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Effects of testosterone and 17, β – estradiol on the polyamine metabolism in cultivated normal rat kidney epithelial cells

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Summary. Ornithine decarboxylase (ODC) and diamine oxidase (DAO) are important enzymes involved in the metabolism of polyamines (putrescine, spermidine and spermine). The influence of testosterone (T) and 17, β – estradiol (E_2) on the activity of ODC and DAO was examined in cultivated normal rat kidney (NRK) epithelial cells. The results showed an increase in enzyme activities 4 hours or 12 hours after hormonal treatment. Both T and E_2 led to a significant increase (1.6-fold) in ODC protein level as compared to the controls. Cellular concentration of spermidine and spermine increased (2.2- and 2.6-fold respectively) 4 hours after T addition. A higher levels in concentrations of putrescine (1.4-fold) and spermine (1.5-fold) 12 hours after E_2 treatment were observed. These results suggest that the biosynthesis and terminal oxidation of the polyamines in NRK epithelial cells are androgen- and estrogen-mediated and depend on the hormonal sensitivity of the cells.

Keywords: Amino acids – Polyamines – Testosterone – 17 β – Estradiol – Ornithine decarboxylase – Diamine oxidase

Introduction

The polyamines putrescine, spermidine and spermine are natural polycations present in all living cells. They are essential for cell growth and differentiation (Tabor and Tabor, 1984; Pegg, 1988; Heby and Persson, 1990). High concentrations of polyamines were found in regenerating organs (Janne et al., 1978), as well as in growing cells of normal and neoplastic tissues (Russell, 1985; Auvinen et al., 1992). Ornithine decarboxylase (ODC, EC 4.1.1.17), which catalyses the formation of putrescine from L-ornithine is also activated in these cells and tissues (Russell, 1985). In most tissues the basal level of ODC is low, but some intracellular factors, like tumor promoters (Janne et al., 1978; Abrahamsen and Morris, 1991), growth factors and hormones (Lima

and Shin, 1985; Scalabrino and Lorenzini, 1991; Scalabrino et al., 1991) can induce rapid increase in its activity. Numerous reports suggest that polyamines are implicated in hormone-dependent cell growth in target organs (Lima and Shin, 1985; Scalabrino and Lorenzini, 1991). For example, the treatment of female mice with testosterone (T) produces significant renal hypertrophy, accompanied by induction of ODC activity (Hoang and Bergeron, 1987; Cymborowska et al., 1997). Increased activity of ODC and polyamine levels in rat uterus (Kaye et al., 1971) and immature chicken oviduct were found (Cohen et al., 1970) after 17, β – estradiol (E_2) administration. The induction of ODC in the cells is usually accompanied by the accumulation of enzyme reaction product – putrescine. Putrescine can serve as a precursor in the synthesis of higher polyamines – spermidine and spermine or it can be oxidized to products which are not related to polyamine biosynthesis.

Diamine oxidase (DAO, EC 1.4.3.6) catalyses the terminal oxidation of polyamines. In previous studies we have demonstrated that T administration of male and female mice stimulates DAO activity in kidney (unpublished results). The stimulatory effect of E_2 on the DAO activity in immature female rat uterus also was observed (Dimitrov et al., 1996). There are data showing that administration of estrogens markedly increased ODC activity in cultured baby hamster kidney cells (Lin et al., 1980).

The influence of male and female sex steroid hormones on the activity of ODC and DAO and the possible relationship of their hormonal regulation are not completely understood. Our aim in the present study was to elucidate the parallel influence of T and E_2 on these two enzymes and thus to determine possible relationship between the regulation of polyamine biosynthesis and terminal catabolism. For the experiments we choose normal rat kidney (NRK) epithelial cells.

Materials and methods

Cell culture

Normal rat kidney epithelial cells were cultivated in DMEM medium Biological Industries, Israel) supplemented with 5% fetal calf serum; 200 mM glutamine (Biological Industries, Israel); ampicillin combined antibiotics (Bio-Lab Ltd., Jerusalem) in an incubator with 5% CO_2 and 95% air.

