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| Sommario | The term “thyroid hormones”, classically referred to both 3,5,3-triiodothyronine (T3) and thyroxine (T4), seems nowadays to be simplistic; some T3 and T4 metabolites, particularly 3,5 diiodothyronine (3,5-T2) and 3-iodothyronamine (T1AM), are independent chemical messengers, with specific metabolic effects. In this PhD thesis we focused on the effects of these derivates on different tissues. In the first project we evaluated the effects of T1AM on the glutamatergic pathway, the main excitatory system in brain. A cancer hybrid cell line of mouse neuroblastoma and rat glioma (NG108-15) and a human glioblastoma cell line (U-87 MG) were used as in vitro model and treated with different T1AM concentration for 24h, alone or in combination with resveratrol 10 µM and/or amyloid peptide (25-35) 10 µM. Firstly, we characterized cell lines for the expression of receptors implicated in glutamatergic pathway with real time PCR (qRT-PCR) and Western blot. Both cell lines expressed AMPA, NMDAR1 and EphB2, but only U-87 MG expressed TAAR1, the putative T1AM receptor. Using LC-MS-MS we discovered that both lines were able to take up T1AM and, rapidly catabolized it to TA1. In both cell lines, T1AM showed a slightly but significant cytotoxic action starting from 0.1 M, that increase is presence of -amyloid (10 µM), but not resveratrol (10 µM) evaluated |

by MTT test. We then evaluate glucose consumption using a glucose assay and observed in the NG108-15 a metabolic effect mediated by T1AM (p<0.05). For protein expression and post-translation modifications Western blot was used and an increase in the phosphorylation of Ca-calmodulin-dependent protein kinase (CaMK) II (pCaMKII/total CaKMII, p<0.05) in NG108-15 cell line was observed. In association with resveratrol T1AM could increase the expression of PKC (p<0.001 vs RSV) in the same cell line, a synergic effect showed exclusively in presence of both T1AM and resveratrol at all tested thyronamine concentration. In U-87 MG T1AM induce the phosphorylation of the transcriptional factor cAMP response element-binding protein (CREB) (PCREB/total CREB p<0.05). Our results indicated that these two nervous cell lines express receptors implicated in glutamatergic system and might be used as biochemical model to study its post synaptic signaling cascade. T1AM had a minimal cytotoxic effect and it was able to induce different post-translational modification in neuronal cell lines. T1AM might activate mechanisms of action which included increasing CaMKII phosphorylation and PKC expression in NG108-15 while in U-87 MG induced the activation of the transcriptional factor CREB. We then focused on another endogenous thyroid hormone derivate, the 3,5-diiodo-l-thyronine (3,5-T2) and its effect on heart which have been poorly investigated so far. It's well understood that 3,5-T2 is able to regulate energy expenditure, resting metabolic rate and oxygen consumption with a mechanism that might involve mitochondria. We decided to evaluate the functional metabolic, and toxic effect of 3,5-T2 using both in vitro and ex vivo models of cardiac preparations. As comparison for our results we also evaluated the response to T3 and T4. As cell culture we selected the H9c2 cells (rat cardiomyoblasts) to determine 3,5-T2, T3, and T4 uptake using LC-MS-MS. We treated cells with 3,5-T2 (0.1 to 10 µM) and evaluated cell viability using MTT test and crystal violet staining. We also investigate a possible 3,5-T2 metabolic effect performing a glucose and hexokinase assay. In the end we measured the cardiac functional effects, perfusing isolated working rat hearts with 3,5-T2, T3, or T4 in Krebs-Ringer buffer and recording hemodynamic variables. H9c2 cells took up 3,5-T2, in cell lysate and the analyte levels increased slowly over time. 3,5-T2 significantly decreased MTT staining at 0.5–10 µM concentration, an effect confirmed by the crystal violet staining only at 10 µM T2, while equimolar T3 and T4 did not share this effect. In cells exposed to 0.1 or 1.0 µM of 3,5-T2 glucose uptake increased by 23% or 30% (p < 0.05). On the opposite side, T3 did not affect glucose consumption which was significantly reduced by 1 and 10 µM T4 (24 and 41%, respectively, p < 0.01 and p < 0.0001). In the isolated perfused rat heart, 10 µM T2 produced a transient and slight reduction in the cardiac output and aortic flow (p<0.05), while thyroid hormone did not induce any hemodynamic change. Our findings demonstrate that 3,5-T2 was taken up by cardiomyoblasts, and in a concentration range between 0.1 µM and 1.0 µM modulated cardiac energy metabolism increasing glucose accumulation. Furthermore, we observed some evidence of cytotoxicity and a transient impairment of contractile performance only at the highest 3,5-T2 concentration tested (10 µM). These effects seem to be specific for 3,5-T2, since they are not reproduced by thyroid hormone. In the end we develop a novel ad-hoc optimized method to quantify T2 isomers using LC-MSMS in human serum. T2 isomers (3,5-T2 and 3,3'-T2) has been detected in human blood using immunological methods,

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| | but until now a reliable assay based on mass spectrometry was not available. We obtained 2 mL of serum samples from 28 healthy subjects. The serum was firstly deproteinized with acetonitrile and then exposed to a solid phase extraction-based procedure. Then samples were furtherly cleaned by hexane washing and subjected to another step of deproteinization with acetonitrile to precipitate residual proteins. Both isomers were then analyzed by high performance liquid chromatography coupled to tandem mass spectrometry. We developed a method with 88–104% accuracy, 95–97% precision, 78% recovery and a matrix effect average of +8%. In the serum sample 3,5-T2 was detected with a concentration averaged (mean ± SEM) 41 ± 5 pg/mL and 133±15 pg/mL for 3,3-T2. Furthermore, we observed a significant correlation between 3,5-T2 and 3,3-T2 concentrations (r = 0.540. p < 0.01), while no significant relation was observed with thyroid hormone. In conclusion, this method can quantify both T2 isomers in human serum using a reliable assay based on LC-MS-MS. The concentrations of these isomers lie in the subnanomolar range and show a significant correlation in healthy subject. |
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