Activation of 5'-AMP activated protein kinase during anaerobiosis in the rock crab, *Cancer irroratus*

Ilka Pinz [1], Dylan J. Perry[2] & Markus Frederich[3]

[1]College of Osteopathic Medicine and [3]Department of Biological Sciences,
University of New England, Biddeford, ME 04005
[2]Carrabassett Valley Academy, Carrabassett Valley ME 04947

5'-AMP-activated protein kinase (AMPK) is a key regulator of energy metabolism in mammals. Depleted cellular energy pools, for example during prolonged exercise or in hypoxic states result in the activation of AMPK [4]. Since AMPK has been highly conserved during evolution [3], its presence in marine crustaceans is likely, although no reports are available yet. Marine crustaceans are often exposed to temperature fluctuations that could lead to hypoxic conditions. At too high and too low temperatures a mismatch of O_2 demand and O_2 supply in the tissues of animals occurs despite sufficient O_2 availability in the environment. These temperature thresholds have been defined as critical temperatures, Tc, and are characterized by the onset of anaerobic metabolism with the accumulation of anaerobic endproducts such as lactate [2, 5]. In addition, the switch from aerobic to anaerobic metabolism causes a change in the cell's energy sources and energy status. Extended exposure to temperatures above high Tc or below low Tc finally leads to death due to energy depletion. It is not known whether AMPK regulates energy metabolism in marine invertebrates under such conditions. Therefore, we tested the hypothesis that AMPK is present in the rock crab *Cancer irroratus* and can be activated during anaerobiosis due to O_2 depletion and temperature stress.

To investigate AMPK activation during hypoxia, *C. irroratus* were incubated in nitrogen equilibrated seawater for 40, 60 and 120 min at 16°C. At the endpoints the crabs were killed by a cut through the cerebral ganglion and the different tissues quickly excised and freeze clamped. During the incubations heart rate was recorded non-invasively using photoplethysmographs glued to the carapace. Lactate in tissues was measured enzymetrically. AMPK activity was measured using the "SAMS" peptide, a synthetic peptide with the specific target sequence for AMPK, synthesized after the AMPK recognition sequence of acetyl-CoA carboxylase from rat liver (amino acid sequence HMRSAMSGLHLVKRR). In this assay AMPK phosphorylates the SAMS peptide using radio-labelled γ^{32}P-ATP. The resulting amount of radioactive SAMS peptide represents therefore AMPK activity, for details see [1].

Fourty minutes of hypoxia resulted in a significant lactate accumulation in heart, hepatopancreas and muscle tissues (Figure 1a). Concomitant with the lactate accumulation in the hepatopancreas AMPK activity increased 5.3 fold. In the heart, 120 min of hypoxia increased lactate 9.1 fold with a 1.7 fold increase in AMPK activity (Figure 1b). The faster and higher activation in the hepatopancreas compared to the heart is probably due to either the difference in metabolic activity or different functions of AMPK in various tissues. The hepatopancreas has a high energy expenditure during hypoxia due to glycogen catabolism supplying the body with glucose. The lesser increase of AMPK activity in the heart despite higher lactate accumulation could reflect the diminished contractile performance of the heart at hypoxic conditions: heart rate dropped from 88.2±10.9 bpm during normoxia to 16.7±9.6 bpm after 40 min hypoxia and 8.5±2.1 bpm after 120 min hypoxia, therefore the energy demand was greatly reduced and the large increase in anaerobic ATP production might have improved the energetic state of the muscle cells. More detailed tissue specific effects of AMPK need to be determined in the future.

