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## Quantification of iodothyronine deiodinases activities, induced in cultured astrocytes by purinergic agonists, with the use of radiometric enzyme assays

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Pavelka S.1,2

1Department of Radiometry, Institute of Physiology, v.v.i., Academy of Sciences of the Czech Republic, Prague and 2Institute of Biochemistry, Masaryk University, Brno, Czech Republic

Described are details of our newly elaborated radiometric methods for extremely sensitive determination of enzyme activities of iodothyronine deiodinases (IDs) of types 1, 2 and 3 in homogenates of cultured mammalian cells. IDs catalyze selective 5'- (outer ring) and 5- (inner ring) mono-deiodinations of iodothyronines and play crucial roles in the bio-transformations of thyroid hormones. The novel radiometric assays for IDs were based on the use of appropriate high-specific-radioactivity <sup>125</sup>I-labeled iodothyronines as substrates; TLC separation of radioactive products from the unconsumed substrates; film-less autoradiography of radiochromatograms using storage phosphor screens; and quantification of the separated compounds with a BAS-5000 (Fujifilm Life Science Co.) laser scanner. The applicability of our sophisticated radiometric methods was demonstrated by quantifying the alterations of IDs activities induced in cultured rat astroglial cells by a series of purinergic agonists (like ATP, ADP, AMP and adenosine), retinoic acid, and their combination. The newly developed radiometric assays proved to be very sensitive and rapid and, at the same time, reliable and robust. Support from the Academy of Sciences of the Czech Republic (Project No. AV0Z50110509) and from the Czech Science Foundation (GACR Grant No. 304/08/0256) is acknowledged.

**Primary author:** Dr PAVELKA, Stanislav (Institute of Physiology, ASCR, Prague/Masaryk University, Brno)

**Presenter:** Dr PAVELKA, Stanislav (Institute of Physiology, ASCR, Prague/Masaryk University, Brno)

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