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INCREASED CONCENTRATIONS OF HAEMOLYMPH Mg^{2+} PROTECT INTRACELLULAR pH AND ATP LEVELS DURING TEMPERATURE STRESS AND ANOXIA IN THE COMMON SHRIMP *CRANGON CRANGON*

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Summary

The effects of temperature (0, 5, 10, 15 and 20 °C) and of anoxia (at 5 °C) on extracellular Mg^{2+} concentration ($[Mg^{2+}]_e$), intracellular pH (pHi) and ATP and lactate levels were investigated in intermoult adults of the common shrimp *Crangon crangon*. All animals caught in summer (summer animals) showed a slight but significant increase in $[Mg^{2+}]_e$ at low temperatures. In contrast, at every temperature tested, a few of the animals caught in winter (winter animals) showed elevated $[Mg^{2+}]_e$ during short-term (4 h) but not during long-term (6 days) incubations. The reasons for the overshoot in Mg^{2+} concentrations in individual animals remain unexplained, but a protective effect of extracellular Mg^{2+} on intracellular pH and on ATP concentrations was visible at haemolymph Mg^{2+} concentrations above 15 mmol l^{-1}. The influence of high extracellular $[Mg^{2+}]$ on pHi and intracellular ATP and lactate levels under normoxic and anoxic conditions was tested using an incubation medium containing 150–250 mmol l^{-1} Mg^{2+}. When haemolymph Mg^{2+} levels were manipulated by exposure of the animal to high levels of Mg^{2+} in the external medium, animals with a haemolymph $[Mg^{2+}]$ below the threshold concentration of 15 mmol l^{-1} had significantly lower values of intracellular pH than animals with haemolymph $[Mg^{2+}]$ above 15 mmol l^{-1}. In addition, the elevation of haemolymph $[Mg^{2+}]$ by incubation in high-$[Mg^{2+}]$ water prevented the drop in pHi and the rise in lactate levels induced by anoxia. The protective effect of high levels of extracellular Mg^{2+} did not depend upon the $[Ca^{2+}]/[Mg^{2+}]$ ratio but only on $[Mg^{2+}]_e$. However, experiments with isolated muscle tissues showed no dependence of muscle intracellular pH on $[Mg^{2+}]_e$ under both normoxic and anoxic conditions, leading to the conclusion that the protective effect is evoked *via* a central, possibly anaesthetising, effect of high $[Mg^{2+}]_e$. The dependence of pHi and muscle [ATP] on extracellular $[Mg^{2+}]$ resembles the protective effect of high Mg^{2+} levels on the post-ischaemic mammalian heart.

Key words: Mg^{2+}, pHi, ATP, muscle, anoxia, Crustacea, *Crangon crangon.*

Introduction

Shrimps are highly active, using their pleopods for steady swimming, but they are also capable of burst escape movements with the help of their well-developed tail muscle. They usually exhibit low extracellular Mg^{2+} concentrations (Hagerman, 1971, 1973, 1978), which accords with a number of publications describing a negative linear relationship between the level of activity and haemolymph $[Mg^{2+}]$ in crustaceans (Katz, 1936; Robertson, 1939, 1949, 1953; Hagerman, 1973; Walters and Uglow, 1981; Morritt, 1989; Spicer *et al.* 1990; Morritt and Spicer, 1993). Although there are a few exceptions to the rule (for example, see Gross, 1964; Tentori and Lockwood, 1990), this inverse correlation between $[Mg^{2+}]_e$ and activity is not surprising since Katz (1936) and Waterman (1941) pointed out that high extracellular levels of Mg^{2+} block neuromuscular transmission in *Carcinus maenas*. Accordingly, artificial sea water with elevated $[Mg^{2+}]$ has been used for decades as a narcotising agent in the immobilisation of marine invertebrates (Pantin, 1946). Decapods with high Mg^{2+} levels in the blood (up to 50 mmol l^{-1}), such as *Maia squinado*, *Hyas araneus*, *Lithodes maia* and *Dromia* sp., are thought to live in a 'semi-narcotised' state (Robertson, 1953). To achieve a high level of activity, $[Mg^{2+}]$ is usually kept low in the haemolymph compared with the sea water, and thus requires active regulation. In decapod crustaceans, Mg^{2+} is excreted *via* the antennal gland (Cornell, 1979), thus maintaining low blood concentrations and elevated levels in the urine.

