Interconnection between cathepsin protein levels and thyroid hormone transporters in human thyroid epithelial cells

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INTRODUCTION

We have previously shown that there is an interconnection between thyroid hormone transporters and thyroid hormone processing enzymes, the cathepsins [1]. Thyroid hormone transporter deficient mice are characterized by altered degradation states of thyroglobulin in comparison to wild type controls. This has been attributed to altered levels of cathepsins, specifically cathepsin B, D and L. In this study, an *in vitro* model was used in order to further verify the interconnection between Tg-processing cathepsins and thyroid transporters, particularly the MCT8. A pharmacological intervention with sulfobromophthalein disodium salt hydrate (BSP) an inhibitor of MCT8 was conducted using the human thyroid epithelial Nthy-ori-3-1 cells.

METHOD

Cultured Nthy-ori 3-1 cells at 80% confluence were treated with fetal bovine serum-free RPMI containing BSP for either 30 minutes or 2 hours at 37°C, after which cells were either fixed in 4% paraformaldehyde or lysed for further immunocytochemical and immunoblotting analyses, respectively.

BSP inhibition triggers difference in morphology

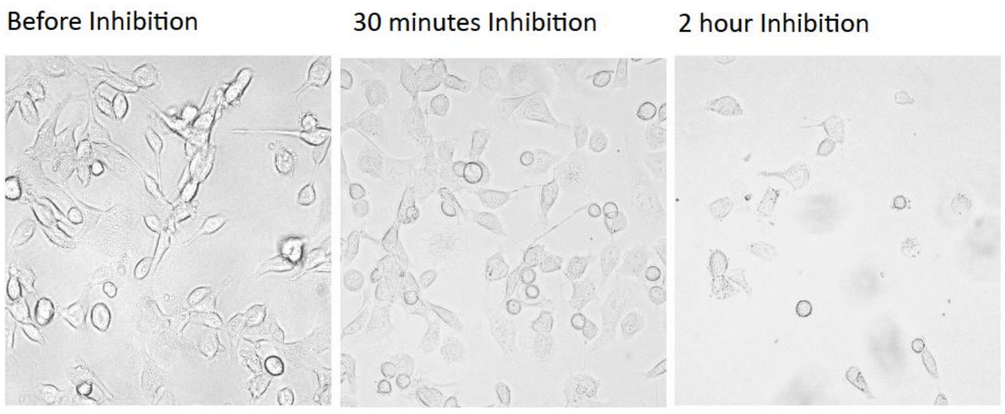


Fig 1. The morphology of Nthy-ori 3-1 cells is altered under BSP inhibition conditions, resulting in detachment of Nthy-ori 3-1 cells from the substratum.

Accumulation of anti-T4 immunopositive signal intracellularly upon BSP inhibition

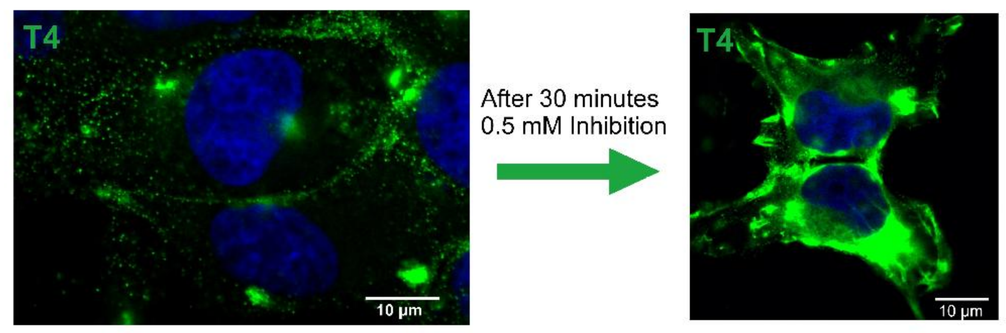


Fig 2. BSP inhibition of MCT8 resulted in increased T4 immuno-positive signal intracellularly, mimicking a thyrotoxic condition.

Thyroglobulin localization differs in BSP inhibited cells

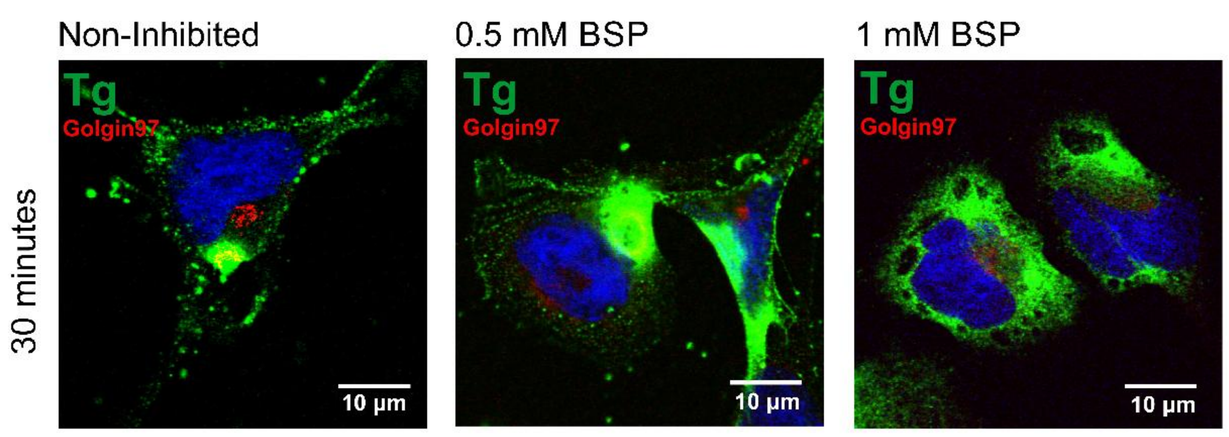


Fig 3. Thyroglobulin was typically observed in peripherally located vesicles and confined to a juxta-nuclear region in non-inhibited Nthy-ori 3-1 cells. Upon BSP-treatment, thyroglobulin was detected to a higher extent in intracellular vesicles.