Cell treatment

The hormone T or E_2 (Sigma Chemicals Co; St. Louis, MO, USA) at a final concentration 1 μg per 10 ml medium (dissolved in ethyl alcohol) were added to the cultures 48 h after seeding. The control cells were cultivated in DMEM medium without the addition of hormone.

Ornithine decarboxylase activity assay

ODC activity was estimated by the radiometric method based on the determination of [^{3}H]-putrescine and using [^{3}H]-ornithine as a substrate (Tabib, 1998).

The cultivated cells were washed twice with 10 ml of cold phosphate-buffered saline (PBS) after different time intervals of hormonal treatment (0, 2, 4, 6, 8 and 12 h) and suspended in ODC buffer containing: 50 mM pyridoxal phosphate (Sigma), 0.1 mM EDTA (BDH), 50 mM Tris-HCl, pH 7.2 (Ultra pure, Sigma), 5 mM dithiothreitol (Sigma). Cells were lysed by freezing and thawing (4 times). The supernatant obtained after centrifugation at 15,000 × g for 20 min at 4°C was used for the assay.

The standard reaction assay mixture contained cell lysate or ODC buffer in the blank (100 μl); 200 mM ornithine (10 μl) (Sigma); 10 μl [^{3}H]-ornithine (55 Ci/mmol; 1 μCi/ml) in final volume of 120 μl. The samples were incubated at 37°C for 1 h. The reaction was stopped by using an ice bath for 2–3 min. The material was than spotted on P81 Whatman phosphocellulose paper and washed with 0.1 M ammonium hydroxide (Djurhuus, 1981). The samples were dried and radioactivity was determined by liquid scintilation caunter.

The protein level was assayed by the method of Bradford (1976).

Western blots

Cells were washed twice with ice cold PBS buffer and suspended in 1 ml PBS. The homogenates were centrifugated at 10,000 × g for 4 min at 4°C. The sediment was resuspended in 100 μl of cell lysis buffer containing: 25 mM Tris pH 7.4; 20 mM $MgCl_2$; 1 mM phenylmethylsulphonylfluoride (PMSF; Sigma); 1% NP-40 (Sigma), 1 mM dithiothreitol (Sigma); 1% aprotein trazytol (Sigma). The cells were lysed by 4-fold freezing and thawing and subsequently the homogenate was centrifugated at 10,000 × g for 15 min at 4°C. The supernatant was used for protein analysis. The proteins were then denaturated and separated by SDS/polyacrylamide gel electrophoresis according to the method of Laemmli (1970). The proteins were blotted on PBDV nitro-cellulose membranes, which were incubated for 12 h at 4°C under shaking with first ODC anti-rabbit antibody (1 : 1,000) dissolved in TBST buffer saline containing: 100 mM Tris, pH 7.4; 1.5 M NaCl; 20 ml Triton-X-100 (Sigma). Thereafter the samples were incubated with second anti-rabbit antibody (1 : 5,000) dissolved in TBST buffer for 1 h at room temperature with shaking.

Determination of polyamines

The cells were suspended in PBS buffer and were treated with 60% perchloric acid in final concentration of 3% and centrifugated. The deproteinated material was used for determination of polyamines by thin-layer chromatography according to the method of Seiler et al. (1983) by using of silica G-60 plastic plaques without fluorescent indicator (20 × 20 cm, thickness 0.2 mm (Merck)). Spots corresponding to the different dansylated derivatives of polyamines were scraped off from the sheet and were extracted with ethyl acetate. The concentration of polyamines was determined fluorimetrically at λ of excitation 360 nm and λ of emission 510 nm.

Diamine oxidase activity assay

The cells were destructed by freezing and thawing after their washing and suspending in PBS buffer. The homogenate was used as an enzyme source for radiochemical assay of DAO by the method of Storer and Ferrante (1998).

Statistical analysis

The statistical significance of differences between two groups of data was determined by the Student t-test. The values were considered significant at $p < 0.05$.

