Mammary Gland Carcinoma-related Increase of Type I Iodothyronine 5'-deiodinase Activity in Sprague-Dawley Rats

D. MACEJOVÁ[1], J. LÍŠKA[2] AND J. BRTKO[1]

1 *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovakia*
2 *Institute of Histology and Embryology, Medical Faculty of Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia*

Key words: Type I iodothyronine 5'-deiodinase — Sprague-Dawley rats — Mammary gland tumours — Histopathology

Abstract. Type I, iodothyronine 5'-deiodinase (5'-DI) catalyses deiodination of the prohormone thyroxine (T_4) to the metabolically active 3,5,3'-triiodo-L-thyronine (T_3). The present study was undertaken to investigate the activity of 5'-DI in rat mammary gland tumours representing various combinations of histologically defined papillary, cribriform or comedo patterns of ductal carcinomas. Female Sprague-Dawley rats were given two doses 50 $mg \cdot kg^{-1}$ 1-methyl-1-nitrosourea (MNU) in abdominal parts on the 52^{nd} day and 113^{th} day of age. We have found that in comparison with non-lactating mammary gland, the activity of 5'-DI in all mammary gland tumours studied was significantly ($p < 0.0001$) increased and that the 5'-DI activity, expressed as pmol of $^{125}I^-$ released per min and per mg of protein, in malignant mammary gland tumours was found to be at least two order higher than that of intact mammary non-lactating gland. From our data, we suggest that thyroid hormone in mammary gland tumours might play a significant role to support high energetic expenditure of neoplastic tissues.

Introduction

The peripheral conversion of the main secretory product of the thyroid, the prohormone thyroxine (T_4) to the active hormone 3,5,3'-triiodothyronine (T_3) or 3,3',5'-triiodothyronine (reverse triiodothyronine, rT_3) is catalyzed by a group of selenoenzymes known as iodothyronine deiodinases (Larsen et al. 1981). Type I iodothyronine 5'-deiodinase (5'-DI) reveals homodimeric structure, i.e. it consists of two identical 27-kDa subunits with one atom of selenium per subunit molecule (Köhrle et al. 1995). 5'-DI is inhibited by 2-propyl-2-thio-uracil (PTU) or thiogold glucose,

Correspondence to: Julius Brtko, Ph.D., Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovakia
E-mail: ueenbrtk@savba.savba.sk

the compounds that serve as an advantageous tools to discriminate 5'-DI and type II iodothyronine 5'-deiodinase (5'-DII) (Köhrle 1994). 5'-DI is expressed predominantly in liver, kidney, thyroid gland, pituitary and central nervous system, but it is also detected in heart, skeletal muscle, lungs, pancreas, spleen, intestine, skin, placental membranes, and mammary gland (Aceves and Valverde 1989; Köhrle 1994). The 5'-deiodination in normal non-lactating mammary gland is markedly lower than that of other tissues (Jack et al. 1994), however, 5'-DI activity in mammary gland increases significantly during lactation (Aceves and Valverde 1989; Valverde and Aceves 1989). Expression of 5'-DI was found to be markedly reduced or even a complete lack of expression of the enzyme was detected in several human cancer tissues, e.g., thyroid, kidney, prostate carcinoma (Schreck et al. 1994; Köhrle 1997; Köhrle 2000). Since mammary gland tumours in rats are very close to human ones, the induction of mammary carcinomas by the application of 1-methyl-1-nitrosourea (MNU) to female rats is one of the most frequently used animal models for the investigation of breast carcinogenesis and mammary tumours therapy (Welsch 1985; Russo et al. 1990; Thompson et al. 1995).

In the present work we examined the activity of 5'-DI in various combinations of histologically defined papillary, cribriform or comedo patterns of ductal carcinomas occurring in rat mammary gland tumours. The aim of the present study was to answer whether 5'-DI, which is believed to act as a "guardian of the gate" to nuclear thyroid hormone receptors and other cellular target sites for thyroid hormone action (Köhrle 1997), is involved in MNU-induced mammary gland tumours of rat.

