

Muscle ammonia stores are not determined by pH gradients

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Abstract

The theory of non-ionic diffusion predicts that ammonia will distribute between intracellular and extracellular tissue compartments according to transmembrane pH gradients. The distribution of ammonia and ¹⁴C-DMO were compared in white muscle and plasma of rainbow trout (*Salmo gairdneri*) at rest, and following exhaustive exercise. Under both experimental conditions, intracellular ammonia levels far exceeded those predicted by transmembrane pH gradients. Calculated equilibrium potentials for NH₄⁺ (E_{NH₄⁺}) were very close to published resting values of membrane potential E_m in fish white muscle. We conclude that NH₄⁺ is permeable across cell membranes and that intracellular ammonia stores are not determined by pH gradients.

Introduction

The theory of nonionic diffusion (Jacobs and Stewart 1936; Milne *et al.* 1958; Pitts 1973) describes the distribution of weak electrolytes, such as ammonia¹, between body and compartments. The theory states that cell membranes are relatively impermeable to NH₄⁺ ions, but highly permeable to the non-ionic forms of ammonia, NH₃. The transfer of ammonia between body compartments, therefore, will depend on NH₃ diffusion (i.e. P_{NH₃}) gradients. NH₃ levels will be larger in a high pH compartment, and hence, ammonia levels in-

crease in a low pH compartment as NH₃ enters, combines with a H⁺ and is trapped in the impermeant NH₄⁺ form. Thus, the distribution of ammonia between tissue compartments will be determined by intracellular-to-extracellular H⁺ ion gradients. In a recent review in this journal, Randall and Wright (1987) analysed the distribution of ammonia and H⁺ ions between trout white muscle and plasma at rest and following exhaustive exercise (data taken from Mommsen and Hochachka 1988, and Milligan and Wood 1986a). From their calculations, ammonia was distributed according to the H⁺ ion distribution in trout muscle, at rest, but not following exercise. The analysis for resting trout muscle fits in well with the accepted theory of non-ionic diffusion (see above). The problem with their analysis is that the trout muscle T_{amm} levels

¹ The term ammonia or T_{amm} will be used to indicate the total ammonia concentration, while NH₄⁺ and NH₃ will refer to ammonium ion and nonionic ammonia, respectively.

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(Mommsen and Hochachka 1988) and muscle pH_i values (Milligan and Wood 1986a) were obtained from two separate studies where fish were raised and maintained under different conditions. When these measurements were performed on a single set of fish, the seawater lemon sole (*Parophrys vetulus*), Wright *et al.* (1988) found that muscle intracellular ammonia levels were 10–20 fold greater than those predicted from transmembrane pH gradients at rest, during hypercapnia, and following exercise. Furthermore, calculated equilibrium potentials for NH_4^+ ($E_{\text{NH}_4^+}$) in sole white muscle were very close to published values of fish white muscle membrane potential (E_m), indicating that NH_4^+ was distributed according to E_m . In the present study, we have measured both the total ammonia and H^+ ion distribution between plasma and white muscle in a single group of rainbow trout at rest and after exercise to determine whether NH_4^+ distributes according to transmembrane pH gradients as predicted by Randall and Wright (1987), or whether trout are similar to lemon sole, where NH_4^+ is distributed according to the membrane potential (Wright *et al.* 1988).

Materials and methods

Rainbow trout (*Salmo gairdneri*; body weight 200–300 g) were anaesthetized, surgically fitted with dorsal aortic catheters (Soivio *et al.* 1972), and left to recover for 48h in individual plexiglass chambers at $15 \pm 1^\circ\text{C}$. Two groups of fish were studied: resting trout and trout swum to exhaustion. In order to determine intracellular pH (pH_i) by the DMO (5,5 dimethyl-2,4-oxazolidinedione) distribution technique (Waddell and Butler 1959), fish were injected with 1 ml.kg^{-1} of $5 \mu\text{Ci.ml}^{-1}$ [^{14}C] DMO and $20 \mu\text{Ci.ml}^{-1}$ of the extracellular marker, [^3H] mannitol in Cortland saline, 12h prior to sampling (Milligan and Wood 1986a,b). Resting fish were left undisturbed in the chambers until sampling, while fish in the exercise group were removed from their chambers, chased for 6 min to exhaustion in a circular tank (500 l), and left to recover for 15 min prior to sampling. This period is adequate to permit DMO re-equilibration in the

