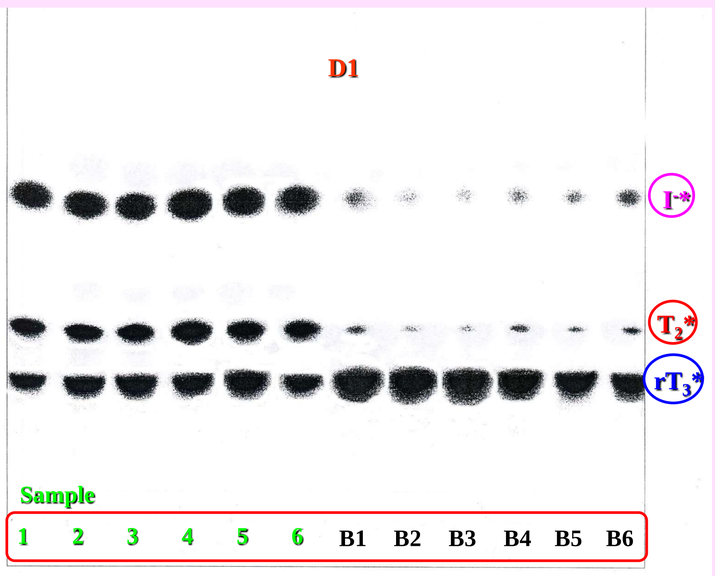
Radiometric Enzyme Assays: Extremely Sensitive Determination of Iodothyronine Deiodinases

S. Pavelka[1,2*]

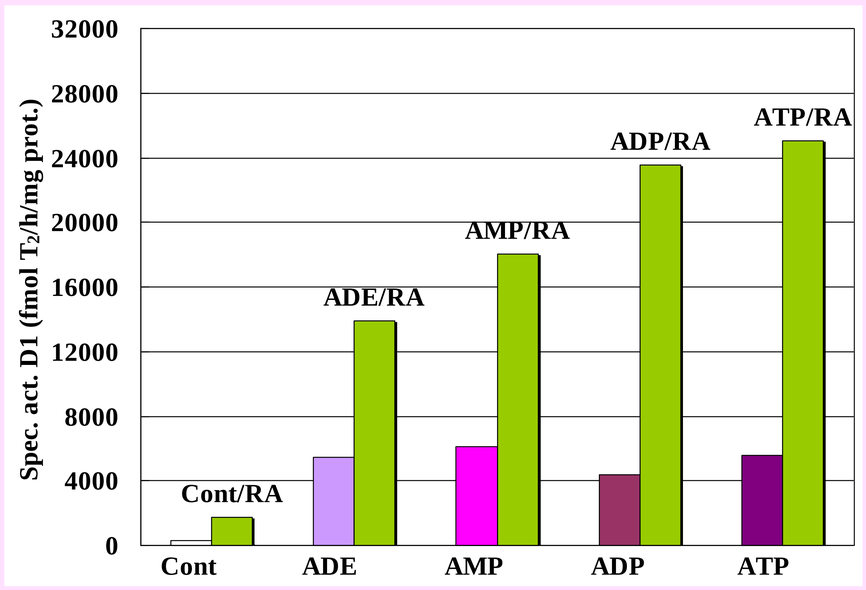
[1] Department of Radiometry, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague and [2]Central-European Technology Institute, Masaryk University, Brno, Czech Republic