Materials and Methods

Animals Female Sprague-Dawley rats, 52 days old, and weighing 170 g were housed 4–5 per cage with free access to food and water, and kept at 23 ± 2°C, 12 h light-dark cycle. On 52nd and 113th day of age, rats ($n = 9$) were given 50 mg·kg^{-1} MNU (Sigma; St. Louis, Mo., U.S.A.) intraperitoneally, alternately in the left and the right abdominal part. The MNU was always dissolved freshly in 0.9% NaCl adjusted to pH 4.0 with acetic acid. Solubility of MNU in water at room temperature was 1.4% w·v^{-1}. The experiment was terminated on the 164th day of age. Animals in control group received 0.9% NaCl adjusted to pH 4.0 with acetic acid All animals were euthanised by decapitation, their skin was examined through translucent light, and all palpable tumours of mammary glands and control mammary glands were removed, frozen in liquid nitrogen and stored at −80°C for subsequent extraction of microsomal proteins.

Histopathology of mammary gland carcinomas. The mammary glands were evaluated for the presence of grossly detectable mammary tumours and the dissected animals with tumours were photographed to provide identification record of the location and gross morphology of lesions (Liska et al. 2000). All palpable tumours were excised and fixed in 10% buffered formalin and processed for histopathological

evaluation Paraffin sections of the excised tissues were stained with hematoxylin and eosin, according to Gomori (1937) and monoclonal antibodies against actine were used Mammary tumours were classified as recommended Russo et al (1990)

Determination of the 5'-DI activity The intact mammary gland or mammary gland tumours were homogenized by sonication (three times for 5 s) in ice-cold homogenization buffer containing 0 25 mol l^{-1} sucrose, 20 mmol l^{-1} Hepes (pH 7 4), 1 mmol l^{-1} EDTA, 1 mmol l^{-1} D,L-dithiothreitol (DTT) 5'-DI activity was determined according to Leonard and Rosenberg (1980) by the release of $^{125}I^-$ from [^{125}I] 3,3',5'-triiodo-L-thyronine (reverse triiodo-L-thyronine, rT_3) using 2 μmol l^{-1} nonradioactive rT_3 and 40 mmol l^{-1} DTT in the absence or presence of 0 1 mmol l ^{1} 6-n-propyl 2 thiouracil (PTU) The fraction of iodide release blocked by PTU was assigned to the 5'-DI activity Specific activity of the 5'-DI was expressed as pmol of $^{125}I^-$ released per min and per mg of protein

Estimation of protein The protein concentration was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard

Statistics Data are expressed as mean ± S E M Statistical significance was assessed using an unpaired Students t-test

Results and Discussion

Mammary glands were evaluated both macroscopically and microscopically for the presence of tumours MNU-induced tumours were encapsulated and of solid consistence The incidence of palpable tumours was 76% Palpable tumours were excised and processed for histopathological evaluation All microscopically evaluated carcinomas were malignant Mammary tumours were classified according to Russo et al (1990) Various combinations of papillary, cribriform or comedo patterns of ductal carcinomas occurred in mammary gland tumours (Figure 1) Russo et al (1990) have shown that papillary carcinomas are the most typical and frequent of the tumours induced in rats Papillary carcinomas in uniform pattern were observed rarely, but they were often dominant in combinations with other components (Table 1) The detailed histological classification assigned to identified lesions is given in our recent work (Liska et al 2000) The percentage of invasive tumours was 44% of the total number of lesions classified Carcinomas effaced architecture of the normal gland (Figure 2) and some of them invaded surrounding tissues Some of invasive tumours in our experiment were characterised by penetration of finger-like projections or duct-like structures or solid sheets of epithelial cells into the surrounding stroma, others were characterised by broken basement membrane and missing myoepithelium only Massive stromal response was often observed, demonstrated by fibrosis and mononuclear infiltration Stromal response frequently observed in carcinomas was more prominent in invasive malignant lesions than those of non invasive or benign ones Many of palpable lesions were invasive, but no