Results

Figure 1 shows the effect of T and E_2 treatment on the ODC activity in NRK epithelial cells. It may be seen that T-treated cells exhibited higher ODC activity as compared to the control cells in all studied time intervals. Four hours after addition of the hormone ODC activity was 8.8-fold higher ($p < 0.001$) and after 12 h of hormonal treatment a 3-fold ($p < 0.001$) increase of enzyme activity was noticed. The treatment with E_2 also caused a significant increase in ODC activity, but not in all time intervals which were studied. A 2.3-fold ($p < 0.05$) increase in ODC activity was observed 4 h after hormone treatment and 1.9-fold ($p < 0.001$) higher enzyme activity at 12 h was measured as compared to the controls.

Changes in the cell content of specific ODC proteins after hormonal treatment with T and E_2 were estimated by Western blots. It may be seen (Fig. 2) that both T or E_2 4 h after administration led to a 1.6-fold ($p < 0.001$) increase in ODC protein levels as compared to that observed in non-treated cells. The effect of E_2 12 h after hormone treatment was nearly the same (1.5-fold increase, $p < 0.001$), but that of T almost disappeared.

The activity of DAO in NRK epithelial cells was also determined after treatment with T or E_2. It is clear from Fig. 3 that the profile of DAO activity differs from that of ODC (Fig. 1). Thus, a 3-fold ($p < 0.02$) increase in enzyme activity was observed 4 h, but not 12 h after E_2 addition. On the other hand, T

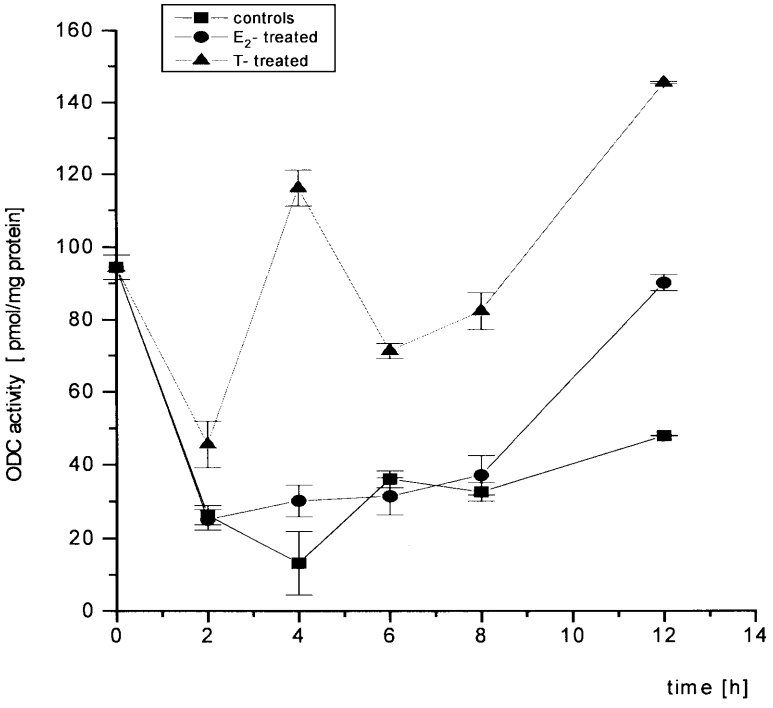

Fig. 1. Effects of estradiol (E_2) and testosterone (T) on the ODC activity in normal rat kidney epithelial cells as a function of time. Values are means ± SE from 3 experiments

