our findings are also valid for other organisms remains to be established. We have discriminated between the effects of the individual intra- and extracellular acid–base parameters on metabolic rate and energy status. A comparable study by Walsh *et al.* (1988) focused on the influence of acid–base parameters on lactate metabolism in rainbow trout hepatocytes. In that study, separation of the effects of pHe and pHi was not possible since pHi changed with pHe. Lactate utilization was depressed by a combined decrease in pHe and pHi as well as by increased P_{CO_2}, while high bicarbonate levels at high pH stimulated lactate metabolism. Barnhart (1992) reported that 1 h of *in vitro* hypercapnia (high P_{CO_2}, low pHe, elevated extracellular $[HCO_3^-]$) failed to depress the oxygen consumption rates of isolated hepatopancreas and mantle tissue from dormant snails *Otala lactea*. As metabolic depression in this species occurs only under hypercapnia *in vivo*, the author concluded that it is mediated by the presence of some factor absent *in vitro*. However, the time span of 1 h that was used in the study appears very short. It should be emphasized that our results were obtained when the tissues had reached a new steady state under the experimental conditions (H. O. Pörtner, A. Reipschläger and N. Heisler, in preparation). Arguably, short-term exposures would have given different results. Acute acidosis, for instance, has been shown to induce an increased rate of oxygen consumption in myocytes of the turtle *Chrysemys picta belli*, but oxygen consumption recovered to the control value within 1 h. The transient increase in the rate of oxygen consumption could be attributed to elevated activities of the Na^+/H^+ exchanger and Na^+/K^+-ATPase (Watson *et al.* 1994). Similarly, transition to steady-state hypercapnia in *S. nudus in vivo* was associated with a transient decrease in the levels of the phosphagen phospho-L-arginine and the free energy change of ATP hydrolysis, indicating a transient rise in energy demand (H. O. Pörtner, A. Reipschläger and N. Heisler, in preparation).

During all treatments, the high-energy phosphates ATP and phospho-L-arginine were maintained at high levels in the body wall musculature preparations. Protons are participants in the arginine kinase and creatine kinase reactions, both of which are considered to operate close to thermodynamic equilibrium. Nevertheless, our results show that changes in pHi were not reflected by significant changes in the concentrations of the other participants of the arginine kinase reaction, i.e. ATP, phospho-L-arginine, L-arginine and ADP. Long-term acidosis also did not influence the free energy change of ATP hydrolysis ($dG/d\xi_{ATP}$) in the isolated body wall musculature of *S. nudus*. This contrasts with recent results obtained for the isolated radula protractor muscle of the gastropod *Busycon canaliculatum* (Combs and Ellington, 1995). Here, extra- and intracellular acidosis were accompanied by a decrease in $dG/d\xi_{ATP}$ and also by a reduced phospho-L-arginine level, both lasting for the total experimental period of 6 h. However, the acidosis employed in the latter study was more severe than in the present study, and the 5,5-dimethyl-2,4-oxazolidinedione (DMO) load of the tissue may have led to elevated energy requirements owing to an increase in proton permeability caused by the weak acid distribution behaviour of DMO. In contrast, proton movements are not involved in the distribution of CO_2 (or NH_3) between body compartments. A decrease in $dG/d\xi_{ATP}$ with acidosis also occurred in various vertebrate muscles during ischaemia (Kammermeier *et al.* 1982). In addition, different studies on both invertebrate and vertebrate muscles reported a pH-induced fall in the tissue levels of phospho-L-arginine or phosphocreatine while ATP levels remained unchanged (Ellington, 1985; Sahlin *et al.* 1983; Zhou *et al.* 1991). Other studies, however, obtained similar results to those reported here for the isolated body wall musculature of *S. nudus*: the high-energy phosphates were maintained at high levels during acidosis in giant muscle fibres of the barnacle (Hamm and Yue, 1987), in the perfused ferret heart (Vandenberg *et al.* 1994) and in cat biceps and soleus muscles (Adams *et al.* 1991). The maintenance of high-energy phosphate levels and $dG/d\xi_{ATP}$ that we observed in the body wall musculature tissue of *S. nudus* during normocapnic and hypercapnic acidosis is very probably related to the simultaneous metabolic depression, which may protect phosphagen levels during periods of acidosis.

Which mechanisms could be involved in our finding that a low extracellular pH leads to a significant depression of aerobic metabolic rate? The reduced energy demand is likely to be associated with ion-regulatory processes since, in isolated tissue, the interaction of extracellular protons with systemic signals affecting intracellular processes as well as effects on muscular activity can be excluded. Effects of extracellular pH on membrane ion channels and/or transport proteins leading to