In spite of the biological relevance of Mg^{2+}, little is known about Mg^{2+} homeostasis in crustaceans. Furthermore, information about temporal changes in the Mg^{2+} content of

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the haemolymph of crustaceans and their biological relevance is scarce. Exposure to low temperatures in the sandhopper *Talitrus saltator* was characterised by elevated $[Mg^{2+}]_e$ and the onset of inactivity, followed or induced by a reduction in oxygen uptake (Spicer *et al.* 1994). This finding is in general agreement with field investigations, where winter animals that remained inactive in high shore burrows had significantly higher Mg^{2+} levels (approximately 15 mmol l^{-1}) than active animals collected during summer (Morritt, 1989; Spicer *et al.* 1990). During the life cycle of crustaceans, periods of relative inactivity are also characteristic of certain states of the moulting cycle, and elevated Mg^{2+} concentrations in the haemolymph are found (Hagerman, 1973) prior to and immediately after ecdysis, when inactivity is required to enable the renewal of the exoskeleton. The elevation of haemolymph $[Mg^{2+}]$ during ecdysis is probably due to an increased uptake of seawater ions in the pre-ecdysis stage (Hagerman, 1973). Although an inverse correlation between activity levels and haemolymph $[Mg^{2+}]$ is well-established, the question of whether $[Mg^{2+}]_e$ initiates the reduction of activity in winter or is the passive consequence of a temperature-induced decrease in metabolic rate remains unanswered. It is possible that the high Mg^{2+} levels observed in winter are a passive consequence of low-temperature inhibition of an active excretion process.

In vertebrates, numerous studies have shown that plasma Mg^{2+} concentration is negatively correlated with cardiovascular diseases such as ischaemia, arrhythmias and hypertension (Altura and Altura, 1981; Iseri and French, 1984; Sheehan and Seelig, 1984; Borchgrevink *et al.* 1989; Murphy *et al.* 1991). Low extracellular Mg^{2+} levels induced deficits in intracellular free $[Mg^{2+}]$, ischaemia, depletion of high-energy phosphates and cardiac failure (Altura *et al.* 1993), while high (approximately 15 mmol l^{-1}) $[Mg^{2+}]_e$ during reperfusion of the ischaemic rat heart enhanced the rate of recovery of ATP and creatine phosphate levels, and of pHi and coronary blood flow (Borchgrevink *et al.* 1989).

The present study was designed to investigate potential relationships between environmental change and the regulation of Mg^{2+} levels in crustaceans by examining fluctuations in Mg^{2+} levels during temperature change and anoxia in the common shrimp *Crangon crangon* and by considering their functional role in whole animals and isolated tissues. The observed fluctuations in $[Mg^{2+}]_e$ gave us the opportunity to examine whether high extracellular Mg^{2+} concentrations have a protective effect on pHi and energy metabolism in the tail muscle as reported for the vertebrate heart.

Materials and methods

Specimens of *Crangon crangon* (L.) were collected from shallow-water areas of the Wadden Sea close to Neuharlingersiel, Lower Saxony (1.5–6 m; water temperature 4 °C in winter, 15 °C in summer). In the laboratory, the shrimps were held at the appropriate temperature in natural sea water (32 ‰ salinity) in large recirculated aquaria containing a 1–2 cm layer of sand. They were fed twice a week with pieces of *Arenicola marina* and fish. The animals were allowed to acclimate for at least 2 weeks prior to experimentation. Females with eggs were not used in the experiments, and the animals were not fed for 3 days prior to anoxic (1 h) treatments, experiments investigating activity and short-term (4 h) incubations at various temperatures. During long-term exposure to individual temperatures (6 days), the shrimps were fed prior to the start of incubation and on the third day, such that sampling always occurred 3 days after feeding stopped. All incubations were performed in darkened aquaria to reduce the spontaneous activity of the animals. Winter animals were exposed to various temperatures (0, 5, 10, 15 and 20 °C) for 4 h (N=5–8) and 6 days (N=5–8). To investigate the elevated haemolymph Mg^{2+} levels found in individual animals and to test the evidence for a link between high extracellular $[Mg^{2+}]$ and pHi, the following series of experiments was performed. (1) Temperature incubations (2, 5, 10, 15, 20, 25 °C) with animals caught in summer (N=5–8). During exposure to changing temperatures, the animals were kept in aquaria with natural, filtered sea water and subjected to a sudden change in temperature by transferring them to an aquarium at the required temperature. (2) To test responses to medium containing a high $[Mg^{2+}]$, the shrimps (summer animals) were incubated in artificial sea water containing elevated Mg^{2+} concentrations (150 or 250 mmol l^{-1}). Water pH was adjusted to 7.8 and the incubations were performed at 15 °C for different periods. (3) The effect of elevated extracellular $[Mg^{2+}]$ on anaerobic metabolism was investigated by anoxic incubation of resting individuals (winter animals, N=5–8) for 1 h in artificial sea water containing 50 mmol l^{-1} or 150 mmol l^{-1} Mg^{2+}, a concentration sufficient to cause a rise in $[Mg^{2+}]_e$ to levels above the threshold concentration of 15 mmol l^{-1} within 1 h. Anoxic conditions were achieved by bubbling a constant stream of gaseous nitrogen through the water beginning 30 min prior to the start of the incubation. (4) To investigate the possible influence of activity on $[Mg^{2+}]_e$, escape swimming was elicited by electrical stimulation as described by Onnen and Zebe (1982). Electrical stimulation (30 V; duration 1 ms; frequency 1 s^{-1}) was applied until the animals were exhausted (winter animals, N=5–8).