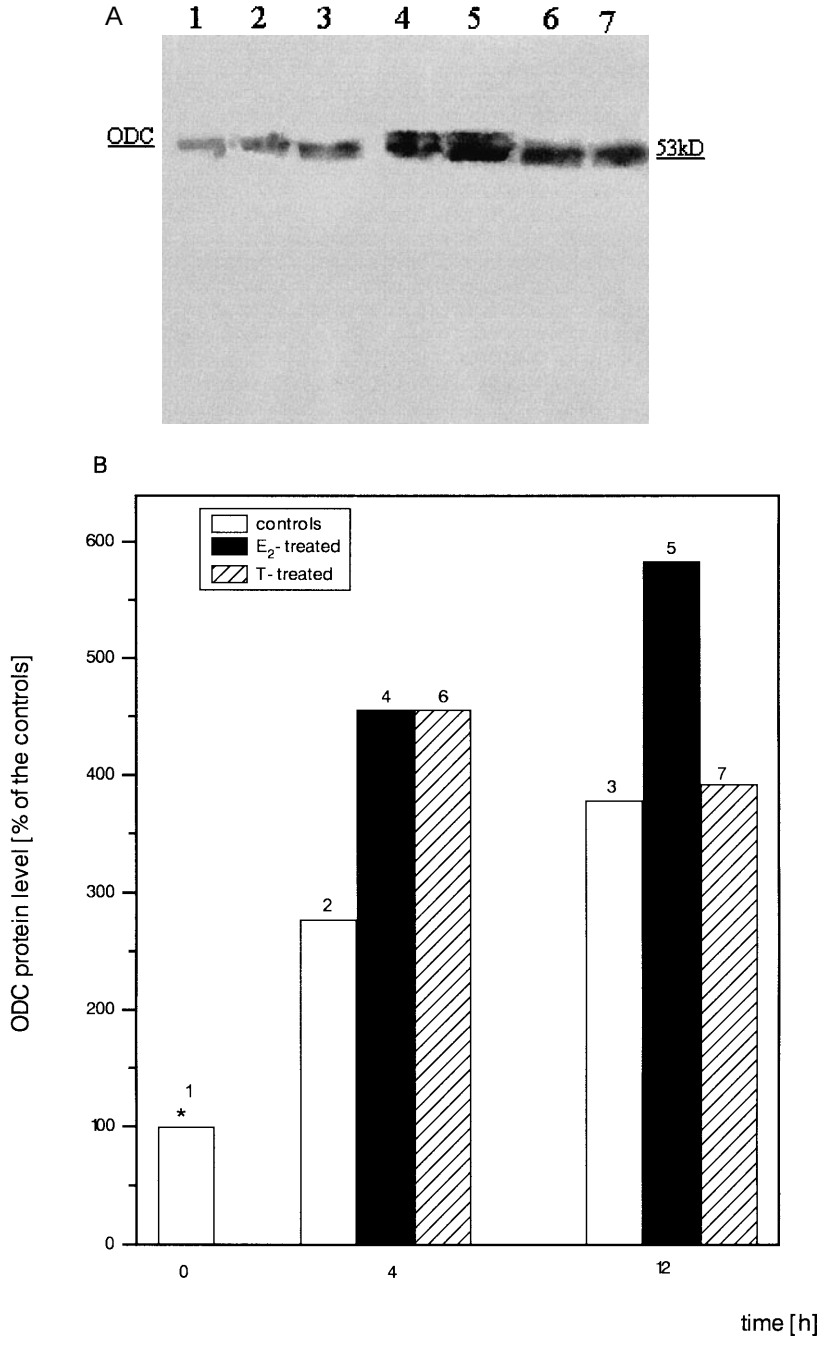

Fig. 2. Effects of estradiol (E_2) and testosterone (T) on the ODC protein level in normal rat kidney epithelial cells, determined by Western blot (**A**) 4 and 12 h after hormone administration. Results (**B**) are based on scanning the bands. *Control protein level which was expressed as 100% of ODC protein concentration. *1*, Controls-(time of incubation-0); *2*, controls-(4 hours of incubation); *3*, controls-(12 hours of incubation); *4*, 4 hours after E_2 addition; *5*, 12 hours after E_2 addition; *6*, 4 hours after T addition; *7*, 12 hours after T addition