***E-mail: pavelka@biomed.cas.cz or pav@chemi.muni.cz**

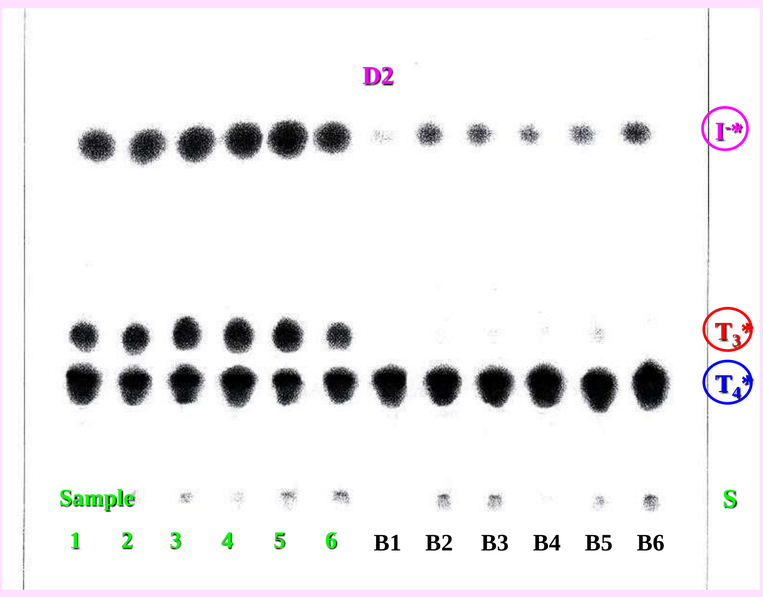
RESULTS



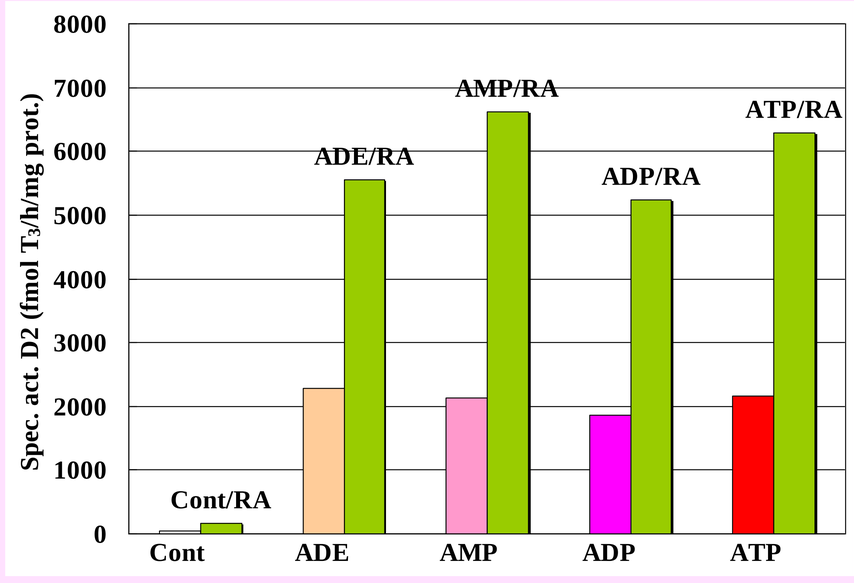
Assay for D1- radiochromatogram



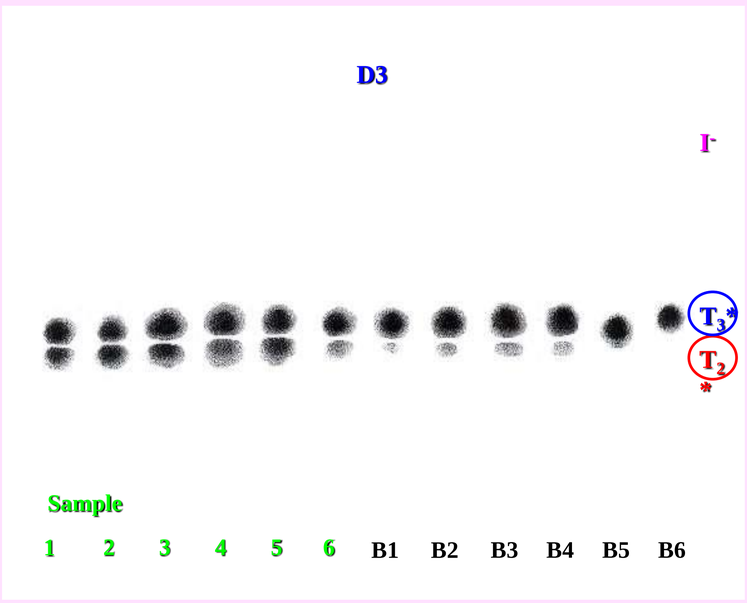
Specific enzyme activity of D1