Prior to haemolymph sampling, the animals were gently dried using a soft paper tissue. Haemolymph samples for the measurement of extracellular $[Mg^{2+}]$ and $[Ca^{2+}]$ were obtained by inserting a glass capillary into the pericardium. After sampling, the tail of the shrimp was cut off and the muscle squeezed out of the exoskeleton as rapidly as possible. It was then divided in two parts, freeze-clamped (Wollenberger *et al.* 1960) and stored under liquid nitrogen until analyzed.

Half the frozen tissue was ground to a fine powder in a mortar cooled by liquid nitrogen before extraction in a four- to fivefold volume of 0.6 mmol l^{-1} perchloric acid (PCA) according to Beis and Newsholm (1975) modified after Pette and Reichmann (1982). The supernatant was neutralised

(pH 7.5) and buffered by adding 5 mmol l^{-1} KOH and a mixture of solid $KHCO_3/K_2CO_3$. After centrifugation, the supernatant was divided into two parts and stored at −80 °C. ATP concentrations were determined enzymatically (Bergmeyer, 1984). Intracellular pH was measured in the second half of the frozen tissue using the homogenate technique described by Pörtner *et al.* (1990).

The levels of Mg^{2+} and Ca^{2+} in the haemolymph of animals were measured using a Dionex Bio LC ion chromatograph (Idstein, Germany) at 28 °C equipped with a CS-3 column equilibrated with 30 mmol l^{-1} HCl and 6 mmol l^{-1} diamino-propionic acid monohydrochloride (DAPHCl). Background conductivity was reduced using a CMMS-1 suppressor perfused with tetrabutylammonium hydroxide (TBAOH, 100 mmol l^{-1}) at 1.7 ml min^{-1}. Mg^{2+} concentrations in the haemolymph of animals incubated with differing levels of external Mg^{2+} were estimated using a photometric kit (Merckotest Magnesium, Merck, Darmstadt) tested to yield results identical to those from ion chromatography.

In the experiments involving isolated muscle tissues, the tail was cut off, the exoskeleton removed and the tissue sliced into pieces. In a preliminary experiment, the size of tissue slice needed to prevent muscle tissue from becoming ischaemic was determined by monitoring intracellular pH over time. Oxygen consumption measurements were carried out in a flow-through respirometer, described by Oeschger *et al.* (1992), and these confirmed that the isolated muscle remained viable for 12 h in aerated artificial saline. During the incubation period, no change in the rate of oxygen consumption was observed. Saline concentrations of inorganic ions were adjusted to the levels found in the haemolymph of control animals (Sartoris and Pörtner, 1996) with the addition of 5 mmol l^{-1} glucose and 5 mmol l^{-1} Hepes at pH 7.8. Concentrations of Mg^{2+} in the saline of between 0 and 50 mmol l^{-1} were chosen for normoxic incubation, and these were adjusted to 5 and 25 mmol l^{-1} during anoxic incubations (1 h). At the end of the incubation period, the muscle was freeze-clamped and stored under liquid nitrogen until analysed.

Changes in the levels of ATP, Ca^{2+}, Mg^{2+} and lactate and in pHi during incubations at various temperatures, under anoxia, in media containing various levels of Mg^{2+} and in animals exercised to exhaustion were tested for significance at the 5% level by one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn *post hoc* test. For a conservative estimate, statistical significance of differences in pHi between individuals with high and low $[Mg^{2+}]_e$ were also tested at the 1% level using one-way ANOVA (Super-Anova, Abacus Concept Inc. Berkeley).

Results

Extracellular $[Mg^{2+}]$ under control conditions (4 °C) was 9.97±6.75 mmol l^{-1} (mean ± S.D., N=7) (Fig. 1A). Incubations at different temperatures for 4 h resulted in an apparent increase in $[Mg^{2+}]_e$ at 0, 10 and 15 °C, while $[Mg^{2+}]$ remained unchanged after 4 h at 20 °C. However, the increase was significant only at 15 °C. The inter-individual variability was rather high. For example, at 15 °C, individual values ranged between 5.5 and 47.3 mmol l^{-1}. After an incubation period of 6 days at 0 and 10 °C, Mg^{2+} levels were typical of control values (Hagerman, 1973, 1978; Sartoris and Pörtner, 1996), with a small inter-individual variability (for example, 5.79±0.93 mmol l^{-1} at 0 °C and 5.55±1.55 mmol l^{-1} at 10 °C, mean ± S.D., N=6) (Fig. 1A).