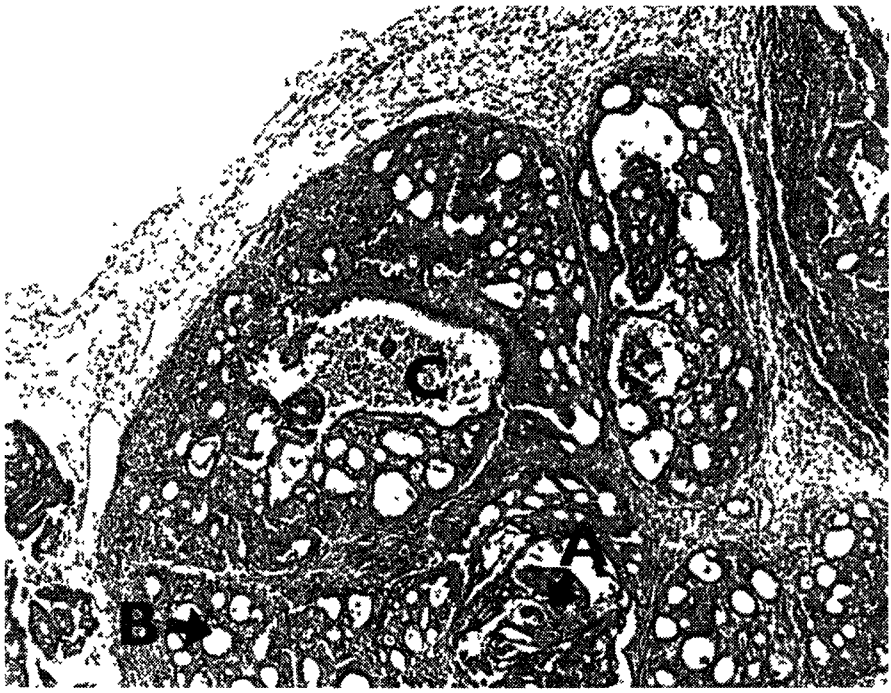

Figure 1. Ductal carcinoma, **A:** papillary, **B:** cribriform, and **C:** comedo pattern Haematoxylin – eosin Magnification 40×

metastases were observed in other organs of MNU treated animals. Metastases are reported when study processed over two or three years (Russo et al. 1990).

Since, predominantly lactating glands were reported to exhibit type I 5'-DI activity (Aceves and Valverde 1989) in order to establish metabolic adaptations to support lactation (Jack et al. 1994), in the present study we have obtained a line of biochemical evidence indicating exhibition of significant 5'-DI activity in the rat mammary gland tumours. As shown in Figure 3, the type I 5'-DI activity, assayed in the same concentration of microsomal proteins per sample, was markedly increased ($p < 0.0001$) in mammary gland tumours, but in contrast, it was nearly negligible in non-lactating mammary glands of mock treated virgin female rats. Low 5'-DI activity in mammary gland of control rats corresponds well with the data on expression of 5'-DI in studies of Jack et al. (1994) and Navarro et al. (1997). The 5'-DI activity in invasive ductal tumours with cribriform patterns marked as IDC-Cr and papillary invasive ductal carcinomas with cribriform and comedo patterns (IDC-PCrCo) was significantly higher than that of in invasive ductal tumour with cribriform and comedo patterns (IDC-CrCo2). At present, we cannot interpret possible differences in 5'-DI among various patterns of those histologically evaluated tumours, but the data of the present study have shown

Table 1 Histological classification of palpable tumours developed by two doses (50 mg kg^{-1}) of 1-methyl-1-nitrosourea (MNU)

Animal No	Number of lesions	Histological classification
1	1	IDC PCr
	1	DCIS Cr
2	1	DCIS-P
	2	IDC-PCrCo
3	2	IDC-CrCO
	1	DCIS-PCr
4	1	IDC PCo
5	1	IDC Cr
	2	DCIS-PCr
6	0	
7	1	DCIS P
8	2	DCIS-PCo
	2	DCIS-CrCo
9	0	

I invasive DC ductal carcinoma, DCIS ductal carcinoma *in situ* P papillary Cr cribriform Co comedo

for the first time the markedly elevated 5'-DI activity in mammary gland tumours induced by MNU

Experimentally MNU induced model of breast carcinogenesis in rat is widely used for studying the biology of breast cancer and for developing and evaluating cancer prevention and control strategies (Rivera et al 1994, Lu et al 1997) The major advantage of this widely accepted animal model is its simplicity and specificity for induction of mammary gland tumours The most highly malignant tumours in rat were found to have some common features with intraduct and infiltrating ductal carcinomas in humans (Russo et al 1990) In general, and as it was confirmed in our experiments, no gross evidence of acute toxicity of MNU on rat that might be linked to the administration of carcinogen, was detected But, it is important to mention that final body weight of MNU treated animals was 16 4% less when compared to control rats

