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Comparative investigations on the effect of environmental salt stress on energy metabolism of amphipods estimated by ^{31}P NMR spectroscopy and microcalorimetry ☆

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Abstract

Potassium pollution of rivers like the Werra, Weser and Rhine directly affects the energy metabolism of indigenous amphipods. After environmental pollution by KCl (10.0 mmol l^{-1}), the heat dissipation of *Gammarus pulex* and *G. tigrinus*, estimated by microcalorimetry, increases significantly in two steps. Using ^{31}P NMR spectroscopy, the metabolic origin of the increasing heat production of *G. pulex* and *G. tigrinus* during KCl pollution of their incubation was investigated. In both species there is an initial increase in aerobic metabolism after the switch to 10.0 mmol l^{-1} K^+ stress. During prolonged K^+ stress an additional increase in heat dissipation, correlated to a simultaneous decrease in phospho-L-arginine in both species, is caused by anaerobic metabolism. In contrast to *G. pulex*, *G. tigrinus* sustains prolonged K^+ stress by using its higher capacity of aerobic metabolism to stabilize energy metabolism.

Keywords: Bioenergetics; *Gammarus*; Metabolism; Microcalorimetry; NMR; Salt pollution

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1. Introduction

Many large rivers in Germany, e.g. the Werra, Weser and Rhine, are intensely salt-polluted by potash mining and the potash industry. In particular, alternating K^+ pollution (0–25 mmol l^{-1}) considerably stresses the freshwater organisms living in these rivers [1–3].

Depending on the extent of salt pollution, the freshwater amphipod *Gammarus pulex* lives in ecological competition with the brackish-water amphipod *Gammarus tigrinus*. During recent decades, *G. tigrinus* has invaded the whole catchment area of the Werra, Weser and Rhine rivers, generating dominant populations among the amphipods [4]. A high capacity to regulate extra- and intracellular homeostasis, especially of K^+, may explain the ecological success of these brackish-water amphipods [1,2]. Compensation of K^+ stress by osmoregulation and ion regulation is associated with considerable energy consumption [5]. Salt pollution should therefore have a significant effect on the energy metabolism of gammarids.

So far the role of energy metabolism during the adaptation of different species to salt-polluted freshwater ecosystems has been incompletely investigated. The present investigation therefore deals with two basic questions: (1) Is the heat dissipation of brackish-water amphipods different from those of freshwater amphipods during K^+ stress? (2) Does the change in heat dissipation during K^+ stress correlate to a simultaneous change in typical parameters of energy metabolism, such as phospho-L-arginine (PLA)?

2. Materials and methods

The heatflow of *G. pulex* and *G. tigrinus* was estimated using microcalorimetry (Thermal Activity Monitor LKB 2277 and perfusion system, Bromma, Sweden) in the absence and presence of external K^+ pollution of 10.0 mmol l^{-1}. During each experiment, 2 animals were incubated in the measuring ampoule (3.5 ml). The flowrate of perfusion was 20 ml h^{-1}. After incubation without K^+ stress, there was a switch to K^+-polluted (10.0 mmol l^{-1} KCl) water. All experiments were carried out at 15°C.

All ^{31}P NMR spectra (Nuclear Magnetic Resonance) were recorded at 81.1 MHz $\pm$ 5000 Hz on a Bruker Biospec 47/40 spectrometer. The pulse width was 60 μs, corresponding to a 37° flip angle. 2048 data points were recorded. Total acquisition time was 0.502 s. For each spectrum, 2400 scans were accumulated within 20 min. First of all 5–7 gammarids were perfused for 1 h at 15°C in the absence of K^+. During the next 3–5 h, the perfusion medium was switched to K^+-polluted water (10.0 mmol l^{-1} KCl). Three 20 min spectra were accumulated within 1 h. The area of the PLA signal of the first spectrum of unpolluted incubation was defined as 100%. The areas of any other PLA signal were given as a ratio (delta%) to this 100% level.

