Study of a possible interaction between Reactive Oxygen Species and the mTORC1 pathway in the regulation of energy balance

Magalie Haissaguerre[1] | Samantha Clark[1] | Emilie Pupier[2] | Antoine Tabarin[1,2] | Daniela Cota [1,2]

[1]Inserm u862, Neurocentre Magendie, Pathophysiology of Energy Balance and Obesity, Bordeaux-F33077, France [2]Université de Bordeaux, Bordeaux-F33077, France

ABSTRACT

Obesity and its consequent metabolic disorders are severe health problems. The mTORC1 pathway and ROS are both involved in the hypothalamic regulation of energy balance. In the present study, we hypothesize that modulation of mTORC1 activity mediates ROS effects on food intake.

To this purpose, we injected C57Bl6J or S6K1 WT/KO mice by ICV administration with the ROS producer H2O2, the ROS scavenger honokiol, alone or in combination with the mTORC1 activator leptin or mTORC1 inhibitor rapamycin and we analyzed changes of food intake (FI) and body weight. Western blots were carried out to study hypothalamic mTORC1 activity. ROS levels were analyzed in POMC neurons using dihydroethidium (DHE) immunofluorescence.

Hypothalamic mTORC1 activity and particularly phosphorylation of mTORC1 downstream target S6K1 were induced by ICV administration of H2O2. H2O2 also increased c-fos expression in the hypothalamic arcuate nucleus and ROS signal in POMC neurons. These effects were associated with a significant decrease in FI over 24h. Conversely, ICV administration of Honokiol increased FI, but had a short-lasting effect. The behavioral effects of H2O2 and Honokiol were not seen in S6K1 KO mice. Pharmacological experiments using ICV co-administration of H2O2 and rapamycin in C57Bl6J mice showed that rapamycin (at a dose not acting on FI) was able to blunt the anorexigenic effect of H2O2. Similarly, ICV honokiol administration combined with an IP leptin injection blocked the anorexigenic effect of leptin.

Our preliminary results confirm that ROS modulators require a functional mTORC1 pathway to regulate FI. Studies are ongoing to better define this relationship at the molecular and neuroanatomical level.

INTRODUCTION

Obesity is a worldwide health problem, representing a risk for metabolic diseases characterized by high morbidity and mortality. The hypothalamus plays a crucial role in energy balance regulation by integrating hormonal and nutrient signals from the periphery. Molecular mechanisms regulating food intake at the hypothalamic level still remain unclear.

The **mammalian/mechanistic target of rapamycin complex 1 (mTORC1) kinase** and its downstream targets such as S6K1 and S6 are critical hypothalamic integrators of both nutrient and hormone actions on food intake regulation.

Oxidative stress is a physiological phenomenon leading to the formation of **Reactive oxygen species** (ROS), such as hydrogen peroxide (H2O2) or superoxide, which can be responsible for cellular toxicity. Nutrient availability and utilization also affect the formation of ROS, regulating neuronal hypothalamic activity and in particular the function of pro-opiomelanocortin (POMC) neurons in the hypothalamic arcuate nucleus. It was recently shown that increased ROS levels in POMC neurons favor satiety while hypothalamic suppression of ROS diminishes POMC neurons activity and food intake.

Little is known about the potential link between ROS and the mTORC1 pathway in energy balance regulation. In the current study we plan to investigate this relationship at the molecular, neuroanatomical and behavioral level.

MATERIALS AND METHODS

- **Animals:** 8 week-old male C57bl/6J and S6K1 mice on standard rodent chow.
- **Intracerebroventricular (ICV) surgery:** cannula implantation into the lateral ventricule (AP: -0,5 mm to bregma; Lat: 1,2 mm to bregma; DV: - 2,1 mm to bregma).
- **Acute ICV drugs injections:** ***H2O2*** (ROS inductor) or ***Honokio***l (ROS scavenger), ***Rapamycin*** (mTORC1 inhibitor) or ***Leptin*** (mTORC1 activator).
- **Food intake (FI)** was recorded in grams (gr) at 1h, 2h, 4h, 8h and 24h after icv injection; Body Weight (BW) was recorded before the icv injection and 24h afterwards.
- **Western blotting (WB):** Immunoblots were performed on hypothalamic samples using rabbit anti phospho and total S6K1 antibodies.
- **Immunohistochemistry (IHC):** 4% paraformaldehyde-fixed brains were cut on a cryostat. Resulting floating sections were used in single DAB staining using a c-fos antibody.
- **ROS production analysis:** IP dihydroethidium (DHE) and ICV H2O2 or saline were injected in C57Bl6J mice before sacrifice and followed by a POMC/DHE immunofluorescence analysis.
- **Statistical analysis:** ANOVA and t test by STATISTICA program. * p<0,05; ** p<0,01 and *** p<0,001. Data are mean ± SEM. Number of animals are in parenthesis.