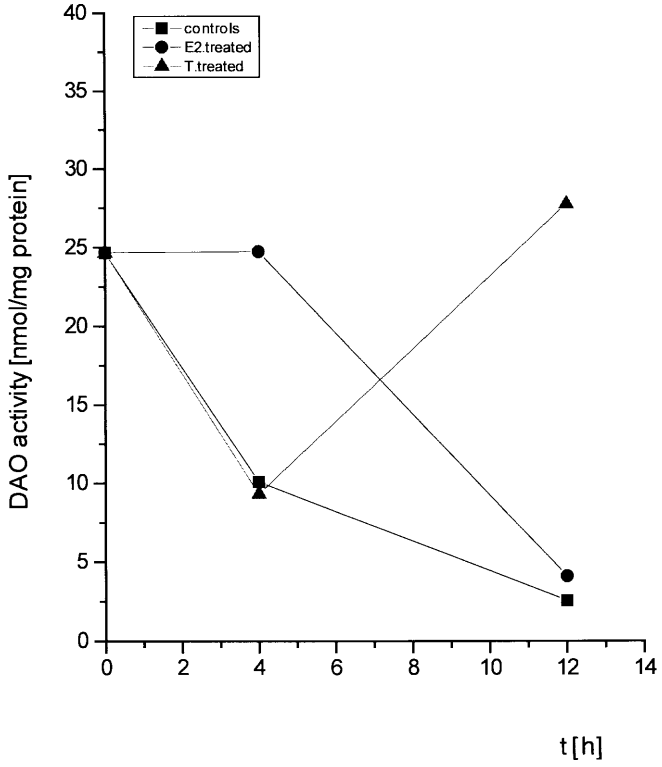

Fig. 3. Effects of estradiol (E_2) and testosterone (T) on DAO activity in normal rat kidney epithelial cells as a function of time. Values are means ± SE from 3 experiments

caused an increase in DAO activity (10-fold, $p < 0.02$) 12 h, but not 4 h after hormone treatment.

The cellular concentration of polyamines is shown on the Fig. 4. The concentration of spermidine and spermine increased 2.2- and 2.6-fold respectively ($p < 0.05$) 4 h after T administration (Fig. 4A), but E_2 did not alter significantly cellular polyamine levels. Twelve hours after hormone treatment T did not affect polyamine levels, while E_2 led to an increase in putrescine (1.4-fold, $p < 0.05$) and spermidine (1.5-fold, $p < 0.05$) levels (Fig. 4B).

Discussion

Data presented in this paper show that both T and E_2 increase the activity of ODC and DAO and thereby modulate the metabolism of polyamines in NRK epithelial cells. The decrease in ODC and DAO activities determined in control cells at the different time intervals (Figs. 1, 3) is due to the ageing of the cell culture. The androgen provokes stronger and faster effect on the activity of key biosynthetic enzyme – ODC, than that described under the influence of estrogen. These data could be one more proof for the presence not only of androgen but also estrogen receptors in kidney cells. The lower