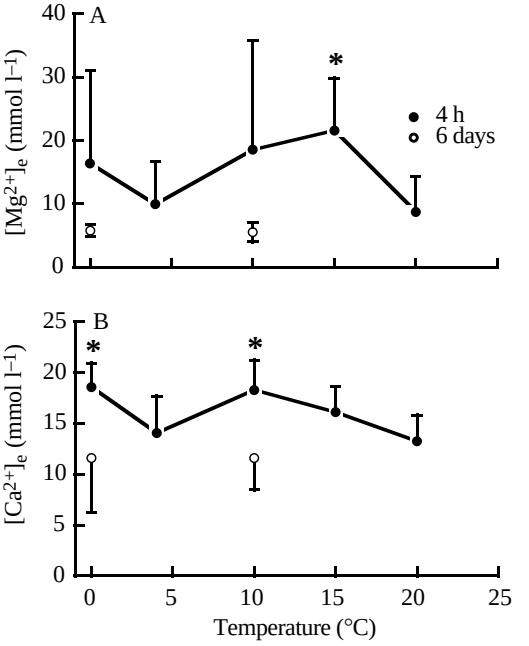

Fig. 1. Extracellular Mg^{2+} (A) and Ca^{2+} (B) concentrations as a function of temperature and incubation time. Each value represents a mean ± S.D. of at least five animals. * indicates a significant difference (P<0.05) compared with control levels at 4 °C.

The response of haemolymph Ca^{2+} levels at different incubation temperatures was similar to the change in $[Mg^{2+}]_e$, although there was a far smaller inter-individual variability after 4 h of exposure (Fig. 1B). The increase in Ca^{2+} levels was significant at 0 and 10 °C, but not at 15 °C, and $[Ca^{2+}]$ remained unchanged at 20 °C. There was an apparent decrease in haemolymph $[Ca^{2+}]$ after 6 days of exposure to 0 and 10 °C, similar to the apparent temperature-induced drop seen for $[Mg^{2+}]$, but this decrease was also not statistically significant.

Since Mg^{2+} is an anaesthetic that depresses neuromuscular transmission by competing with Ca^{2+} for binding sites, the $[Ca^{2+}]/[Mg^{2+}]$ ratio rather than $[Mg^{2+}]$ itself might be relevant. Owing to the high inter-individual variability in haemolymph $[Mg^{2+}]$, an evaluation of individual ratios appeared more useful than an evaluation of mean values. Fig. 2A shows $[Ca^{2+}]/[Mg^{2+}]$ in relation to pHi. Although there is a trend for the ratio to increase with pHi, there is no significant correlation between these parameters. This is in contrast to the relationship between $[Mg^{2+}]_e$ and pHi shown

in Fig. 2B, in which pHi remains relatively constant at approximately pH 7.4 at $[Mg^{2+}]$ values above 15 mmol l^{-1}. This is true both for exposure to various temperatures and for incubations in media with high $[Mg^{2+}]$ (Fig. 2B). Owing to the large inter-individual variability, temperature incubations were repeated with a second group of animals that differed from the first group only in being caught in summer (water temperature approximately 15 °C). In contrast to Fig. 1, $[Mg^{2+}]$ remained below 10 mmol l^{-1} (Fig. 3A). In animals exercised to exhaustion by electrical stimulation, extracellular Mg^{2+} levels increased significantly (P<0.05) compared with the controls, but still remained below 10 mmol l^{-1} (Fig. 3B). Anoxic incubation had no effect on $[Mg^{2+}]_e$ in animals incubated in sea water containing 50 mmol l^{-1} Mg^{2+}, whereas incubation in water containing 150 mmol l^{-1} Mg^{2+} resulted in elevated extracellular Mg^{2+} levels of approximately 24 mmol l^{-1} (Fig. 3B). Since a large fraction of the intracellular Mg^{2+} is bound to adenylates, we examined whether ATP levels and extracellular $[Mg^{2+}]$ are also correlated. A positive correlation (Fig. 4, y=0.073x+2.514, r=0.447, N=44, P<0.01) exists, but a threshold concentration of $[Mg^{2+}]$ as demonstrated for the relationship between pHi and $[Mg^{2+}]$ could not be determined.