RESULTS : Effects of icv H2O2 (ROS activator) in C57bl/6J mice *in vivo* and *ex vivo*

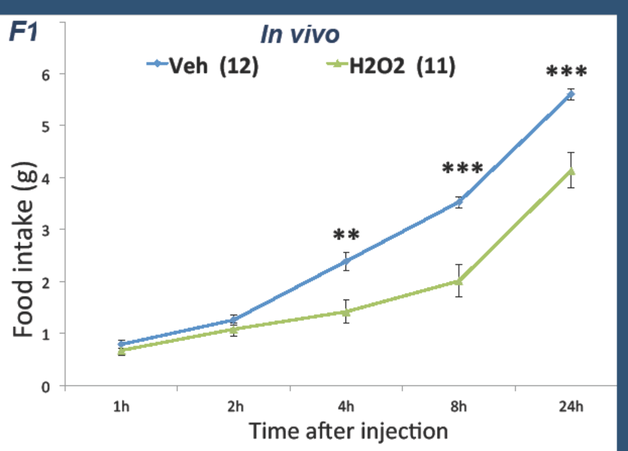

Figure 1. Icv H2O2 decreases food intake in C57 chow-fed mice.

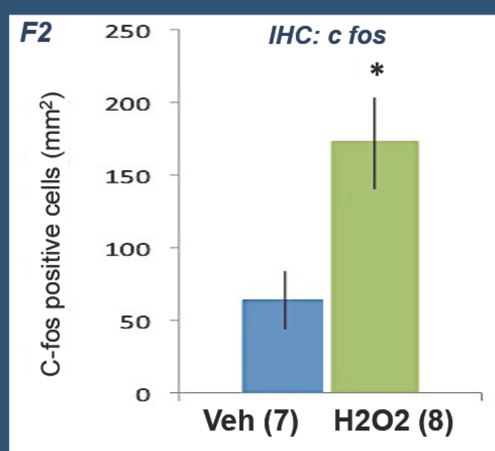

Figure 2. Icv H2O2 increases c-fos staining in the arcuate nucleus.

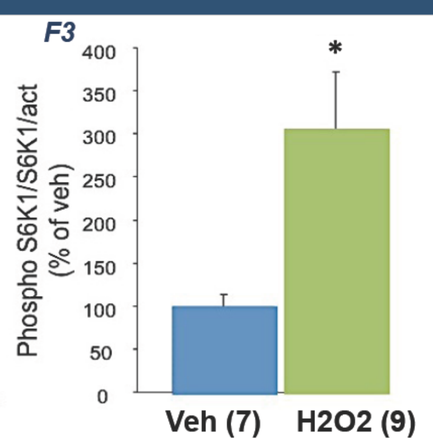

Figure 3. Icv H2O2 increases the activity of S6K1 in the hypothalamus.