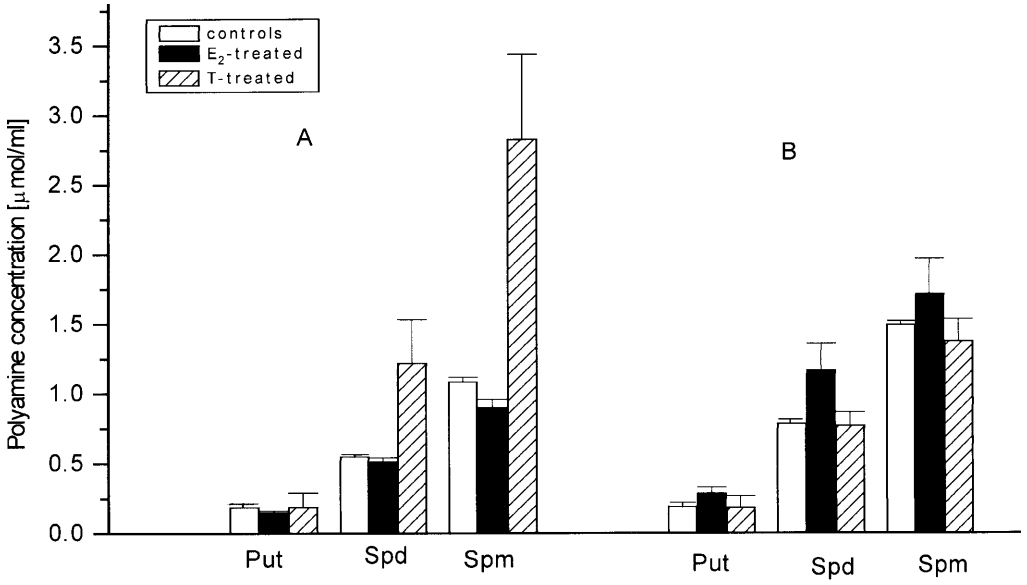

Fig. 4. Effects of estradiol (E_2) and testosterone (T) on the polyamine concentrations in normal rat kidney epithelial cells after 4 h (**A**) and after 12 h (**B**) of the hormone treatment. Values are means ± SE from 2 experiments

estrogen effect on the ODC activity corresponds to the lower anabolitic activity of the estrogens in general, compared to that observed for the androgens. The increase of ODC activity after hormone administration was accompanied by a significant increase in the cell enzyme protein content. Therefore, the increase of ODC activity could be related to the gene activation and new protein synthesis in accordance with the well-known model of steroid hormone action in target cells. An involvement of new mRNA and protein synthesis in the T and E_2 stimulated ODC activity have been reported (Berger et al., 1984; Thomas and Thomas, 1994; Cintron et al., 1996). It is possible that the stimulatory effect of both T and E_2 on DAO activity may involve such molecular mechanism, but its confirmation requires further investigation.

Numerous data associate the rapid growth with an increase in ODC and DAO activities in normal and pathological conditions (Perin et al., 1985; Linsalata et al., 1993; Wolvekamp and Bernin, 1994; Cymborowska et al., 1997). The induction of ODC leads to accumulation of the reaction product-putrescine. The increase of DAO activity at the same time results in remuval of the putrescine, which may directly inhibit the ODC and decrease the polyamine synthesis. Our data show that this mechanism of regulation is really possible. The activity of DAO increases under the influence of T and E_2 along with the stimulation of ODC. T enhances significantly the ODC activity on the 4^{-th} h after its administration, but its stimulatory effect on DAO is observed about the 12^{-th} h. This changeable correlation between the activity of the two enzymes could explain the increased cellular level of polyamines at the 4^{-th} h, and the equalisation of their content with the control at the 12^{-th} h

after the T-treatment. In contrast to T, E_2 increases the activity of the two enzymes 4 h after administration. At the 12-th h the hormone stimulatory effect on ODC is relatively high, while no effect on DAO activity is noticed. The cellular polyamine level corresponds to the enzyme dynamics-unchanged at the 4-th h and increased at the 12-th h.

It could be difficult to explain the transient decrease of stimulatory T-effect on ODC activity 6–8 h after hormonal treatment. As a speculative reasoning it could be reminded that in the ODC regulation an antizyme takes part, which inhibits the enzyme protein (Muranami et al., 1994; Nilsson et al., 1997), as well as an anti-antizyme, which restores the enzyme activity (Cohen, 1998). If we assume that T provokes the synthesis or the activation of these regulatory factors, it would be possible to explain the fluctuation in ODC activity, observed in the period of 6–8 h after hormone administration.

In summary we have shown that the biosynthesis and terminal oxidation of the polyamines in NRK epithelial cells are probably T- and E_2-mediated processes. The estrogen and androgen influence is different and depend on very likely the hormonal sensitivity of the cells. It seems that ODC and DAO are hormonally regulated enzymes. Their regulation is co-ordinated and determines an optimal polyamine level needed by the cells.