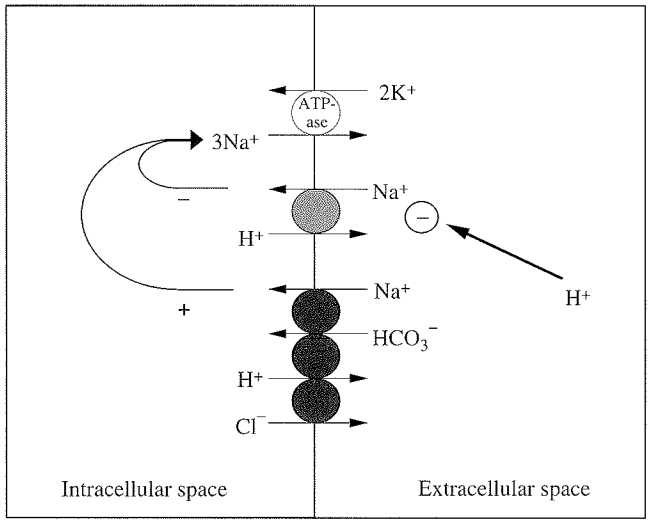

Fig. 4. Model proposed to account for a reduced energy turnover rate at low extracellular pH. Extracellular protons inhibit (–) the Na^+/H^+ exchanger. As a consequence, the $Na^+/H^+/Cl^-/HCO_3^-$ exchanger becomes increasingly involved in pHi regulation. This exchanger transports two moles of acid–base equivalents per mole of Na^+. The reduced requirement of acid–base regulation for Na^+ leads to a reduction in Na^+/K^+-ATPase activity. The $Na^+/H^+/Cl^-/HCO_3^-$ exchanger represents one mechanism of electroneutral Na^+-dependent Cl^-/HCO_3^- exchange, and, in the present context, it is functionally equivalent to the other exchange mechanisms that can account for electroneutral Na^+-dependent Cl^-/HCO_3^- exchange, i.e. (i) $Na^+ + 2HCO_3^-$ for Cl^-, (ii) $Na^+ + CO_3^{2-}$ for Cl^- and (iii) the $NaCO_3^-$ ion pair for Cl^-.

changes in membrane conductance and/or ion transport rates may be involved in metabolic depression in our preparation of isolated body wall musculature. As a working hypothesis, we propose a model that could explain a lowered energy demand of pHi regulation and a thus decreased metabolic rate during extracellular acidosis (Fig. 4). pHi regulation under steady-state conditions is achieved by ion exchange between intra- and extracellular compartments. The transport systems that continuously extrude protons from the cell, thus maintaining pHi, all operate against the electrochemical gradient. With a few exceptions, such as H^+-ATPase, H^+ exchangers are driven by the Na^+ gradient and are energetically balanced by the action of Na^+/K^+-ATPase. The stoichiometry of transported acid–base equivalents and transported Na^+ differs and is characteristic for each transporter. This also implies differences in the ATP demand of each transport system. A shift in transport activities from the ubiquitous Na^+/H^+ exchanger (Madshus, 1988) to a Na^+-dependent Cl^-/HCO_3^- exchanger such as the $Na^+/H^+/Cl^-/HCO_3^-$ exchanger (described for many invertebrates, Thomas, 1977; Boron *et al.* 1981; Thomas and Schlue, 1986; Galler and Moser, 1986; Wheatly and Henry, 1992) could lead to an immense reduction of the ATP cost of pHi regulation (up to 50 %) and might be involved in metabolic depression under hypercapnia and, generally, under conditions of decreased pHe. The shift can be induced by low pHe since the Na^+/H^+ exchanger has been reported to be inhibited by high extracellular H^+ concentrations in a competitive or mixed manner, or even in a non-competitive manner (Grinstein and Rothstein, 1986). We are aware that except for the evidence supporting a crucial role of pHe in metabolic depression our data do not provide direct support for the above model. Instead of kinetic modifications of ion transport rates, transporter densities could be down- or up-regulated. Future studies must show whether this model holds true.

Nevertheless, previous data concerning pHe and pHi regulation during anaerobiosis in *S. nudus* might indicate a possible benefit of keeping pHe low. Individuals exhibiting different anaerobic metabolic rates (mainly due to seasonal variations), all exposed to 24 h of anaerobiosis, revealed the following pattern of pH regulation at different anaerobic metabolic rates. Intracellular pH decreased as a result of metabolic proton formation, but did not fall below a minimum value. At the same time, the amount of protons transferred to the extracellular space increased linearly with metabolic rate, and extracellular pH fell progressively depending on the metabolic rate. The transfer of proton equivalents from the extracellular space to the ambient water has been shown to be small and independent of metabolic rate under anaerobiosis (Pörtner *et al.* 1991; see Pörtner, 1987). Taken together, these results indicate that pHi is regulated (at a set point approximately 0.2–0.3 units below the normoxic value) at the expense of pHe (Pörtner, 1993). Extracellular pH drops and is poorly regulated even at high anaerobic energy turnover rates. The reduction in pHe could counteract high acidifying rates of anaerobic metabolism and very probably supports the reduction of ATP turnover observed during anaerobiosis (Hardewig *et al.* 1991). In contrast to *S. nudus*, pHi drops to a larger extent during anoxia in some other facultative anaerobes, with an exceptionally large decrease from 7.7 to 6.7 in anoxic *Artemia franciscana* embryos (Kwast *et al.* 1995). Such a large drop in pHi may then contribute to metabolic depression.

In summary, we provided evidence for a key role of extracellular pH in metabolic depression in *S. nudus*. In support of our findings, the importance of extracellular pH in the regulation of systemic and cellular functions is becoming increasingly clear: Tian *et al.* (1995) reported that, in isolated rat cerebral arteries, vasodilation in response to hypercapnia is induced by extracellular and not by intracellular acidosis. Extracellular pH has also been shown to modulate membrane potential in the tubular cells of frog kidney (Belachgar *et al.* 1995). Future studies must reveal whether the importance of extracellular pH changes for metabolic regulation is as high in other systems as we have demonstrated for *Sipunculus nudus*.

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