face of the exercise-induced pH change (Milligan and Wood 1985). At sampling, 2 ml of blood was withdrawn from each fish and immediately analysed for extracellular pH (pH_e); plasma was separated by centrifugation (2 min), acidified (8% HClO_4), and stored for later determination of plasma total ammonia concentration (T_{amm}). In less than 1 min after blood withdrawal, fish were removed from the water, killed by a blow to the head, and a thin slice of white muscle tissue was immediately frozen with freeze-clamp tongs, cooled in liquid N_2 . The frozen samples were stored in liquid N_2 for later determination of intracellular T_{amm} , while the unfrozen tissue samples were later analysed for [^{14}C] DMO, [^3H] mannitol, and water content. For details of the analytical techniques and calculations the reader is referred to Wright *et al.* (1988). Ammonia concentrations (T_{amm}) are expressed per litre of extracellular or intracellular water, as appropriate.

Data are presented as means \pm 1 SEM. Student's two-tailed t-test was used to determine the significance of difference between mean values ($P < 0.05$).

Results and discussion

Exhaustive exercise resulted in an intracellular and extracellular acidosis comparable to that seen previously in rainbow trout (Milligan and Wood 1986b). There was a three-fold increase in plasma and muscle T_{amm} levels relative to control values (Table 1). The increase in muscle ammonia concentration following exercise was lower than that found by Mommsen and Hochachka (see Randall and Wright 1987) in the same species, but greater than that in lemon sole (Wright *et al.* 1988). The relative increase in plasma ammonia levels in the present study was similar to that found by Mommsen and Hochachka, whereas, in sole, plasma T_{amm} did not change (Wright *et al.* 1988).

Measured muscle pH_i and T_{amm} values are compared to values predicted by the theory of non-ionic diffusion in Table 1 assuming that P_{NH_3} is in equilibrium across the membrane and NH_4^+ is impermeant (see Wright *et al.* 1988). In both resting

Table 1. Measured pH_e , muscle pH_i , plasma and muscle T_{amm} , and the plasma: muscle T_{amm} ratio (ammonia concentration ratio) in trout at rest (control) and following exercise. The measured muscle pH_i (DMO method) is compared to pH_i calculated with the Henderson-Hasselbalch equation employing the assumption that plasma $P_{\text{NH}_3} = \text{muscle } P_{\text{NH}_3}$ (theory of non-ionic diffusion). The measured muscle T_{amm} is similarly compared with the muscle T_{amm} predicted by the non-ionic diffusion theory model where plasma $P_{\text{NH}_3} = \text{muscle } P_{\text{NH}_3}$. $E_{\text{NH}_4^+}$ was calculated from the Nernst equation.

	Control	Exercise
Measured: pH_e	7.82 ± 0.02	$7.56 \pm 0.02^*$
pH_i (DMO)	7.25 ± 0.02	$6.77 \pm 0.02^*$
plasma T_{amm} ($\mu\text{M.l}^{-1}$)	77 ± 15	$209 \pm 16^*$
muscle T_{amm} ($\mu\text{M.l}^{-1}$)	2170 ± 652	$6790 \pm 803^*$
ammonia concentration ratio	0.04	0.03
Predicted: pH_i	$6.39 \pm 0.16^{**}$	$6.05 \pm 0.08^{**}$
muscle T_{amm} ($\mu\text{M.l}^{-1}$)	$297 \pm 68^{***}$	$1285 \pm 136^{***}$
Calculated: $E_{\text{NH}_4^+}$ (mV)	-81 ± 9	-85 ± 4