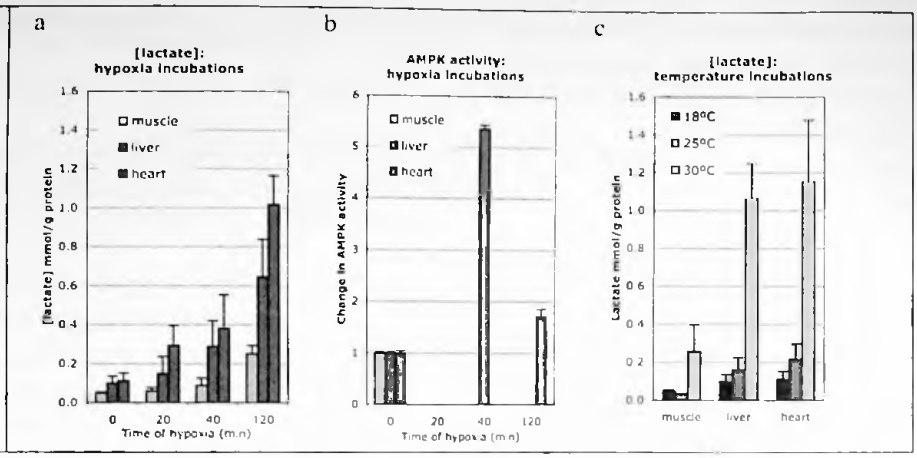

Fig. 1. Lactate accumulation (a) and respective increase in AMPK activity (b) in various tissues of *Cancer irroratus*. Exposure to high temperatures leads to lactate accumulation comparable to prolonged exposure to hypoxia (c) and indicates the upper critical temperature between 25 and 30°C.

To test whether AMPK also plays a role in thermal tolerance, the critical temperatures (Tc) of *C. irroratus* had to be determined. Exposure of the animals to various temperatures and subsequent tissue sampling and lactate analysis showed a significant increase in lactate between 25 and 30°C (Figure 1c). Tc is therefore reached in this temperature range. The animals died above 30°C. The lactate accumulation at 30°C in the leg muscle is comparable with an exposure to 40 min of hypoxia. The lactate accumulation at 30°C in the heart is comparable with an exposure to 120 min of hypoxia. The various degrees of anaerobiosis are again a function of the metabolic activity of the respective tissues. Further experiments will test the activation of AMPK as well as the AMPK gene expression after the onset of anaerobiosis due to temperature stress.

We conclude that the highly conserved metabolic master switch, AMPK, is present in *C. irroratus* and is activated during hypoxia. Additional measurements are currently done to characterize the described AMPK activation in more detail. The specific effect of AMPK activity on downstream targets, such as acetyl-CoA carboxylase, malonyl-CoA carboxylase, glycogen synthase, and others still needs to be determined for *C. irroratus*. An enhanced understanding of the integrative regulation of energy metabolism during environmental stress could lead to a better understanding of the animal's tolerance of various stressors.

This research was supported by an MDIBL New Investigator Award and a faculty development grant from the University of New England to M.F. and a Maine High School Research Fellowship supported by the Betterment Fund to D.P.

1. **Frederich, M., Balschi, J.A.** The relationship between AMP-activated protein kinase activity and AMP concentration in the isolated perfused rat heart. *J Biol Chem* 277/3: 1928-1932, 2002.
2. **Frederich, M., Pörtner, H.O.** Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab Maja squinado. *Am J Physiol* 279: R1531-R1538, 2000.
3. **Gao, G., Widmer, J., Stapleton, D., The, T., Cox ,T., Kemp, B.E., Witters, L.A.** Catalytic subunits of the porcine and rat 5'-AMP-activated protein kinase are members of the SNF1 protein kinase family. *Biochim Biophys Acta* 1266(1): 73-82, 1995.
4. **Kemp, B.E., Stapleton, D., Campbell, D.J., Chen, Z.P., Murthy, S., Walter, M., Gupta, A., Adams, J.J., Katsis, F., van Denderen, B., Jennings, I.G., Iseli, T., Michell, B.J., Witters, L.A.** AMP-activated protein kinase, super metabolic regulator. *Biochem Soc Proc* 31/1: 162-168, 2003.
5. **Pörtner, H.O.** Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. *J Exp Biol* 205: 2217-2230, 2002.