The influence of thyroid status on the progression of carcinogen-induced rat mammary carcinomas is not clear yet, numerous contradictory studies have been reported (Welsch 1985) It has been shown that hyperthyroid status in rats rendered by the administration of thyroxine or 3,5,3'-triiodo-L-thyronine can enhance (Cave et al 1977, St Gerard et al 1980), inhibit (Jull and Huggins 1960, Newman and Moon 1968) or unalter (Grice et al 1966, Gruenstein et al 1968) development and growth of carcinogen-induced mammary carcinomas On the other hand, it has been reported that induced hypothyroidism by thyroidectomy or by administration of an array of goitrogens or ^{131}I can also enhance (Grice et al 1967, Shellabarger 1969, Eskin 1970, Milmore et al 1982), inhibit (Jull and Huggins 1960, Helfenstein et al 1962, Kellen 1972, Goodman et al 1980) or have no effect (Eskin et al 1968, David-

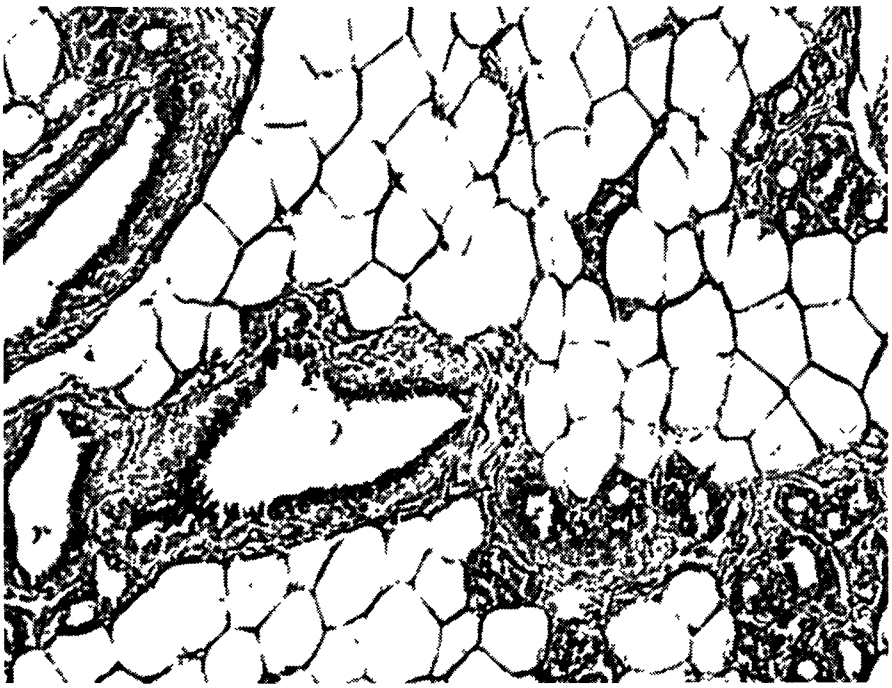

Figure 2. Normal adult mammary gland of virgin female rat – control group Haematoxylin – eosin. Magnification. 40×.

son et al. 1969; Cave et al. 1979; Rose and Mountjoy 1983) on development and growth of these neoplasms. Milmore et al. (1982) have reported that mild propylthiouracil treatment of rats previously treated with MNU results in enhancement of mammary tumour development. In other studies, hypothyroidism failed to influence mammary tumour development in rats previously treated with MNU (Cave et al. 1977; Rose and Mountjoy 1983). Within the past few years, several studies have provided convincing data showing that lactation is accompanied by a characteristic euthyroid sick-like syndrome, characterized by decreased concentration of circulating iodothyronines and increased concentration of thyroid-stimulating hormone (TSH) (Fukada et al. 1980). It has also been shown that human breast cancer could be accompanied by a variety of thyroid disorders (Rasmusson et al 1987; Giani et al. 1996; Limanova et al. 1998). On the other hand, reduction or even a complete lack of 5'-DI was confirmed in human thyroid, kidney or prostate carcinomas (Schreck et al. 1994; Köhrle 1997; Köhrle 2000). Tissue-specific activation or inactivation of ligands of thyroid hormone nuclear receptors, belonging to the steroid retinoid-thyroid hormone superfamily of transcription factors (Glass and Holloway 1990; Rollerova et al. 2000; Zhang and Lazar 2000), represents an

