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INSULIN TREATMENT MODULATES THE RESPONSE OF HYPOTHALAMIC FATTY ACID SENSORS IN RAINBOW TROUT

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Introduction

In previous studies (Librán-Pérez et al., 2012; Soengas, 2014) we have characterized in rainbow trout the presence and functioning in central and peripheral places of fatty acid (FA) sensor systems based on FA metabolism, FA transport through FAT/CD36, and mitochondrial activity, which are related to the control of food intake (hypothalamus), hormone relase (Brockmann bodies) or metabolic homeostasis (liver). Metabolic hormones such as insulin and leptin and gastrointestinal hormones such as CCK, ghrelin or GLP-1 are known to modulate the activity of metabolic sensors in mammalian hypothalamus (Levin et al., 2011). In fish, we have already demonstrated their effects on glucosensing systems (Polakof et al., 2011, Soengas, 2014) but to date there is no evidence available regarding their possible modulator role on the activity of hypothalamic FA sensing systems. Therefore, we aimed to evaluate the possible modulatory role of insulin in the capacity of different FA sensing systems in rainbow trout as a model of teleost fish.

Material and Methods

24h fasted rainbow trout were anesthezided and IP injected with 10 mL.Kg^{-1} of saline alone (control) or containing insulin (2 mg.Kg^{-1}), oleate (30 µg.kg^{-1}), or insulin+oleate. To safely deliver FA they were solubilized in 45% hydroxypropyl-β-cyclodextrin (HBP) to a final concentration of 17 mM. 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 anesthezided, blood was collected and fish were sacrificed by decapitation, and hypothalamus were taken and stored. Parameters were assessed as previously described (Librán-Pérez et al., 2012). Comparisons among groups were carried out using one-way ANOVA followed by Sudent-Newman-Keuls test.

Results

Innsulin treatment alone decreased levels of glucose in plasma and mRNA levels in hypothalamus. Oleate treatment alone increased FA levels in plasma and decreased in hypothalamus CPT-1 activity and mRNA levels of ACLY, FAS, and SREBP1c and increased mRNA abundance of FAT/CD36. The joint treatment of insulin and oleate returned parameters to values similar to those of the controls and different than those of oleate treatment alone for FA levels in plasma, and mRNA abundance of FAT/CD36, FAS, and SREBP1c in hypothalamus. In other hypothalamic parameters the joint treatment of I+O resulted in values in parameters similar to those of the group such as for FA levels, CPT-1 activity or mRNA abundance of ACLY and UCP2a in hypothalamus

Table I. Levels of metabolites, enzyme activities (mU.mg^{-1} protein), and gene expression of parameters related to different FA sensing systems in plasma and hypothalamus of rainbow trout after intraperitoneal (IP) injection of 10 mL·Kg^{-1} body weight of saline alone (control, C) or containing 2 mg bovine insulin·kg^{-1} body mass insulin (insulin, I) or 30 µg.kg^{-1} oleate (O) or insulin+oleate (I+O) for 6 hours. Data represent mean ± SEM of 10 (metabolites and enzyme activities) or 5 (gene expression) measurements. Gene expression results are referred to control group and are normalized by β-actin expression Different letters indicate significant differences (P<0.05) from different groups.

Discussion and Conclusion

Changes observed in parameters related to putative FA sensing systems in hypothalamus after oleate treatment agree in general with those already described in the same species (Librán-Pérez et al., 2012). Moreover, insulin treatment alone was effective in reducing plasma glucose levels. These results validate the experimental design. Insulin treatment alone decreased mRNA abundance of ACLY in hypothalamus suggesting that this hormone is modulating the FA sensing system related to FA metabolism in that tissue. More important the presence of insulin was able to modify the response to oleate of the parameters related to the different FA sensing systems assessed (FA metabolism, FA transport through FAT/CD36, and mitochondrial activity).

In conclusion, these preliminary results suggest that insulin has a modulatory role in the capacity of different FA sensing systems in rainbow trout.

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Parameter	Treatment C	I	O	I+O
Plasma				
Fatty acid (mM)	0.18±0.02 a	0.14±0.02 a	0.27±0.02 b	0.15±0.01 a
Glucose (mM)	4.5±0.18 a	1.2±0.05 b	4.5±0.25 a	1.2±0.08 b
Hypothalamus				
Fatty acid (μmol.g^{1})	0.14±0.01 a	0.15±0.01 a	0.18±0.01ab	0.20±0.02 b
Triglyceride (μmol.g^{1})	0.47±0.06 ab	0.56±0.09 ab	0.63±0.05 a	0.48±0.02 b
CPT-1 activity	9.6±0.97 a	8.9±0.97 a	5.6±0.63 b	4.9±0.91 b
ACLY mRNA	1.0±0.14 a	0.61±0.09 b	0.63±0.10 b	0.57±0.10 b
FAT/CD36 mRNA	1.0±0.07 a	1.2±0.12 ab	1.3±0.12 b	1.2±0.12 ab
FAS mRNA	1.0±0.09 a	0.95±0.10 ab	0.70±0.02 b	0.95±0.04 ab
SREBP1c mRNA	1.0±0.10 a	0.78±0.06 ab	0.61±0.07 b	0.79±0.05 ab
UCP2 mRNA	1.0±0.09 a	1.3±0.24 ab	1.5±0.33 ab	2.2±0.52 b

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