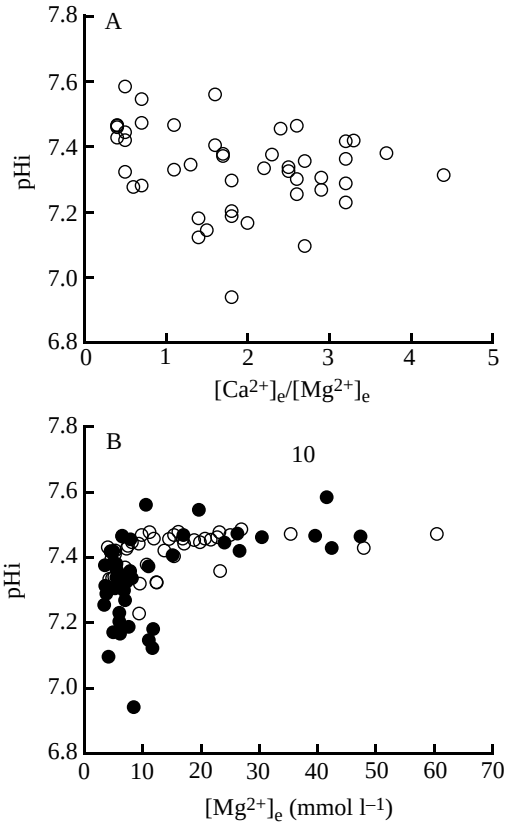

Fig. 2. (A) Relationship between intracellular pH values and the $[Ca^{2+}]/[Mg^{2+}]$ ratio in the haemolymph of *Crangon crangon*. No significant correlation was detected (P>0.05, r=0.261, N=45). (B) Relationship between extracellular $[Mg^{2+}]$ and intracellular pH in whole animals (filled circles, temperature incubation series; open circles, incubation in media with elevated $[Mg^{2+}]$, 150–250 mmol l^{-1} Mg^{2+}). Both incubation series revealed a threshold level of Mg^{2+} at approximately 15 mmol l^{-1} above which pHi values were significantly different from the control (P<0.01).

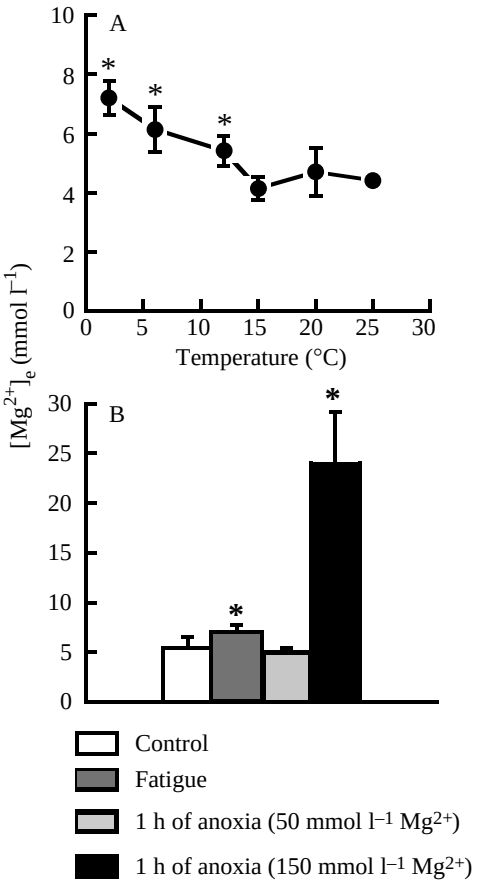

Fig. 3. (A) Extracellular $[Mg^{2+}]$ as a function of temperature after 4 h of incubation. Each value represents a mean ± S.D. of at least five animals. * indicates a significant difference (P<0.05) compared with control levels at 15 °C. (B) Extracellular $[Mg^{2+}]$ as a result of different treatments. Electrical stimulation was applied until the animals were exhausted (fatigue), whereas anoxic incubations were performed in different media for 1 h. Each value represents a mean + S.D. of at least five animals. * indicates a significant difference (P<0.05) compared with control levels.

Compared with the controls at 4 °C, ATP levels tended to decrease at high and low temperature, but this decrease was only significant at 20 °C (Fig. 5A). ATP concentrations and pHi were positively correlated, as shown in Fig. 5B (y=0.0429x+7.196, r=0.598, N=47, P<0.001). Anoxic incubation caused intracellular ATP content to increase significantly (Fig. 6B) in medium with a high $[Mg^{2+}]$ and in medium with a low $[Mg^{2+}]$. Intracellular lactate levels were significantly increased in animals incubated in the 'low'-$[Mg^{2+}]$ saline (50 mmol l^{-1} Mg^{2+}) and, as a consequence, pHi decreased significantly in these animals owing to proton production by anaerobic glycolysis. In contrast, pHi and lactate levels remained unchanged under anoxic conditions in animals incubated in high-$[Mg^{2+}]$ saline (150 mmol l^{-1} Mg^{2+}) (Fig. 6A,C). Unlike the dependence of pHi on

