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Effects of peripheral iodothyronine deiodinases on thyroid hormone economy: Insights from the overexpressing mice

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Background: In peripheral tissues, triiodothyronine (T3) production and consequent thyroid hormone actions are mainly regulated by iodothyronine deiodinases (DIOs) classified into 3 types: D1, D2, and D3. Although thyroid hormone economy in the DIO-deficient mice and patients carrying genetic variants of DIOs was well clarified, a condition of DIOs upregulation was not enough elucidated. We aimed to investigate effects of peripheral DIOs on thyroid hormone economy and consequent thyroid hormone action using novel DIO-overexpressing mice that were not yet generated. **Methods:** We cloned coding sequences of human DIOs with FLAG-tag and HiBiT-tag sequences into a pcDNA3.1 vector. To obtain full-length proteins, we modified these vectors by cloning selenocysteine insertion sequence of each DIO (SECIS vectors). Western blot analyses and HiBiT lytic assay using HEK293T cells revealed that SECIS vectors expressed full-length proteins with substantial activity. Subsequently, in vivo transfections of pLIVE-based SECIS vectors into male C57BL/6J mice were performed by hydrodynamic gene delivery to generate mice overexpressing DIOs (D1, D2, and D3 mice). **Results:** First, we verified that DIOs were successfully overexpressed in the livers using HiBiT lytic assay as well as RT-PCR. Analyses of multiple organs of D3 mice assured that full-length protein was not significantly expressed in the heart, the kidney, and the skeletal muscle. RT-PCR analyses also suggested that the pituitary glands and the thyroid glands were not transfected. We evaluated thyroid hormone economy of DIO-overexpressing mice compared to Empty mice transfected with pLIVE-Empty vector. D1 mice did not have changes in serum thyroid hormone levels, whereas D2 mice had higher serum fT3 levels. Clearer changes were found in D3 mice. They had hypothyroidism with higher serum rT3 levels. Correspondingly, in their livers, rT3 concentration was higher and T3-responsive genes were downregulated. We elucidated by adding the cohort with levothyroxine (LT4) administration

because DIO-overexpressing mice had liver T4 insufficiency probably due to consumption by DIOs. Comparisons between the DIO-overexpressing mice with and without LT4 administration revealed that D2 mice presented upregulation of T3-responsive genes by LT4 administration distinct from Empty mice. **Conclusions:** We generated DIO-overexpressing mice that expressed human DIOs predominantly in the liver. They provided physiological evidence to integrate the phenotypes of the knockout mice and specific clinical situations. D2 mice seemed to recruit T3 into the circulation as well as in the liver. D3 mice had an overt phenotype characterized by consumptive hypothyroidism. In addition, D3 mice are expected to be a novel hypothyroidism model that does not involve disruption of thyroidal hormone secretion. DIO-overexpressing mice can be generated by single injections of plasmid vectors of interest and can contribute to progress in the field of thyroid hormone economy and action.

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