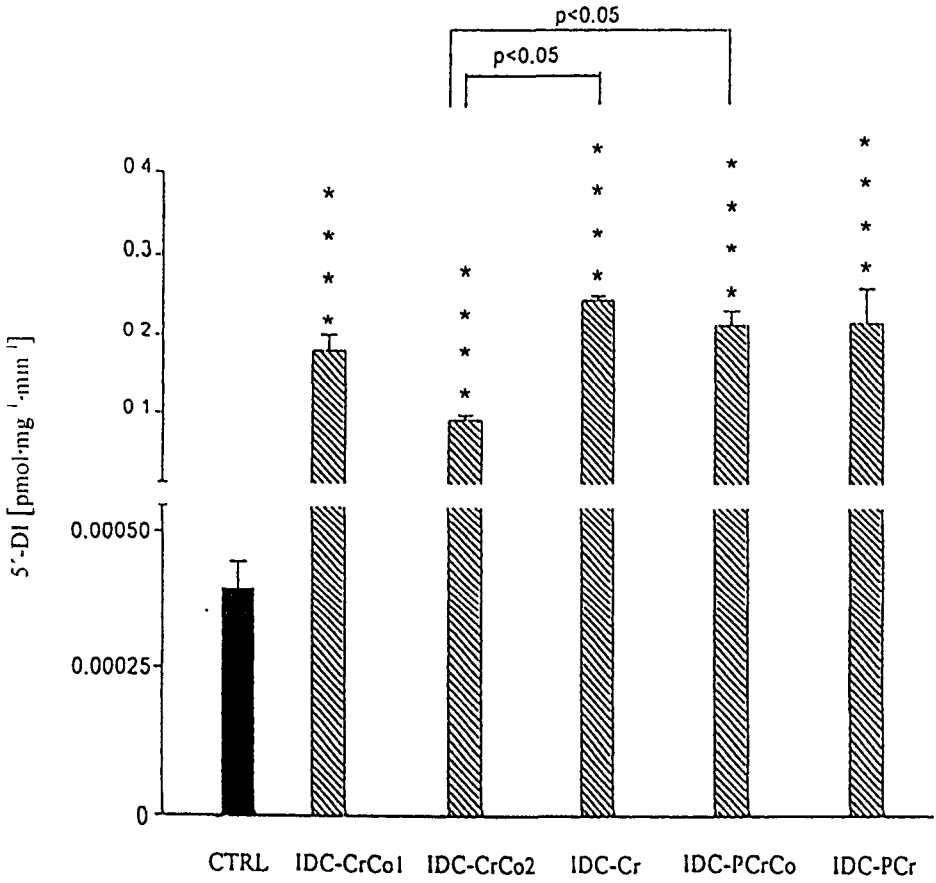

Figure 3. Activity of type I iodothyronine 5'-deiodinase (5'-DI) in rat mammary gland tumours CTRL – normal mammary gland, Ductal carcinomas IDC-CrCo – invasive cribriform and comedo, IDC-Cr – invasive cribriform, IDC-PCrCo – papillary invasive cribriform and comedo, IDC-PCr – papillary invasive cribriform

important principle of tissue-specific local modulation of thyroid hormone action (Kohrle 1999)

Despite the relatively large number of studies yielding assorted data on the role of thyroid hormone on development of neoplasms in mammary gland, there is still an unanswered question· Do thyroid hormones play a considerable role in development, growth, and progression of carcinogen-induced rat mammary carcinomas?

Summing up, from our data we suggest that in rat, type I 5'-DI activity, responsible for monodeiodination of thyroxine to biologically active 3,5,3'-triiodo-

L-thyronine, is markedly increased in mammary gland carcinomas when compared to that of intact mammary gland, probably, in order to support high energetic expenditure necessary for the growth of mammary gland tumours Further work on the type I 5'-DI expression in various types of mammary gland carcinoma is warranted

Acknowledgements. The authors wish to acknowledge the technical assistance of Maria Danihelová, Maria Mičincová and Zuzana Némethová for providing microphotographs of mammary gland tissues Supported in part by the grant of VEGA No 2/6085/99