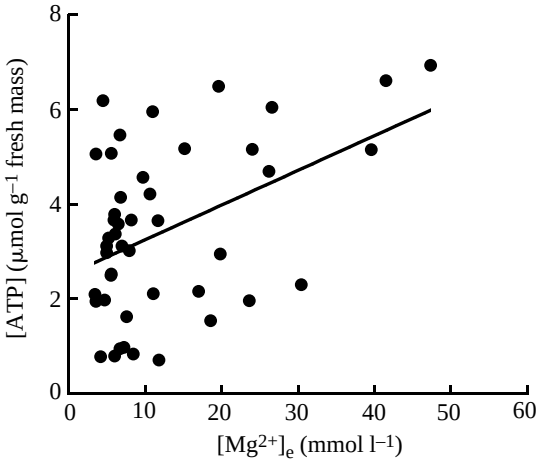

Fig. 4. Tissue ATP levels as a function of extracellular Mg^{2+} concentration measured during temperature incubations. Linear regression yielded the following significant relationship: y=0.073x+2.514 (r=0.447, P<0.01, N=44).

$[Mg^{2+}]_e$ in intact animals, isolated muscle did not show any correlation between the Mg^{2+} concentration in the saline and pHi after 4 h of normoxic or 1 h of anoxic exposure (Table 1).

Discussion

Short-term exposure to various temperatures in winter animals caused extracellular Mg^{2+} levels to rise to exceptionally high levels in some specimens, giving an enormous inter-individual variability (Fig. 1A). This variability was greatly reduced in summer animals, and the general trend for $[Mg^{2+}]_e$ to rise at low temperatures was confirmed (Fig. 3A). Information about temporal changes in $[Mg^{2+}]_e$ in crustaceans is very scarce and cannot provide any explanation for the large individual differences found in the winter animals. However, since summer animals did not show such variability, it is possible that seasonal variations in metabolic rate, as reported for *Palaemon serratus* by Thebault and Raffin (1991), could explain our results. An increase in $[Mg^{2+}]_e$ has been reported during the moulting cycle both prior to and immediately after ecdysis (Hagerman, 1973; Towle and Mangum, 1985) and as a consequence of adaptation to low temperature (Spicer *et al.* 1994). In this study, we have only used animals that are in the intermoult stage, as indicated by a hard exoskeleton, and so the stage of the moulting cycle could not account for observations of high $[Mg^{2+}]$. In contrast to the findings of Spicer *et al.* (1994) in *Talitrus saltator* and the results collected from the summer animals, the increase in Mg^{2+} levels at all temperatures was a consequence of the temperature change itself rather than a result of acclimation to low temperature. The results obtained from summer animals confirm that it is unlikely for *Crangon crangon* to experience torpor in winter, a result supported by the fact that the animals caught in winter were actively swimming. In addition, no differences in extracellular Mg^{2+} levels could be detected

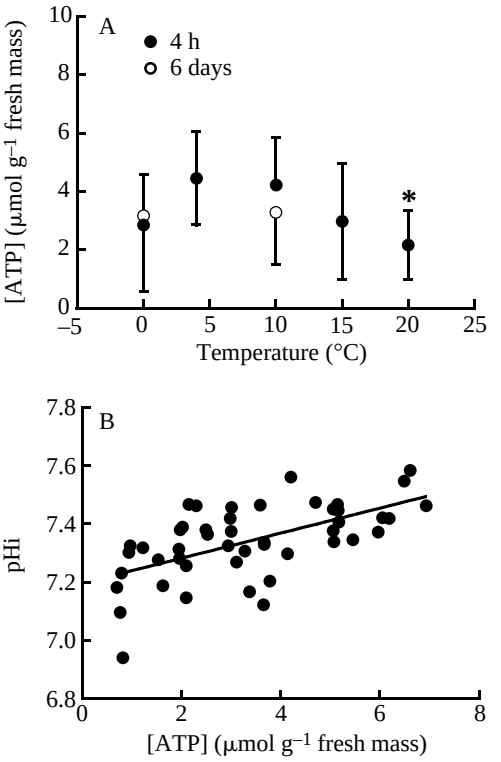

Fig. 5. (A) Levels of ATP in tail muscle as a function of the incubation temperature. Each value represents a mean ± S.D. of at least five determinations. * indicates significant difference (P<0.05) compared with control levels at 4 °C. (B) Relationship between intracellular pH and ATP levels (y=0.0429x+7.196, r=0.598, P<0.001, N=47).

between summer and winter animals. Neither exposure to anoxia nor exhaustive activity resulted in haemolymph Mg^{2+} levels comparable to those observed in the temperature incubations of winter animals. Nevertheless, extracellular $[Mg^{2+}]$ was significantly increased in fatigued animals, although the absolute values remained below 10 mmol l^{-1} (Fig. 3B). It is widely accepted that the level of extracellular Mg^{2+} is the result of an active excretion process, so an increase in Mg^{2+} content could reflect an increased uptake from the ambient water ($[Mg^{2+}]_w$=50 mmol l^{-1}), the partial inhibition of the active exclusion mechanism or an increased permeability of epithelia to Mg^{2+}.