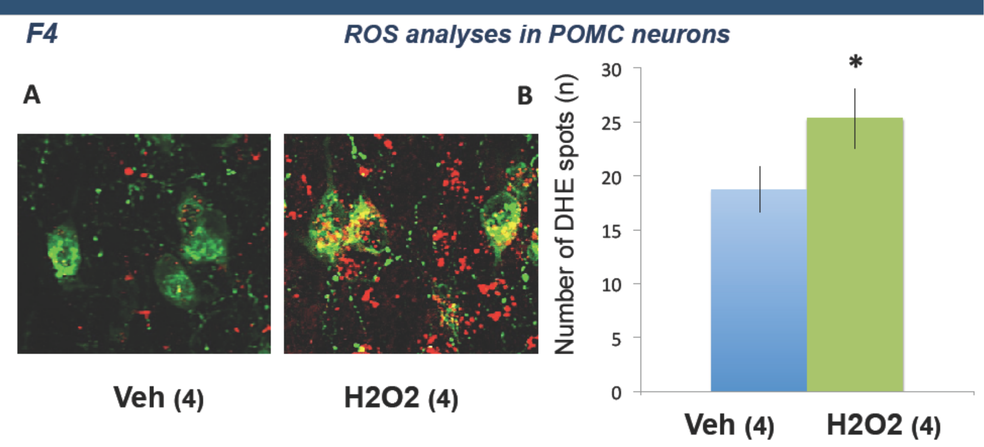

Figure 4. Icv H2O2 increases DHE signal (in red) (x63) (A) and number of spots (B) in POMC neurons from H2O2 icv treated mice.

Effects of icv H2O2 on FI in S6K1 mice

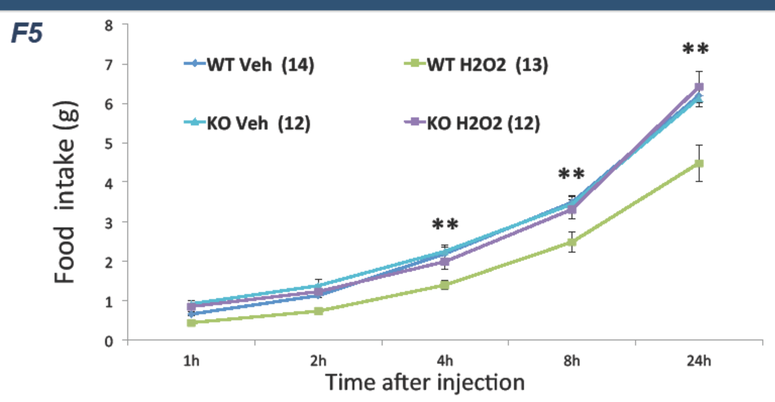

Figure 5. Icv H2O2 decreases food intake in S6K1-WT, but not in S6K1-KO mice.

Effects of icv Honokiol (ROS scavenger) on FI in C57bl/6J and S6K1 mice

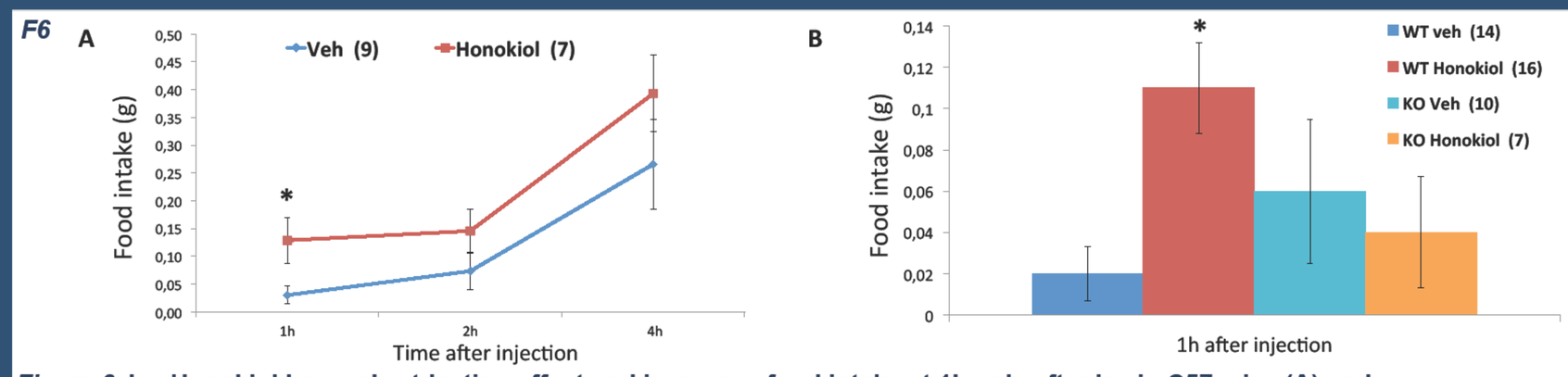

Figure 6. Icv Honokiol has a short-lasting effect and increases food intake at 1h only after icv in C57 mice (A) and S6K1-WT mice, but not in S6K1-KO mice (B).

