PROTECTION OF SKELETAL MUSCLE AGAINST ISCHEMIA BY INACTIVATION OF THE OXYGEN SENSOR PHD1: AN IN VIVO 31P AND IN VITRO 1H MRS STUDY

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Abstract

The prolyl HIF-hydroxylases PDH1, -2 and -3 regulate the stability of the hypoxia-inducible factors HIF-1α and -2α in an oxygen dependent manner. Despite the presumed importance of these oxygen sensors, their precise biological role in health and disease has remained unclear.

Here we report that loss of PHD1 protects skeletal muscle against ischemic insult induced by femoral occlusion. In vivo ^{31}P MRS spectra of the lower limb showed that PCr and ATP content were rapidly declined after ischemia in wild type mice, whereas both were markedly preserved in PHD1$^{-/-}$ mice Additionally, the lactate and succinate profile, determined by 1H MRS in muscle extracts, suggest a preserved Krebs cycle activity in the PHD1$^{-/-}$ ischemic musculature.

Objective

Under hypoxic conditions, cells increase the expression of proteins involved in the physiological adaptation to low oxygen environments. This compensatory response depends on the activation of hypoxia inducible factors (HIF)[1-3]. In normoxia, HIF-α subunits are degraded by interaction with the von Hippel-Lindau (VHL) tumour suppressor[4]. In hypoxia, however, HIF-α subunits lose their ability to interact with VHL, and are subsequently stabilized[5]. Recent evidence indicated that a novel family of mammalian proline hydroxylases named PHD1, PHD2 and PHD3 enzyme is involved in hydroxylation of the proline residues in the HIF-α subunits[6]. Since PHD-dependent hydroxylation of HIF- α subunits requires oxygen, under hypoxic conditions, HIF activaty is increased.

The present study aimed at the in vivo evaluation of the effect of PHD1 deficiency in a low-flow ischemia model in a mouse skeletal muscle by determining the energy metabolites with non-invasive ^{31}P MRS.

As hypoxia plays a significant role in ischemic cardiovascular disease, cancer and numerous other diseases, understanding the role of each PHD enzyme is of critical importance.

Materials and Methods

In vivo ^{31}P NMR spectroscopy experiments on lower limbs were performed in a 4.7 Tesla BIOSPEC horizontal magnet (Bruker), using a 10 mm diameter, 6 turns, solenoid transmit-receive coil. Anaesthetized (pentobarbital; i.p.) mice (WT n=3; PHD1$^{-/-}$ n=3) were placed on a Perspex plate such that the lower limb was positioned directly inside the coil. Serial ^{31}P NMR spectra were acquired every 3 min during 1.5 h, starting twenty minutes post ligation, and again after 24 h (30 degree pulse, TR = 1.4 s, NA = 128; no proton decoupling; T=36°C). The peak intensities of PCr, Pi and ATP resonances were quantified with the jMRUI software package. The experiments were approved by the Local Ethical Committee for Animal Experiments, in agreement with international guidelines.

In vitro ^{1}H MRS analyses of lactate and succinate content in gastrocnemius perchloric acid extracts from operated limbs in WT and PHD1$^{-/-}$ mice were performed at 8.4 Tesla in a high resolution AMX 360 (Bruker). Specific settings (278 K) were, TR = 12 s, NA = 256, 32k points, selective water presaturation. Peak intensities of lactate (lact), succinate and total creatine (tCr) were quantified by integration after baseline correction, using the spectrometer processing software.

Results & Discussion

In acute ischemia, skeletal muscle ATP levels are initially maintained through a rapid hydrolyzation of PCr to generate ATP from ADP. In WT mice, PCr levels progressively declined within 30-120 minutes after onset of ischemia (Figure 1A), while inorganic phosphate (Pi) levels steadily increased, reflecting continuous ATP utilization and insufficient ATP regeneration. In contrast, in PHD1$^{-/-}$ mice, muscular PCr contents decreased only during the initial 30 minutes after onset of ischemia, but subsequently stabilized and even tended to increase again during prolonged ischemia (Figure 1B). Collectively, these data indicate that loss of PHD1 results in a striking resistance of skeletal muscle to ischemia-induced energy exhaustion.

Furthermore, in vitro ^{1}H NMR spectroscopy revealed that acute ischemia provoked a rapid glycolytic response with accumulation of skeletal muscle lactate in both genotypes: 1 hour post-ligation the lact to tCr ratio was 80 ± 14 % in WT mice *versus* 79 ± 3 % in PHD1$^{-/-}$ mice (n = 5; P = n.s.). Strikingly, however, at 6 hours post-ligation lactate in PHD1$^{-/-}$ muscle returned to baseline levels, whereas elevated lactate levels persisted in ischemic WT muscle: lact to tCr ratio was 82 ± 28 % in WT mice *versus* 15 ± 4 % in PHD1$^{-/-}$ mice (n = 6; P = 0.04). We additionally determined succinate levels by ^{1}H MRS. In WT mice, prolonged ischemia for 6 hours caused a marked accumulation of muscle succinate levels. By contrast, succinate levels were not increased in ischemic PHD1$^{-/-}$ limbs, suggesting that mitochondria were capable of normally converting succinate to fumarate in the Krebs cycle (6 hours post-ligation: succinate to tCr: 1.8 ± 0.4 % in WT mice *versus* 0.5 ± 0.05 % in PHD1$^{-/-}$ mice; n = 6; P = 0.015).

References

(1) Semenza. *Trends Mol Med* **8**, S62-7 (2002).
(2) Pugh and Ratcliffe *Nat Med* **9**, 677-84 (2003).
(3) Wiesener and Maxwell *Ann Med* **35**, 183-90 (2003).
(4) Maxwell *et al.* Nature 399, 271-5 (1999).
(5) Huang *et al.* J Biol Chem 271, 32253-9 (1996).
(6) Ivan *et al.* Science 292, 464-8 (2001).