References

Aceves C , Valverde C (1989) Type I, 5'-monodeiodinase activity in the lactating mammary gland Endocrinology **124**, 2818—2820

Cave W T , Dunn J T , MacLeod R M (1977) Effects of altered thyroid states on mammary tumor growth and pituitary gland function in rats J Natl Cancer Inst **59**, 993—999

Cave W T , Dunn J T , MacLeod R M (1979) Effects of iodine deficiency and high-fat diet on N-nitrosomethylurea-induced mammary cancers in rats Cancer Res **39**, 729—734

Davidson A , Owen J , Thomas C G (1969) Further studies on the role of altered thyroid function on experimentally induced breast cancer in Sprague-Dawley rats Proc Am Assoc Cancer Res **10**, 17

Eskin B A , Murphey S A , Dunn M R (1968) Induction of breast cancer in altered thyroid states Nature **218**, 1162

Eskin B A (1970) Iodine metabolism and breast cancer Trans NY, Acad Sci **32**, 911—942

Fukada H , Ohshima K , Mori M , Kobayashi I , Greer M A (1980) Sequential changes in the pituitary-thyroid axis during pregnancy and lactation in the rat Endocrinology **107**, 1711—1716

Gerard S S , Gardner B , Patti J , Husain V , Shouten J , Alfonso A E (1980) Effect of triiodothyronine and reserpine on induction and growth of mammary tumors in rats by 3-methylcholanthrene J Surg Oncol **14**, 213—218

Giani C , Fierabracci P , Bonacci R , Ghiotti A , Campani D , De Negri F , Cecchetti D , Martino E , Pinchera A (1996) Relationship between breast cancer and thyroid disease relevance of autoimmune thyroid disorders in breast malignancy J Clin Endocrinol Metab **81**, 990—994

Glass C K , Holloway J M (1990) Regulation of gene expression by the thyroid hormone receptor Biochim Biophys Acta **1032**, 157—176

Grice O D , Faircloth S , Thomas C G (1966) The effect of hypothyroidism on induced cancer of the breast Proc Am Assoc Cancer Res **7**, 26

Grice O D , Faircloth S , Thomas C G (1967) The effect of hypothyroidism on induced cancer of the breast – further observations Proc Am Assoc Cancer Res **8**, 23

Gruenstein M , Meranze D R , Acuff M and Shimkin M B (1968) The role of the thyroid in hydrocarbon-induced mammary carcinogenesis in rats Cancer Res **28**, 471—474

Goodman A D , Hoekstra S J , Marsh P S (1980) Effects of hypothyroidism on the induction and growth of mammary cancer induced by 7,12-dimethylbenzanthracene in rat Cancer Res **40**, 2336—2342

Gomori G (1937) Silver impregnation of reticulum in paraffin sections Amer J Pathol **13**, 993—1002

Helfenstein J E , Young S , Currie A R (1962) Effect of thiouracil on the development of mammary tumours in rats induced with 9,10-dimethyl-1,2-benzanthracene Nature **196**, 1108

Jack L J , Kahl S , St Germain D L , Capuco A V (1994) Tissue distribution and regulation of 5'-deiodinase processes in lacting rats J Endocrinol **142**, 205—215

Jull J W , Huggins C (1960) Influence of hyperthyroidism and thyroidectomy on induced mammary cancer Nature **188**, 73

Kellen J A (1972) Effect of hypothyroidism on induction of mammary tumors in rats by 7,12-dimethylbenzanthracene J Natl Cancer Inst **48**, 1901—1904

Kohrle J (1994) Thyroid hormone deiodination in target tissues – a regulatory role for the trace element selenium? Exp Clin Endocrinol **102**, 63—89

Kohrle J , Schomburg L , Drescher S , Fekete E , Bauer K (1995) Rapid stimulation of type I 5'-deiodinase in rat pituitaries by 3,3',5-triiodo-L-thyronine Mol Cel Endocrinol **108**, 17—21

Kohrle J (1997) Thyroid carcinoma Interrelationships between thyroid hormone metabolism by the type I 5'-deiodinase and the expression of thyroid hormone receptors and other thyroid hormone receptors and other thyroid-specific (de-)differentiation markers Curr Top Pathol **91**, 83—116