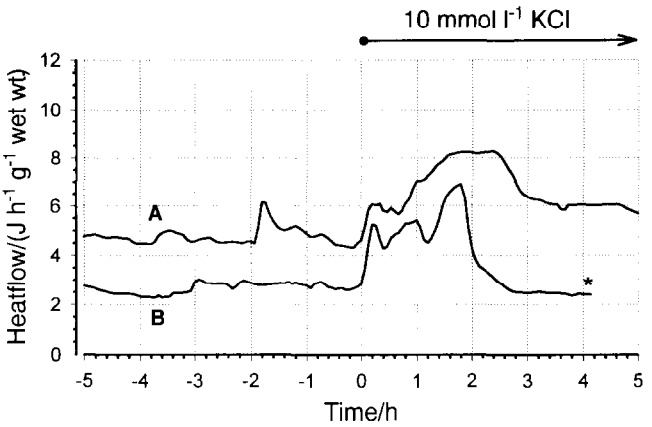

Fig. 1. Typical microcalorimetry experiment showing the heat dissipation of *G. tigrinus* (A) and *G. pulex* (B) without and during KCl stress of 10.0 mmol l^{-1}; * indicates termination of incubation after death of *G. pulex*.

3. Results

3.1. Heat dissipation of gammarids during external KCl-stress of 10.0 mmol l^{-1}

10 mmol l^{-1} KCl pollution significantly increases the heat dissipation of both species. In Fig. 1, the heat dissipation during a typical experiment with *G. tigrinus* (A) and *G. pulex* (B) without and during 10 mmol l^{-1} potassium stress is shown. During unpolluted incubation, the heat flow of the animals was not constant. Both species show a basic level of heatflow and more or less periodical occurrences of activity.

10.0 mmol l^{-1} KCl pollution increases the heat dissipation of *G. pulex* (Fig. 1B) in two steps. In the first 10–20 min, the heat dissipation increases by nearly 60% of the control level. During continual K^+ stress (1.2 h) the heat dissipation is held at a level of about 4.3 J h^{-1} g^{-1} wwt. After 1.2 h, KCl stress causes an additional increase in heat dissipation by 50–60% of the first step. The maximum heat flow is reached after 1.4 h of KCl stress, being 6.9 J h^{-1} g^{-1} wwt. Then the heat dissipation rapidly decreases, reaching the value of unpolluted controls after 2.8 h of stress incubation. At this time the animals die.

After the switch to KCl pollution (10.0 mmol l^{-1}), the heat dissipation of *G. tigrinus* (Fig. 1A) increases by 20% of the control level within 10–20 min, reaching a level of about 5.7 J h^{-1} g^{-1} wwt. After 40 min of continuous KCl stress, the heat dissipation increases again up to a level of about 8.2 J h^{-1} g^{-1} wwt. During this state, the maximum heat dissipation is 72% above the unpolluted rate. This high rate of heatflow is not stable. After 2.5 h of incubation, the heat dissipation decreases, reaching a steady state level of (5.8 ± 0.3) J h^{-1} g^{-1} wwt after 3 h of incubation. This steady state level still remains 20% above control level. After 5 h of KCl stress, the animals show normal locomotor activity.

