Abnormal post-ischemic reperfusion patterns in the skeletal muscle of serotonin deficient mice identified by arterial spin labeling coupled to SSFSE imaging

D. Bertoldi[1], Y. Fromes[2], C. Fligny[3], F. Cote[3], G. Vodjdani[3], P. G. Carlier[1]

[1]Laboratoire RMN, AFM-CEA, Institut de Myologie, Paris, France, [2]INSERM U582, Institut de myologie, Paris, France, [3]Laboratoire de Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératifs, UMR 7091, Paris, France

Introduction

Perfusion provides nutriments for cellular metabolism and drains catabolites. It is therefore a variable of paramount importance and is finely regulated. This is the case during an exercise when perfusion is drastically increased to match tissue metabolic demand. Also, many factors including nitric oxide, catecholamine, adenosine, prostaglandins, kinins, EDRF and stretch are known to regulate skeletal muscle vasodilatation, at least in vitro. In this study, we investigated whether serotonin, which was not supposed to play a major role in this regulation, might be implicated in the control of post-ischemic hyperemia in the skeletal muscle. To do this in vivo, NMR imaging combined with arterial spin labeling (ASL) is the method of choice. It is indeed totally non invasive, quantitative, with decent spatial and high temporal resolutions.

Materials and methods

Two groups of mice were studied. The first group was control mice, the second one was mice in which tryptophan hydroxylase 1 (tph1) had been inactivated [1]. The tph1 enzyme controls the rate-limiting step of peripheral serotonin synthesis and the tph1 mutant have extremely low blood serotonin concentrations (less than 10% of normal levels). Reactive hyperemia was studied twice, after periods of ischemia lasting 5 and 30 minutes. The interval between the two measurements was one week. Calf muscle perfusion was quantified using SATIR, the arterial spin labeling variant developed in our laboratory [2]. Temporal resolution was 10s. All measurements were performed on a 4 Tesla magnet retrofitted with 200 mT. m^{-1} gradients with rise time of 260 µs. A home built volume coil was used for transmission and an actively decoupled surface coil (Rapid Biomedical) was used for reception. Regions of interest were traced in the gastrocnemius muscles and signal intensities were measured in the stack of positively and negatively tagged images acquired during 20 min post-reperfusion.

The time-courses of the hyperemic responses were characterized by the following variables: maximal peak perfusion, time to peak perfusion, time to return to baseline and perfusion-time integral.

Results

The analysis of the hyperemic time-courses demonstrated that the absence of peripheral serotonin synthesis, in the tph1 mutant was associated with abnormal skeletal muscle reperfusion patterns as compared to control mice after 5 and 30 minutes of ischemia (figure 1 and 2). In addition, the perfusion time integrals were different between control and mutant mice after 5 and 30 minutes of ischemia ($p<0.05$) (see figure 3).

After 5 min and 30 min ischemia, peak perfusion(figure 4) and time to peak perfusion were identical in the two groups.

The hyperemia duration was shorter in the serotonin deficient mice, the times to return to baseline were 194 ± 47 s 273 ± 93 vs in control mice after 5 minutes of ischemia ($p<0.05$) and 586 ± 159 s vs 871 ± 191 s in controls after 30 minutes ischemia (figure 5). The prolonged hyperemic response seen in the control animals was blunted in the tph1 mutants ($p<0.05$).

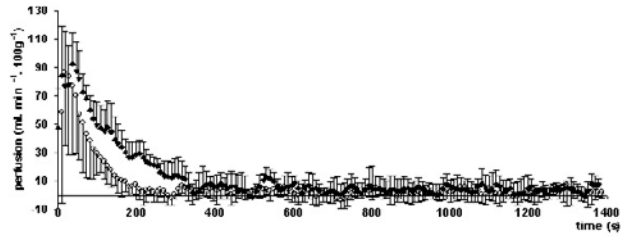

Figure 1: Temporal evolution of the calf muscle perfusion after an ischemic period of 5 minutes (♦ control mice; ◊ TPH1-KO mice)

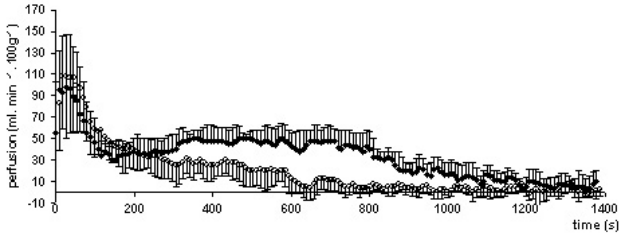

Figure 2: Temporal evolution of the calf muscle perfusion after an ischemic period of 30 minutes (♦ control mice; ◊ TPH1-KO mice)

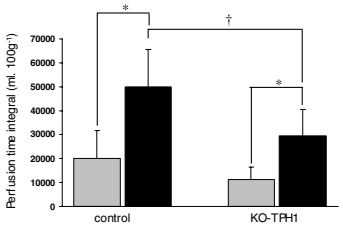

Figure 3: Bar graph of perfusion- time integrals after (■) 5 and (□) 30 minutes of ischemia
∗ : $p < 0.05$ between strains, † $p < 0.05$ between protocols

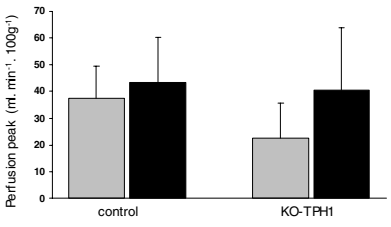

Figure 4: Bar graph of peak perfusion after (■) 5 and (□) 30 minutes of ischemia

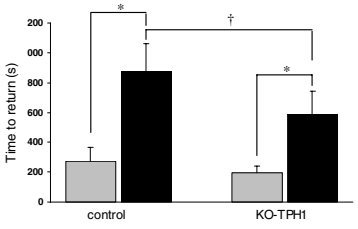

Figure 5: Bar graph of time to return to baseline after (■) 5 and (□) 30 minutes of ischemia
∗ : $p < 0.05$ between strains, † $p < 0.05$ between protocols

Discussion

These results indicate that serotonin might be involved in the regulation of the post-ischemic vasodilatation in the skeletal muscle. Serotonin is likely to participate to the control of the duration of the hyperaemic response while peak hyperemia seems to be unaffected by the absence of this molecule. The stimuli responsible for serotonin release are totally speculative at the moment. Hypoxia might be one of those [3].

More generally speaking, this study illustrates how the ASL technique can help to characterize the functional consequences of mutations and genetic manipulations.

References

1. Cote, F., et al., *Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function.* Proc Natl Acad Sci U S A, 2003. **100**(23): p. 13525-30.
2. Raynaud, J.S., et al., *Determination of skeletal muscle perfusion using arterial spin labeling NMRI: validation by comparison with venous occlusion plethysmography.* Magn Reson Med, 2001. **46**(2): p. 305-11.
3. Burnstock, G., et al., *Serotonin is localized in endothelial cells of coronary arteries and released during hypoxia: a possible new mechanism for hypoxia-induced vasodilatation of the rat heart.* Experientia, 1988. **44**(8): p. 705-7.