The observed fluctuations in $[Mg^{2+}]_e$ are unlikely to be due to analytical errors, given the significant correlation between $[Mg^{2+}]$ and other parameters such as pHi and [ATP]. These relationships provide an interesting insight into how metabolic rate and acid–base status may depend upon extracellular Mg^{2+} levels (Figs 2, 4, 6). To our knowledge, this is the first study to address these relationships in crustaceans. Overall, the results show that intracellular pH is preserved at high levels when $[Mg^{2+}]_e$ exceeds 15 mmol l^{-1} (Fig. 2B). High pHi values appear to reflect a complete resting state of the tissue. Since, as first reported by Katz (1936) and Waterman (1941), high $[Mg^{2+}]$ blocks neuromuscular activity, it appears that the conservation of high pHi and ATP levels is a consequence of

partial anaesthesia, leading to a reduction in the level of activity. Elevated haemolymph Mg^{2+} levels reduce ATP turnover to a minimal level so that no ATP depletion or glycolytic proton production occurs. This conclusion is confirmed by the results of anoxic incubations where elevated $[Mg^{2+}]_e$ prevented both lactate production and a fall in pHi to below control levels (Fig. 6). Our observations confirm that anoxic incubation leads to reduced activity levels in animals exposed to control levels (50 mmol l^{-1}) of Mg^{2+} in the ambient water. As a result of this, the elevated ATP levels observed in such animals may reflect 'resting' levels under anaerobic conditions. However, anaerobic metabolism resulted in intracellular acidification caused by an increase in intracellular lactate concentration and glycolytic proton production. The positive correlation between pHi and [ATP] (Fig. 5B) suggests that a Mg^{2+}-induced increase in intracellular pH causes the rate of ATP synthesis to exceed the rate of ATP hydrolysis until a new steady state is reached. This is valid even without the contribution of anaerobic metabolism. Future investigations are needed to determine whether the rise in metabolic ATP turnover at low $[Mg^{2+}]_e$ is associated with reduced steady-state levels of intracellular pH and ATP. Do crustaceans characterised by high $[Mg^{2+}]_e$, e.g. *Hya arenaeus*, exhibit elevated intracellular ATP levels and pHi values compared with highly active species such as *Crangon crangon*? Unfortunately, no information concerning ATP levels and pHi in species with high $[Mg^{2+}]_e$ is, at present, available. It would also be interesting to compare $[Mg^{2+}]_e$, energy status and acid–base balance in animals with different seasonal activity patterns. Morritt and Spicer (1993) reported that $[Mg^{2+}]_e$ in the common sandhopper *Talitrus saltator* varied with the level of activity, depending on the season, but they presented no information on ATP values or pHi. However, our observation that a threshold Mg^{2+} concentration of 15 mmol l^{-1} is required to maintain high pHi and ATP levels agrees well with observations in *Talitrus saltator*, where haemolymph Mg^{2+} levels above 15 mmol l^{-1} are required to induce or maintain the torpid state in winter animals (Morritt and Spicer, 1993). It would be of particular interest to measure pHi in torpid *Talitrus saltator* since inactivity is usually accompanied by a decrease in pHi (Nuccitelli and Heiple, 1982; Thebault and Raffin, 1991).

Robertson (1953) reported that a high $[Ca^{2+}]/[Mg^{2+}]$ ratio rather than low $[Mg^{2+}]$ alone is crucial to maintain the excitability of the nervous tissue in crustaceans. A small decrease in this ratio may be sufficient to depress neuromuscular transmission. Although we found a trend for $[Ca^{2+}]/[Mg^{2+}]$ to decrease in our study, this was not significant (Fig. 2A). Our data clearly demonstrated that high extracellular Mg^{2+} levels on their own are sufficient to support the conservation of high intracellular pH and ATP levels (Figs 2, 4). This may indicate that the observed effects are due not only to the failure of muscle innervation, which should correlate with $[Ca^{2+}]/[Mg^{2+}]$, but also to the direct effects of Mg^{2+}

Table 1. *pHi of isolated muscle tissues as a function of the Mg^{2+} concentration in the medium after incubation for 1 h in anoxia or for 4 h in normoxia*

	pHi	
$[Mg^{2+}]$ (mmol l^{-1})	Anoxic 1 h	Normoxic 4 h
0	–	7.369±0.040
5	7.099±0.033	7.399±0.070
25	7.082±0.038	7.368±0.030
50	–	7.401±0.080

Each value represents the mean ± S.D. of five determinations.
No significant differences occurred (one-way analysis of variance; ANOVA).