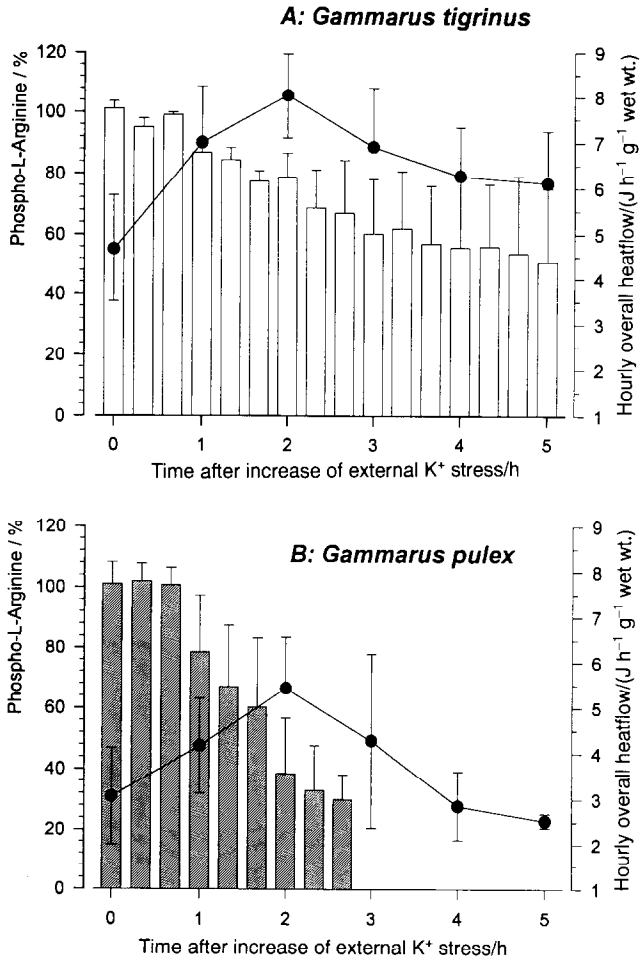

Fig. 2. Influence of 10.0 mmol l^{-1} KCl on phospho-L-arginine steady state (bars, left ordinate) and hourly overall heat flow (black circles, right ordinate) of *G. tigrinus* (A) and *G. pulex* (B).

The increase in heat dissipation was not caused by KCl mixing enthalpy. After the switch to 10.0 mmol l^{-1} KCl, no increase in heat dissipation was measured in the absence of the animal. The switch to 22.0 mmol l^{-1} KCl increases the heat dissipation during the first 30 min by 0.5% at most.

3.2. Change of hourly mean heatflow during K^+ stress

In Fig. 2(A,B), the average heat flow over 1 h ($n = 4$) during K^+ stress of 10.0 mmol l^{-1} is shown as the change in hourly overall heat flow of *G. pulex* and *G. tigrinus* during potassium stress. For the unpolluted situation (without K^+ stress at

$t = 0$, $n = 16$) the hourly overall heatflow was (3.1 ± 1.1) J h^{-1} g^{-1} wwt for *G. pulex* and (4.7 ± 1.2) J h^{-1} g^{-1} wwt for *G. tigrinus*.

In *G. tigrinus* the overall heat flow significantly increases by 50% up to (7.0 ± 1.2) J h^{-1} g^{-1} wwt during the first hour of stress incubation ($t = 1$). After 2 h, the heat dissipation reaches a maximum value of (8.0 ± 1.0) J h^{-1} g^{-1} wwt, which is an increase of 70% compared with the control value at $t = 0$. Within the third hour of K^+ stress, the overall heat dissipation decreases. In contrast to *G. pulex*, the heat dissipation of *G. tigrinus* does not decrease down to the unpolluted control level at $t = 0$. The heat dissipation of *G. tigrinus* is stabilized at (6.1 ± 1.1) J h^{-1} g^{-1} wwt until the end of incubation ($t = 5$).

During the first 2 h of K^+ stress, the overall heat flow of *G. pulex* increases significantly by 67% up to (5.4 ± 1.1) J h^{-1} g^{-1} wwt. During further K^+ stress, the heat flow of *G. pulex* decreases continuously. At $t = 4$ h, the heat dissipation amounts to (2.9 ± 0.8) J h^{-1} g^{-1} wwt. At this state, the heat dissipation remains below the overall heat flow at $t = 0$, but is comparable to the overall heat flow of the unpolluted control level. At $t \geq 1.8$ h, the fitness of the animals was reduced irreversibly. At $t = 5$ h, mortality was 100%.

