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Effects of environmental hypercapnia on animal physiology: A ^{13}C NMR study of protein synthesis rates in the marine invertebrate *Sipunculus nudus*

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Abstract

Global climate change is associated with a progressive rise in ocean CO_2 concentrations (hypercapnia) and, consequently, a drop in seawater pH. However, a comprehensive picture of the physiological mechanisms affected by chronic CO_2 stress in marine biota is still lacking. Here we present an analysis of protein biosynthesis rates in isolated muscle of the marine invertebrate *Sipunculus nudus*, a sediment dwelling worm living at various water depths. We followed the incorporation of ^{13}C-labelled phenylalanine into muscular protein via high-resolution NMR spectroscopy. Protein synthesis decreased by about 60% at a medium pH of 6.70 and a consequently lowered intracellular pH (pHi). The decrease in protein synthesis rates is much stronger than the concomitant suppression of protein degradation (60% versus 10–15%) possibly posing a threat to the cellular homeostasis of structural as well as functional proteins. Considering the progressive rise in ocean CO_2 concentrations, permanent disturbances of cellular protein turnover might seriously affect growth and reproductive performance in many marine organisms with as yet unexplored impacts on species density and composition in marine ecosystems.

Keywords: Hypercapnia; Protein synthesis; ^{13}C NMR spectroscopy; Marine invertebrate; Respiratory acidosis; Metabolic depression

1. Introduction

Growth of living organisms is usually defined as an increase in cell number and/or cell size in parallel with a positive change in caloric content (Mommsen and Moon, 2001). This long-term process largely depends on cellular protein synthesis, builds a key prerequisite for successful reproduction and thus supports the maintenance of populations. During individual growth, protein synthesis will exceed protein breakdown. Once net growth has ceased, protein turnover is in a steady state where most functional proteins are continually replaced. The energetic expenditure that is required to fuel this process is enormously high: calculations by Hawkins (1991) demonstrated a minimal fraction of about 20% for the contribution of protein synthesis to whole body maintenance metabolism in a wide range of species, comprising mammals, fish and mussels. Numerous biotic and abiotic factors control the partitioning of resources into growth ranging from the individuals' genetic background through animal density and food quality to temperature and environmental pollutants (Mommsen and Moon, 2001).

Under conditions of environmental stress, many organisms display specific strategies of metabolic energy conservation and extended passive survival that involve the depression of both energy producing and energy consuming cellular processes (for a review see Hand and Hardewig, 1996). In the light of its large contribution to maintenance costs protein synthesis is one target for inhibition during metabolic depression. Oxygen limitation, for instance, has been demonstrated to depress protein synthesis in rat liver (Surks and Berkowitz, 1971). Protozoan cells like *Tetrahymena* decrease protein synthesis by up to 70% during starvation (Cuny et al., 1985).

Hypercapnia (elevated CO_2 partial pressure) correlates with adverse factors like hypoxia and elevated temperature and, until now, represents a transient stress factor in some marine environments like the sediments of the intertidal zone (Diaz and Rosenburg, 1995), hypoxic bottom waters (Knoll et al., 1996) and

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intertidal pools (Truchot and Duhamel-Jouve, 1980). Currently rising concentrations of CO_2 in atmosphere and surface waters (Haugan and Drange, 1996; Brewer, 1997; Caldeira and Wickett, 2003) as well as anticipated scenarios of anthropogenic CO_2 disposal in the deep sea (Marchetti, 1979; Auerbach et al., 1996) indicate that hypercapnia will play a more important and permanent role in shaping the structure and functioning of marine ecosystems. Understanding the effects of CO_2 requires a thorough understanding of the physiological mechanisms through which CO_2 exerts its effects on organismic, population and thus, ecosystem functioning (Pörtner et al., 2004, 2005).

In marine invertebrates, hypercapnia has been found to elicit metabolic depression (through an acidification of body fluids; Reipschläger et al., 1997; Pörtner et al., 1998, 2000) as well as growth reductions, observed by Shirayama (2002), Michaelidis et al. (2005). An extreme case of hypercapnia and/or anoxia induced hypometabolism can be found in brine shrimp (*Artemia franciscana*) embryos associated with an almost complete shutdown of protein synthesis due to global arrest of translation and a drastic reduction of transcription (Hofmann and Hand, 1994; Van Breukelen et al., 2000). However, the effects of hypercapnia alone on protein synthesis have neither been studied in adult brine shrimp nor in marine ectotherms in general and the effective physiological parameters have not been identified. In this context, the question needs to be addressed, whether the effects of CO_2 on growth occur through an imbalance between whole body protein synthesis and degradation.

The present paper addresses the effects of elevated CO_2 levels on protein synthesis in the marine invertebrate, *Sipunuclus nudus*, a model species in previous efforts to investigate CO_2 effects in a non-calcifying organism. *S. nudus* is adapted to regular CO_2 oscillations in some of its natural habitats. Previous studies have focused on short to medium-term effects such as acid–base regulation and metabolic rate (see above) or changes in aerobic energy metabolism of specific tissues (Langenbuch and Pörtner, 2002, 2003). Long-term consequences include enhanced mortality (Langenbuch and Pörtner, 2004), possibly due to disturbances of protein metabolism. A decrease in N-excretion and in O/N ratios of isolated muscle tissue paralleled metabolic depression at reduced extracellular pH (pHe) and suggested associated changes in amino acid catabolism (Langenbuch and Pörtner, 2002).