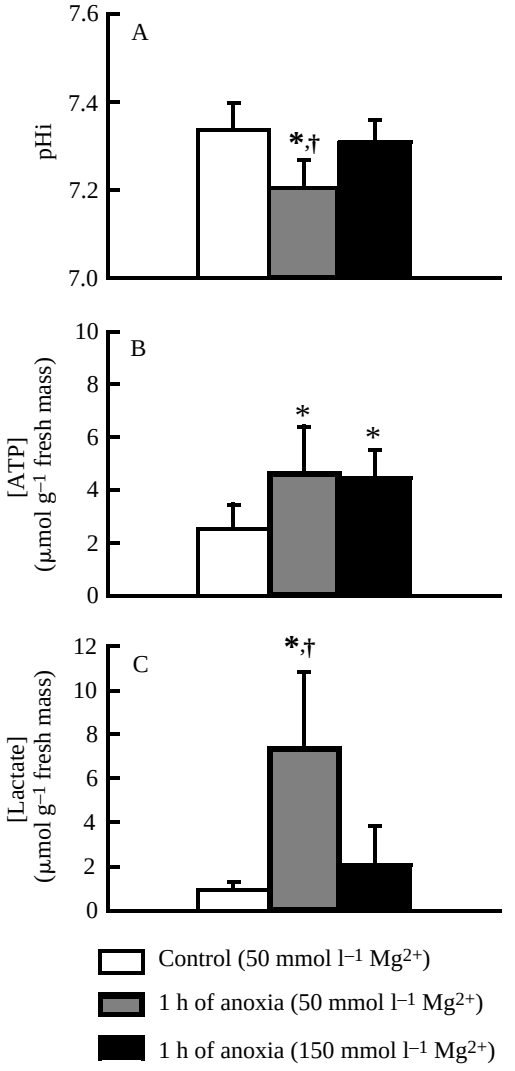

Fig. 6. Influence of 1 h of anoxia on intracellular pH (A), and ATP (B) and lactate (C) concentrations. Each value represents a mean + S.D. of at least five animals. * indicates a significant difference (P<0.05) compared with control levels; † indicates a significant difference (P<0.05) between animals incubated for 1 h in anoxic sea water containing 50 mmol l^{-1} Mg^{2+} and animals incubated in anoxic sea water containing 150 mmol l^{-1} Mg^{2+} for the same period.

mediated by transmembrane ion-exchange processes. We therefore also investigated the effect of high $[Mg^{2+}]_e$ on isolated muscle preparations. The results of these experiments do not appear to support the theory that transmembrane Mg^{2+} exchange in muscles is important for the preservation of pHi and ATP levels (Table 1).

The beneficial effects of elevated $[Mg^{2+}]_e$ are not restricted to crustaceans. Borchgrevink *et al.* (1989) reported that reperfusion of the ischaemic rat heart with elevated $[Mg^{2+}]$ ($15\,mmol\,l^{-1}$) enhanced the rate of recovery of ATP and creatine phosphate levels, and of pH and coronary blood flow, while high Mg^{2+} levels had no effect in control hearts. These authors pointed out that this protective effect is due to a variety of mechanisms, including conservation of myocardial K^+ levels, reduced cellular energy consumption, improvement in cellular energy production and the enhancement of coronary flow. Furthermore they suggested that extracellular Mg^{2+} probably has direct metabolic effects unrelated to the extracellular $[Ca^{2+}]/[Mg^{2+}]$ ratio. Similar mechanisms may be responsible for the effects we observed in *Crangon crangon*, although further investigations at the cellular level are required to confirm this.

In conclusion, we have demonstrated a protective effect of high concentrations of extracellular Mg^{2+} on intracellular pH and ATP levels in *Crangon crangon in vivo*. This effect is mostly due to high Mg^{2+} levels in the haemolymph and is not related to the $[Ca^{2+}]/[Mg^{2+}]$ ratio. Elevated $[Mg^{2+}]_e$ may be an adaptation in crustaceans allowing them to undergo prolonged periods of inactivity or to recover from environmental or metabolic stress. The marine environment contains sufficiently high levels of Mg^{2+} for the animals to have only to reduce the active elimination of the ion to gain the potential benefits of elevated extracellular $[Mg^{2+}]$.

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