Contribution ID: **326** Type: **Verbal**

Radiometric determination of enzyme activities, exemplified by several key enzymes of thyroid hormone metabolism

Thursday, 22 April 2010 10:30 (15 minutes)

Radiometric enzyme assays are based on the conversion of radio-labeled substrates to labeled products, and on the measurement of radioactivity of either products or residual substrate after their quantitative separation. In the present studies, we aimed to establish valid assay conditions for radiometric determination of the activities of several enzymes, which are involved in the metabolism of thyroid hormones (TH): Thyroid peroxidase (TPO), the key enzyme in the biosynthesis of TH in the thyroid gland, and uridinediphospho-glucuronyl-transferase (UDPGT) and iodothyronine sulfotransferases (ST), the enzymes responsible for biotransformations of TH in peripheral tissues. Further, with the aid of such developed assays, we attempted to follow the effects of some exogenous substances - bromide and perchlorate ions as supposed goitrogenic agents, and an antidepressant drug fluoxetine (Prozac) - on the metabolism of TH in the rat.
The procedure of the radiometric assay for TPO in vitro was based on the ability of TPO to oxidize [¹³¹I]-iodide in the presence of H₂O₂, generated in situ by glucose oxidase, and to catalyze subsequent iodination of specific tyrosyl residues in the added thyroglobulin. The measure of TPO activity in microsomal fractions of the thyroids was the amount of radio-iodine incorporated into thyroglobulin. This was determined either after TLC separation of the incubated samples, or simply after precipitation of radio-labeled protein and measurement of ¹³¹I radioactivity in separated fractions.
In the radiometric assay for UDPGT in rat liver microsomes, the rate of conjugation of phenolic group of L-3,3',5'-[¹²⁵I]-triiodothyronine ([¹²⁵I]-rT3) with glucuronic acid, which was catalyzed by UDPGT was measured. Reaction mixtures, containing uridinediphospho-glucuronic acid, the substrate [¹²⁵I]-rT3 and samples of microsomes, were analyzed after the incubation by chromatography on micro-columns of lipophilic Sephadex LH-20.
The basis for radiometric determination of ST activity in liver cytosolic fractions was the transfer of sulfonate moiety from the "active sulfate", 3'-phosphoadenosine 5'-phosphosulfate, to the substrate [¹²⁵I]-rT3. The extent of conversion was determined after the separation of the sulfated product from the unmodified [¹²⁵I]-rT3, again by Sephadex LH-20 chromatography.
With the use of the described radiometric assay for TPO, we found that the influence of exogenous bromide on the TPO activity in the rat thyroids was biphasic, with regard to the extent of bromide intake in the animals. Administration of fluoxetine alone caused a significant (about 2-fold) increase in UDPGT activity. In contrast, the radiometric determination of ST did not demonstrate any significant effects of the application to the rats of fluoxetine alone, or together with T3, on the induction of these enzyme activities.

Acknowledgments: This work was supported by the Academy of Sciences of the Czech Republic (Research project No. AV0Z50110509), by the Ministry of Education of the Czech Republic (Research project No. MSM0021622413) and by the Czech Science Foundation (Grant No. 304/08/0256).

Primary author: Dr PAVELKA, Stanislav (Dept. of Radiometry, Inst. of Physiolgy, ASCR Prague; and Inst. of Biochemistry, Masaryk Univ. Brno, Czech Rep.)

Presenter: Dr PAVELKA, Stanislav (Dept. of Radiometry, Inst. of Physiolgy, ASCR Prague; and Inst. of Biochemistry, Masaryk Univ. Brno, Czech Rep.)

Session Classification: Nuclear Methods in Medicine, Radiopharmaceuticals and Diagnostics, Labelled Compounds 2

Track Classification: Nuclear Methods in Medicine, Radiopharmaceuticals and Radiodiagnostics, Labelled Compounds