In the present study we analysed the cellular background of metabolic depression at high water PCO_2 with a focus on the possible down-regulation of protein biosynthesis. To this end we measured the incorporation of ^{13}C-labelled phenylalanine (Phe) into the cellular protein pool. We report a pH-dependent decline in protein biosynthesis rates at high CO_2 levels. Results are

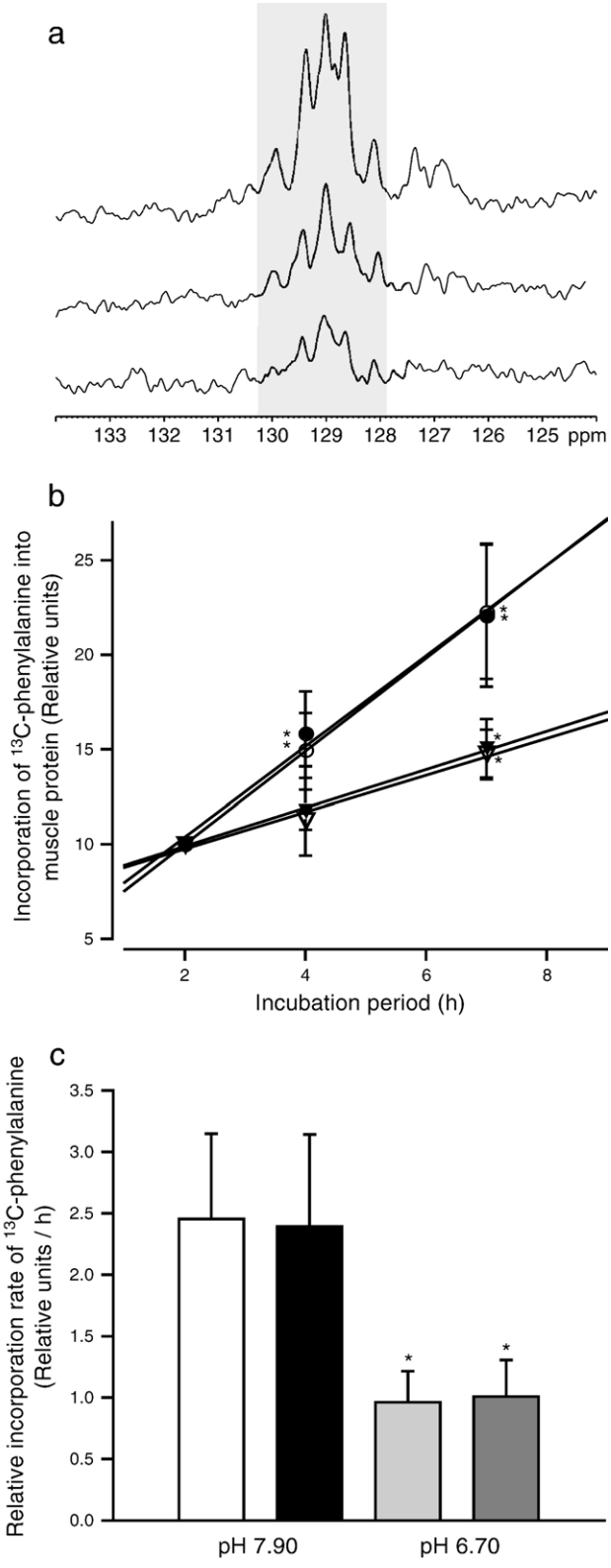

Fig. 1. Impact of PCO_2 and pHe on incorporation of ^{13}C labelled phenylalanine into muscle protein. a) Intercepts of ^{13}C NMR spectra of protein extracts from tissue samples of one animal depicting increasing signals of aromatic carbon nuclei from incorporated ^{13}C-Phe after labelling periods of 2 h, 4 h and 7 h, respectively. Peak area has been integrated over the range of the shaded area. b) Incorporation of labelled Phe into muscle protein over time. The relative amount of ^{13}C-Phe as computed from peak area integration of protein ^{13}C NMR spectra is plotted over time. Different symbols represent results for various experimental pH/PCO_2 conditions (i.e. filled circle, pH 7.90, PCO_2 0.01 kPa; hollow circle, pH 7.90, PCO_2 1.01 kPa; filled triangle, pH 6.70, PCO_2 0.03 kPa, hollow triangle, pH 6.70, PCO_2 1.01 kPa). * Indicates values of incorporated ^{13}C-Phe significantly different from the respective control value after 2 h of labelled substrate incubation. c) Relative incorporation rates of ^{13}C-Phe into muscle protein as calculated from regression lines in Fig. 1b. White and black bars represent values at control pH 7.90 and low (0.03 kPa) or high (1.01 kPa) PCO_2, respectively; light and dark grey bars depict the respective data for pH 6.70. Note that the significant 60% decrease (indicated by *) in incorporation rates is (as in b) directly attributable to the pH treatment without any influence of the respective PCO_2 level.