Kohrle J (1999) Local activation and inactivation of thyroid hormones the deiodinase family Mol Cell Endocrinol **151**, 103—119

Kohrle J (2000) The selenoenzyme family of deiodinase isozymes controls local thyroid hormone availability Rev Endocr Metab Disorders **1**, 49—58

Larsen P R , Silva J E , Kaplan M M (1981) Relations between circulating and intracellular thyroid hormones physiological and clinical implications Endocr Rev **2**, 87—102

Leonard J L , Rosenberg I N (1980) Iodothyronine 5'-deiodinase from rat kidney substrate specificity and the 5'-deiodination of reverse triiodothyronine Endocrinology **107**, 1376—1383

Limanova Z , Barkmanova J , Friedmanova Z (1998) [Frequent incidence of thyropathies in women with breast carcinoma] Vnitr Lek **44**, 76—82 (in Czech)

Liska J , Galbavy S , Macejova D , Zlatos J , Brtko J (2000) Histopathology of mammary tumours in female Sprague-Dawley rats treated with 1-methyl-1-nitrosourea Endocr Reg **34**, 91—96

Lowry O H , Rosebrough N J , Farr A L , Randal, R J (1951) Protein measurement with the Folin phenol reagent J Biol Chem **193**, 265—275

Lu J , Pei H , Kaeck M , Thompson H J (1997) Gene expression changes associated with chemically induced rat mammary carcinogenesis Mol Carcinog **20**, 204—215

Milmore J E , Chandrasekaran V , Weisburger J H (1982) Effects of hypothyroidism on development of nitrosomethylurea-induced tumors of the mammary gland, thyroid gland, and other tissues Proc Soc Exp Biol Med **169**, 487—493

Navarro L , Landa A , Valverde C , Aceves C (1997) Mammary gland type I iodothyronine deiodinase is encoded by short messenger ribonucleic acid Endocrinology **138**, 4248—4254

Newman W C , Moon R C (1968) Chemically induced mammary cancer in rats with altered thyroid function Cancer Res **28**, 864—868

Rasmusson B , Feldt-Rasmussen U , Hegedus L , Perrild H , Bech K, Hoier-Madsen M (1987) Thyroid function in patients with breast cancer Eur J Cancer Clin Oncol **23**, 553—556

Rivera E S , Andrade N , Martin G , Cricco G , Mohamad N , Davio C , Caro R , Bergoc R M (1994) Induction of mammary tumors in rat by intraperitoneal injection of NMU histopathology and estral cycle influence Cancer Lett **86**, 223—228

Rollerová E , Gáspárová Z , Wsólová L , Urbančíková M (2000) Interaction of acetochlor with estrogen receptor in the rat uterus Acetochlor – possible endocrine modulator? Gen Physiol Biophys **19**, 73—84

Rose D P , Mountjoy K G (1983) Influence of thyroidectomy and prolactin suppression on the growth of N-nitrosomethylurea-induced rat mammary carcinomas Cancer Res **43**, 2588—2591

Russo J , Russo I J , Rogers A E , Van Zweiten M J , Gusterson B (1990) Tumours of mammary gland In Pathology of tumours in laboratory animals Vol 1, Tumours of the rat (Eds V Turusov, U Mohr), pp 47—78, IARC Scientific Publications, Lyon

Schreck R , Schnieders F , Schmutzler C , Kohrle J (1994) Retinoids stimulate type I iodothyronine 5'-deiodinase activity in human follicular thyroid carcinoma cell lines J Clin Endocrinol Metab **79**, 791—798

Shellabarger C J (1969) Hypothyroidism and DMBA rat mammary carcinogenesis Proc Am Assoc Cancer Res **10**, 79

Thompson H J , McGinley J N , Rothhammer K , Singh M (1995) Rapid induction of mammary intraductal proliferation, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea Carcinogenesis **16**, 2407—2411

Valverde C , Aceves C (1989) Circulating thyronines and peripheral monodeiodination in lacting rats Endocrinology **124**, 1340—1344

Welsch C W (1985) Host factors affecting the growth of carcinogen-induced rat mammary carcinomas a review and tribute to Charles Brenton Huggins Cancer Res **45**, 3415—3443

Zhang J , Lazar M A (2000) The mechanism of action of thyroid hormones Annu Rev Physiol **62**, 439—466

Final version accepted June 7, 2001