Data are shown as mean \pm sem; $n = 8$ for control and 7 for exercised group; * significantly different from control ($P < 0.05$); ** significantly different from measured pH_i ($P < 0.05$); *** significantly different from measured muscle T_{amm} ($P < 0.05$)

and exercised fish, predicted pH_i was significantly lower than pH_i measured by the DMO method and predicted muscle T_{amm} was significantly lower than measured muscle T_{amm} . In other words, there is far more T_{amm} in muscle than predicted by the non-ionic diffusion model. These results indicate that ammonia is *not* distributed between plasma and muscle tissue according to transmembrane pH gradients in trout either at rest or following exercise. As a result, there is a substantial P_{NH_3} gradient from muscle to plasma not only after exercise, as predicted by Randall and Wright (1987), but also at rest (Fig. 1). Interestingly, the magnitude of the gradient changed little between resting and post-exercise situations because the intracellular acidosis counteracted the increased intracellular T_{amm} , resulting in unchanged intracellular P_{NH_3} .

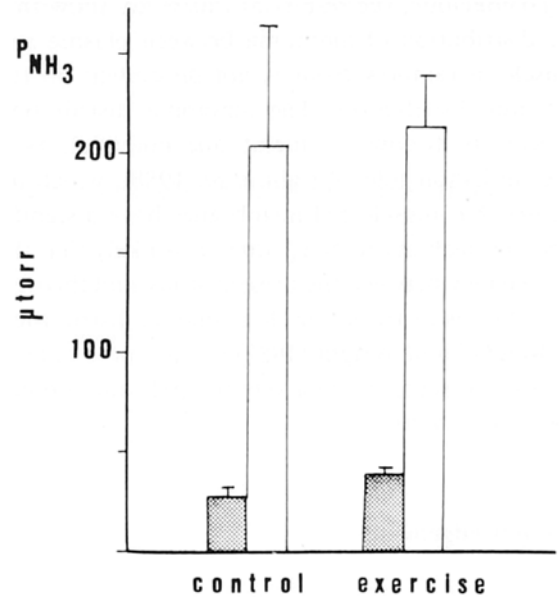


Fig. 1. Partial pressure of NH_3 (P_{NH_3}) in trout white muscle (open bars) and in plasma (stippled bars) under control and post-exercise conditions. Values are shown as means \pm 1 SEM.

Wright *et al.* (1988) presented a model to explain the maintenance of similarly high P_{NH_3} gradients from tissues to plasma in resting and exercised lemon sole. In brief, if cell membranes have a significant permeability to NH_4^+ (though not necessarily as large as their permeability to NH_3), then ammonia will distribute according to the transmembrane potential, resulting in large standing gradients of P_{NH_3} from intra-to-extracellular fluids. The calculated resting $E_{\text{NH}_4^+}$ value for trout muscle in the present study (-81 mV, Table 1) is in agreement with calculated resting sole $E_{\text{NH}_4^+}$ (-83 mV, Wright *et al.* 1988) and published measured E_m values for fish white muscle (-80 to -85 mV; Hagiwara and Takahashi 1967; Hidaka and Toida 1969; Yamamoto 1972). During exercise there is an increase in muscle ammoniogenesis (Suyama *et al.* 1960; Fraser *et al.* 1966; Driedzic and Hochachka 1976; Dobson and Hochachka 1987; Wright *et al.* 1988). The fact that $E_{\text{NH}_4^+}$ following exercise in this study is still within the expected range of muscle E_m ($E_{\text{NH}_4^+} = -85$ mV, Table 1), implies that the distribution of ammonia remains close to electrochemical equilibrium even after accelerated intracellular ammonia production.

To conclude, the results of this study show that the distribution of ammonia between plasma and muscle in rainbow trout is not dependent on the H^+ ion distribution. The ammonia distribution appears to follow the membrane potential, as it does in lemon sole (Wright *et al.* 1988), which indicates that muscle cell membranes have a significant permeability to NH_4^+ ions. It is likely that the discrepancy between the present study and theoretical analysis of trout muscle ammonia distribution by Randall and Wright (1987) is due to the latter's use of two separate data sets for pH_c and ammonia concentrations.

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