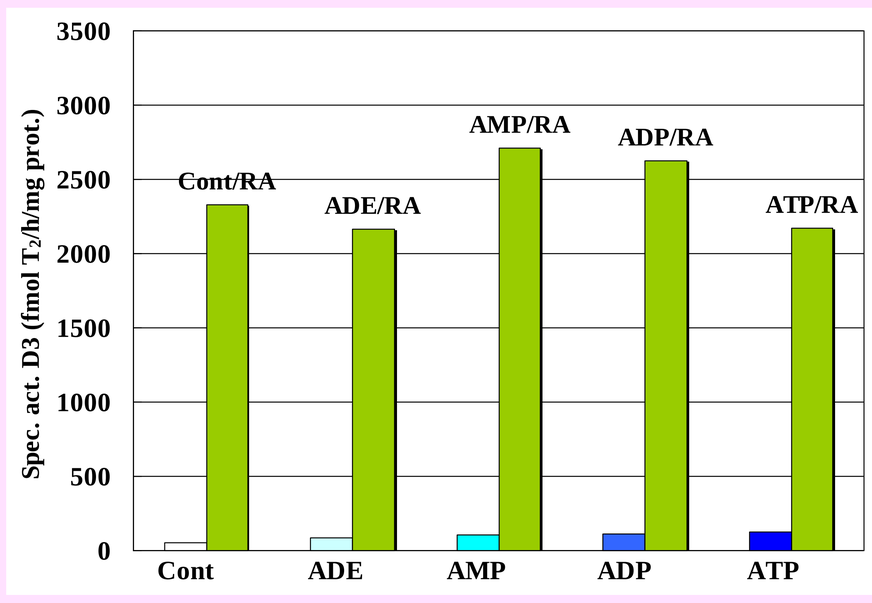
Assay for D2- radiochromatogram



Specific enzyme activity of D2



Assay for D3- radiochromatogram



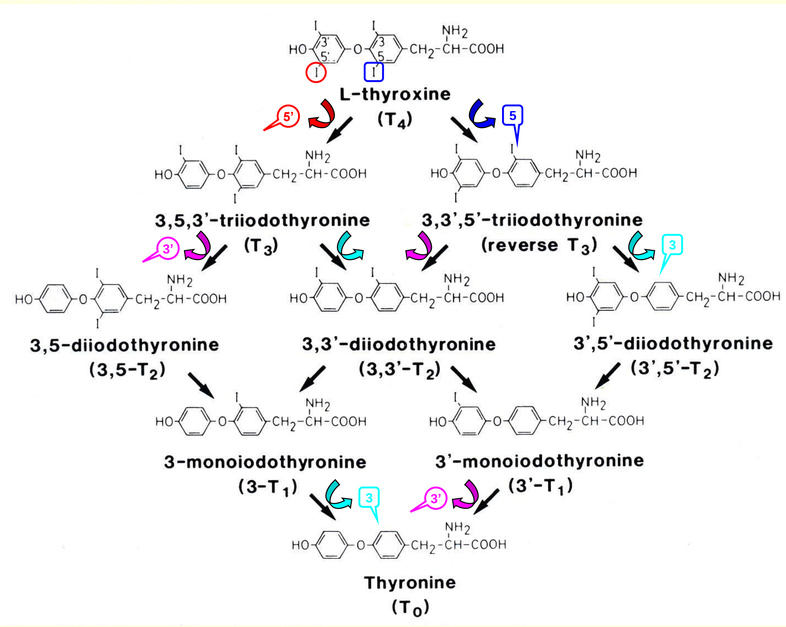
Specific enzyme activity of D3

Laser scanner BAS-5000 for Imaging Plates (Fuji)

