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# ABSTRACTS

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# PRELIMINARY EVIDENCE ON THE PRESENCE AND FUNCTIONING OF ALTERNATIVE GLUCOSENSOR SYSTEMS IN RAINBOW TROUT HYPOTHALAMUS

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## Introduction

In previous studies (Polakof et al., 2011; Soengas, 2014) we have characterized in rainbow trout the presence and functioning in central and peripheral places of glucosensor systems based on glucokinase related to the control of food intake (hypothalamus and hindbrain), hormone relase (Brockmann bodies) or metabolic homeostasis (liver). Other glucosensor systems have been characterized in mammalian hypothalamus but there is no evidence of their presence and functioning in fish tissues yet (Soengas, 2014). Therefore, we aimed to evaluate the presence in rainbow trout hypothalamus of alternative glucosensor systems based on: SGLT-1, LXR, taste receptors or mitochondrial activity. Moreover, we have also evaluated the response of these systems to changes in the levels of glucose.

## Material and Methods

24h fasted rainbow trout were anesthezized and IP injected with 5 $mL.Kg^{-1}$ of saline alone (normoglycaemic) or containing insulin (4 mg/kg; hypoglycaemic) or glucose (500 mg/kg; hyperglycaemic). Immediately after injection, fish were returned to their tanks where remained for 6h without any access to food. Fish in all tanks were sampled after 6h. In each group 10 fish were used for the assessment of metabolite levels and enzyme activities and 5 fish were used for the assessment of gene expression. In each sampling, fish were anesthezized, blood was collected and fish were sacrificed by decapitation, and hypothalamus was taken and stored. Parameters were assessed as previously described (Polakof et al., 2010; Librán-Pérez et al., 2012). Comparisons among groups were carried out using one-way ANOVA.

## Results

Glucose levels changed in parallel with the treatment used both in plasma and in hypothalamus. In hypothalamus, levels of glucose and glycogen as well as the activities of GK and HOAD and the expression of GK increased in parallel with the increase of glucose levels in plasma. The expression of PK, FBPase, COX4 and T1R3 decreased in parallel with the increased glucose levels. No significant changes were noted for LXR and SGLT-1 mRNA levels in hypothalamus.

## Discussion and Conclusion

The experimental design was appropriate since changes observed in glucose levels both in plasma and in hypothalamus changed according to the glycaemic treatment. In response to treatment changes observed in parameters related to the classical glucosensing system based on GK changed as expected reinforcing the validity of the experimental design. Changes observed in parameters related to alternative glucosensing systems displayed in general changes supporting the presence and functioning of alternative glucosensing systems based on LXR (changes in FBPase mRNA though no changes were apparent in LXR mRNA), taste receptors (changes in T1R3 mRNA levels), and mitochondrial activity (changes in HOAD activity and COX4 mRNA abundance). In contrast, no apparent changes were noted in the parameter assessed related to a putative SGLT-1 glucosensing-based system.

In conclusion, these preliminary results support, for the first time in fish, the presence in rainbow trout hypothalamus of glucosensing systems alternative to that mediated by GK. The role of these putative glucosensor systems has to be assessed in further studies. We can hypothesize that it could be related to the control of food intake in a way comparable to that described for the glucosensor system based on GK.

Table 1. Levels of metabolites, enzyme activities and gene expression of parameters related to different glucosensing systems in plasma and hypothalamus of rainbow trout under different glycaemic conditions elicited by intraperitoneal (IP) injection of 5 $ml\cdot kg^{-1}$ body weight of saline alone (normoglycaemic) or containing 4 mg bovine $insulin\cdot kg^{-1}$ body mass insulin (hypoglycaemic) or 500 mg $D\text{-}glucose\cdot kg^{-1}$ body mass (hyperglycaemic) for 6 hours. Data represent mean ± SEM of 5 (gene expression) or 10 measurements. Gene expression results are referred to normoglycaemic group and are normalized by β-actin expression. Different letters indicate significant differences ($P<0.05$) among glycaemic conditions.

*(Continued on next page)*

| | Treatment | | |
|---|---|---|---|
| **Parameter** | Hypoglycaemic | Normoglycaemic | Hyperglycaemic |
| *Plasma* | | | |
| Glucose (mM) | 1.95±0.23 **a** | 4.99±0.27 **b** | 11.20±0.51 **c** |
| *Hypothalamus* | | | |
| Glucose (μmol.$g^{-1}$) | 2.15±0.26 **a** | 2.49±0.25 **a** | 7.83±1.01 **b** |
| Glycogen (μmol.$g^{-1}$) | 1.02±0.25 **a** | 1.72±0.36 **b** | 1.38±0.29 **ab** |
| GK activity | 0.17±0.04 **a** | 0.74±0.21 **b** | 1.12±0.40 **b** |
| HOAD activity | 97.7.±13.9 **a** | 124.7±14.5 **a** | 273.8±16.5 **b** |
| LXR mRNA | 0.68±0.09 | 1±0.15 | 0.61±0.08 |
| PK mRNA | 1.66±0.18 **a** | 1±0.21 **ab** | 0.69±0.12 **b** |
| FBPase mRNA | 1.52±0.16 **a** | 1±0.19 **ab** | 0.82±0.06 **b** |
| COX4 mRNA | 1.43±0.23 **a** | 1±0.16 **ab** | 0.69±0.11 **b** |
| T1R3 mRNA | 5.16±0.56 **a** | 1±0.05 **b** | 0.58±0.18 **c** |
| SGLT-1 mRNA | 1.10±0.15 | 1±0.30 | 0.95±0.16 |

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