Effects of the icv co-administration of ROS modulators and mTORC1 modulators on FI in C57bl/6J mice

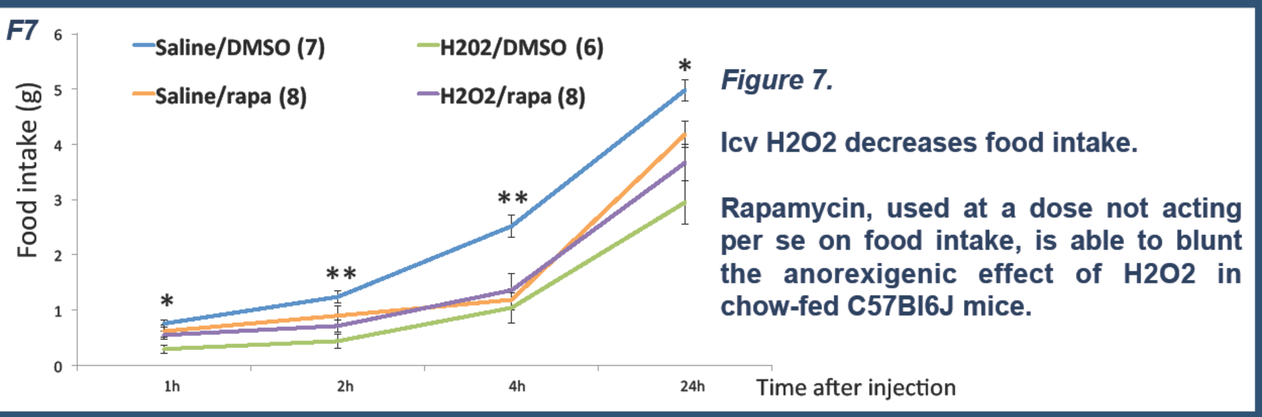

Figure 7.

Icv H2O2 decreases food intake.

Rapamycin, used at a dose not acting per se on food intake, is able to blunt the anorexigenic effect of H2O2 in chow-fed C57Bl6J mice.

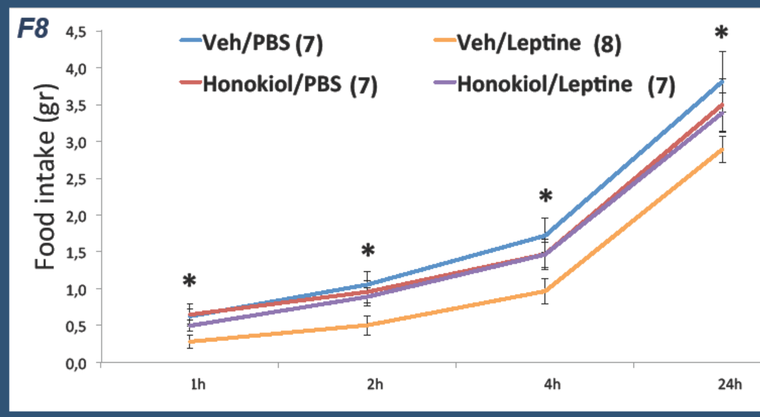

Figure 8.

Ip Leptin decreases food intake.

Honokiol, used at a dose not acting per se on food intake, is able to block the anorexigenic effect of Leptin in chow-fed C57Bl6J mice.

CONCLUSION

Our data demonstrate that ROS activation by central H2O2 administration is able to decrease food intake and body weight in chow-fed C57bl6/J mice. This is associated with an increase in ROS levels in POMC neurons. Conversely, central ROS inhibition by ICV Honokiol administration in chow-fed C57bl6/J mice increases food intake.

These effects depend upon a functional mTORC1 pathway, since they are not observed in mice lacking the mTORC1 downstream target S6K1 or in case of co-administration with rapamycin, a mTORC1 inhibitor. Molecular and neuroanatomical studies are ongoing to further elucidate the relationship between ROS and the mTORC1 pathway in the hypothalamic control of food intake.

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Magalie Haissaguerre

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