INTRODUCTION

We have developed novel, very sensitive and rapid radiometric enzyme assays for iodothyronine deiodinases (IDs) of types 1, 2 and 3 (D1, D2 and D3, respectively). All the three IDs are integral membrane selenoproteins requiring for their catalytic activity a thiol cofactor. Outer ring 5'(3')-deiodinations (ORD) as well as inner ring 5(3)-deiodinations (IRD), carried out by these enzymes, play a crucial role in the metabolism of thyroid hormones (TH) – *see* the chart below.

The objectives of the present study were: 1) to find out correct assay conditions, allowing determination of true enzyme activities under the optimum concentration conditions; 2) to perform some applications of these adapted radiometric assays, e.g. to follow the changes in IDs activities, caused by short-time incubation of cultured rat astroglial cells with different concentrations of effectors, purinergic agonists, without and with retinoic acid (RA) pre-incubation.



Stepwise deiodinations of TH

METHODS and RESULTS

Astroglial cells obtained from cerebrocortical hemispheres of 2-day-old rats were grown to confluence (about 10-15 days) in DMEM medium containing 10 % fetal calf serum. Confluent cells were cultured for 3 additional days in a chemically defined medium (CDM) supplemented with insulin, and for another 2 days in CDM with an addition of hydrocortisone, transferrin, and selenite. After treatment with purinergic agonists, the medium was removed, the monolayer of cells washed and scraped in an assay buffer. IDs activities were measured in sonicates of cells containing 0.4-40 μg of protein in a final volume of 40 μl.

During the incubation of samples (30 min at 37°C), optimized amounts of nonradioactive and of the corresponding radioactively labeled iodothyronine substrates were also present together with 10-40 mM dithiothreitol. Aliquots of incubation mixtures were analyzed by TLC – *see* images of radiochromatograms above.

Culturing of astroglial cells (for 2-12 h) in the presence of various concentrations (20-500 μM) of ATP, ADP, AMP, adenosine (ADE) and a series of their analogues caused a more or less marked induction of D1 and D2 activities (up to 30-fold basal level), depending on the structure of the agonists. Activity of D3 was also induced by these substances but to a lesser extent (only 3- to 7-fold increase in comparison with the control cells). Induction of IDs activities was time- and concentration-dependent.

In another experiments, confluent cells were cultured for 2-3 days in the presence of 1 μM retinoic acid (RA) before addition of purinergic agonists. Preincubation of cells with RA had a crucial influence on the degree of induction of D1 and D2 activities by the action of purinergic agonists (up to 42-fold increase in D2) – *see* the graphs above.

CONCLUSIONS

- **The newly developed radiometric enzyme assays, based on**
  - **the use of ^{125}I-labeled iodothyronines of high specific activity as substrates**
  - **TLC separation of radioactive products from the unconsumed substrates**
  - **film-less autoradiography of radiochromatograms using storage phosphor screens (Imaging Plates BAS-IP MS 2025)**
  - **quantification of the separated compounds with the BAS-5000 laser scanner**

  proved to be very sensitive and rapid and, at the same time, reliable.
- **With the aid of these assays, a new signaling pathway in the multiregulation of IDs activities in astroglial cells was proved. It included the participation of endogenous purines (ATP, ADP, AMP and adenosine) and some subtypes of P_2 purinoceptors and A_{2A} adenosine receptor.**
- **Preincubation of the cells with retinoic acid synergistically influenced the degree of induction of IDs by purines, especially in the case of D1 and D2. The activity of D3 was markedly elevated already by retinoic acid itself and was not affected significantly by the presence of purinergic agonists.**

ACKNOWLEDGEMENTS

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).