3.3. Influence of 10.0 mmol l^{-1} KCl on the steady state of phospho-L-arginine

Fig. 2(A,B) shows the changes in phospho-L-arginine (PLA) steady-state values of *G. pulex* (below) and *G. tigrinus* (above) estimated by ^{31}P NMR spectroscopy. During the first 40–60 min after switching to KCl stress, the PLA level in *G. pulex* remains constant. Values are stabilized at the control level of (101.0 ± 7.2)%. During prolonged stress incubation, PLA in *G. pulex* decreases continuously. After 2 h of stress incubation, the PLA level is 30% of the control level. In this state, the animals are totally exhausted. As in *G. pulex*, in *G. tigrinus* the steady state of PLA remains at (101.0 ± 2.6)% during the first 40–60 min after switching to external K^+ pollution. During prolonged KCl stress, as with *G. pulex*, at first the PLA pool of *G. tigrinus* decreases. In contrast to *G. pulex*, the decrease of PLA in *G. tigrinus*, however, is not continuous, down to the exhaustion level of 20–30% of the control level. After 3 h the PLA pool of *G. tigrinus* is stabilized at 60% of unpolluted control level until the end of incubation.

4. Discussion

During unpolluted incubation, the hourly overall heat flow of the freshwater amphipod *G. pulex* amounts to (3.1 ± 1.1) J h^{-1} g^{-1} wwt, and that of the brackish-water amphipod *G. tigrinus* amounts to (4.7 ± 1.2) J h^{-1} g^{-1} wwt. Therefore, without K^+ stress, the energy metabolism capacity of *G. tigrinus* is 50% higher than that of *G. pulex*. Marine invertebrates have comparable heat dissipations between 2.5 and 7.8 J h^{-1} g^{-1} wwt [6].

Though *G. pulex* was dead after the decrease in heat dissipation during prolonged K^+ stress, the heat dissipation is still measurable (Fig. 1B). This phenomenon may

be explained by intracellular degradation processes starting after death. Additional energy-consuming processes may be caused by microorganisms. However, no significant heat dissipation is measured in the absence of dead gammarids. Therefore further experiments are necessary to explain this phenomenon.

PLA is a phosphagen widespread among invertebrates. At the beginning of environmental or functional hypoxia, a decrease in PLA rapidly provides phosphate-bond energy and acts as an effective energy buffer during transition from aerobic to anaerobic metabolism [7–9]. In analogy, a decrease in the PLA pool of the gammarids during KCl stress was used to indicate the transition from aerobiosis to anaerobiosis. In both species, changes in PLA during K^+ stress correlate well to changes in heat dissipation. Depletion of the PLA pool in both species occurs only during the second burst of heat dissipation. Therefore, the first increase of heat dissipation in both amphipods is provided by aerobic metabolism. During prolonged K^+ stress, however, the capacity of aerobic metabolism is not high enough to compensate K^+ stress by active, energy-consuming ion regulation. An increase in anaerobic metabolism is necessary to supplement the high energy demand of K^+ regulation. As a consequence, heat dissipation increases further and reaches a maximum. The final decrease of heat dissipation and PLA in *G. pulex* suggests that in this species the total capacity of aerobic and anaerobic metabolism is not high enough to reach ion homeostasis again. In contrast, *G. tigrinus* does not require its anaerobic capacity during the whole period of K^+ stress. After a temporary period of anaerobiosis, *G. tigrinus* is able to reach ion homeostasis again and energy metabolism becomes stabilized. But maintaining ion homeostasis still creates a high energy demand. *G. tigrinus* is able to satisfy this energy demand by a continually high aerobic energy production. The high aerobic capacity that satisfies increased energy demands during prolonged K^+ stress over a period of many hours is the decisive difference in the energy metabolism of *G. tigrinus* and *G. pulex*. These results correlate well to simultaneous changes in pH, lactate accumulation and increase in inorganic phosphate in the whole animal during prolonged K^+ stress (Koop, unpublished results).

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