Thyroglobulin degradation state upon BSP inhibition

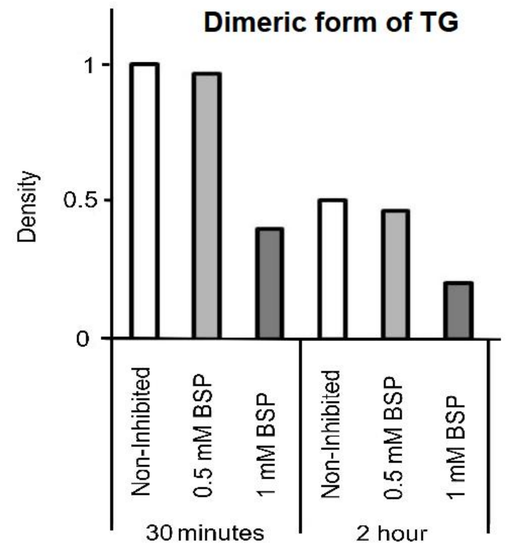


Fig 4. Immunoblotting of thyroglobulin revealed less dimeric thyroglobulin at long-term-treatment with BSP.

Cathepsin L localization differs in BSP inhibited cells

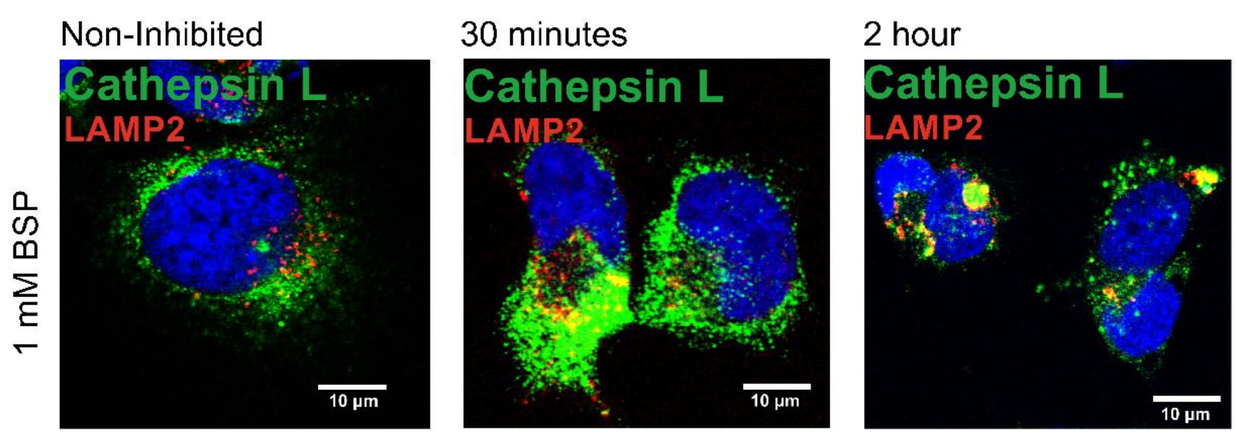
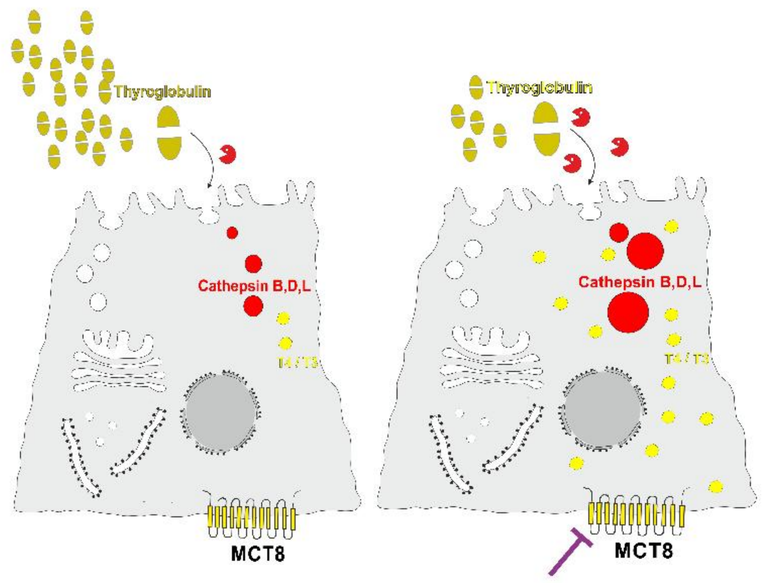


Fig 5. Cathepsin L is located in different compartments of the secretory and endocytic pathways in Nthy-ori 3-1 cells under non-inhibition conditions (left). BSP inhibition resulted in enlargement of cathepsin L-immunopositive vesicles.

CONCLUSION



Upon BSP-mediated inhibition of MCT8 function, altered localization and different degradation states of thyroglobulin are observed in Nthy-ori 3-1 cells, which correlates with upregulated cathepsin L levels.

1. Correlation of the expression and localization of thyroid hormone transporters with thyroglobulin-processing cathepsins in mouse thyroid epithelial cells. J. McInnes, J. Weber, M. Rehders, P. Saftig, C. Peters, T. Reinheckel, U. Schweizer, H. Heuer, E.K. Wirth, K. Brix Eur Thyroid J 2013, 2(suppl 1), p. 90, OP45