References

Abrahamsen MS, Morris DR (1991) Perspectives on cellular regulation: from bacteria to cancer. Wiley-Liss, New York, pp 101–119

Auvinen A, Paasinen A, Andersson LC, Holtta E (1992) Ornithine decarboxylase activity is critical for cell transformation. Nature 360: 355–358

Berger FG, Szymanski P, Read E, Watson G (1984) Androgen-regulated ornithine decarboxylase mRNAs of mouse kidney. J Biol Chem 259: 7941–7976

Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-due binding. Anal Biochem 72: 248–254

Cymborowska MM, Chmurzynska W, Peska M, Sztabert BG (1997) Tumour effects on arginine/ornithine methabolic relationship in hypertrophyc mouse kidney. Mol Cell Biochem 168: 51–57

Cintron JR, Asadi FK, Malanouti S, Abcarian A (1996) Androgen induces ornithine decarboxylase gene expression in colonic cell line HT-29. Dis Colon Rectum 39: 406–409

Cohen SC (1998) A guide to the polyamines. Oxford University Press, Oxford, pp 238–239

Cohen SC, O'Malley BW, Stastny M (1970) Estrogen induction of ornithine decarboxylase in vivo and in vitro. Science 170: 336–338

Djurhuus R (1981) Ornithine decarboxylase (E.C.4.1.1.17) assay based upon the retention of putrescine by cation-exchange paper. Anal Biochem 113: 352–355

Dimitrov O, Pavlov V, Jotova I (1996) Effects of female sex hormones on polyamine – oxidizing enzyme activities and polyamine concentration in immature rat uterus and liver. Experientia 52: 795–798

Janne J, Poso H, Raina A (1978) Polyamines in rapid growth and cancer. Biochim Biophis Acta 473: 241–293

Heby O, Persson L (1990) Molecular genetics of polyamine synthesis in eucariotic cells. Trends Biochem Sci 15: 153–158

Hoang T, Bergeron MD (1987) Androgen modulation of ornithine decarboxylase and compensatory renal growth. Growth 51: 202–212
Kaye AM, Icekson I, Lindner HR (1971) Stimulation by estrogens of ornithine and S-adenosil methionine decarboxylases in immature rat uterus. Biochim Biophis Acta 252: 150–159
Laemmli UK (1970) Clavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
Lima G, Shin R (1985) Role of polyamines in estradiol-induced growth of human breast cancer cells. Cancer Res 45: 2466–2470
Lin VC, Loring JM, Villee CA (1980) Diethylstilbestrol stimulates ODC in kidney cells in culture. BBRC 95: 1393–1403
Linsalata M, Russo F, Cavallini A, Bercolo P, Di Leo A (1993) Polyamines. DAO and ODC activity in colorectal cancer and in normal surrounding mucosa. Dis Colon Rectum 36: 662–667
Muranami Y, Matsufuji S, Miyazaki Y, Hayashi S (1994) Forced expression of antizyme abolishes ornithine decarboxylase activity, suppresses cellular levels of polyamines and inhibits cell growth. Biochem J 304: 183–187
Nilsson J, Koskiniemi S, Persson K, Grahn B, Holm I (1997) Polyamines regulate both transcription and translation of gene encoding ODC antizyme in mouse. Eur J Biochem 250: 223–231
Pegg AE (1988) Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 48: 759–774
Perin A, Sessa A, Desiderio MA (1985) In: Mondovi B (ed) Structure and function of amine oxidases. CRC Press, Boca Raton, pp 179–186
Russell DH (1985) Ornithine decarboxylase: a key regulatory enzyme in normal and neoplastic growth. Drug Metab Rev 16: 1–88
Scalabrino G, Lorenzini EC (1991) Polyamines and mammalian hormones. Part II. Mol Cell Endocrinol 77: 37–56
Scalabrino G, Lorenzini EC, Ferioli ME (1991) Polyamines and mammalian hormones. Part I. Mol Cell Endocrinol 77: 1–35
Seiler N (1983) Thin layer chromatography and thin layer electrophoresis of polyamines and their derivatives. Methods Enzymol 94: 3–25
Storer RJ, Ferrante A (1998) In: Morgan DML (ed) Methods in molecular biology: polyamine protocols, vol 73. Humana Press, Clifton, pp 91–95
Tabib A (1998) In: Morgan DML (ed) Methods in molecular biology: polyamine protocols, vol 73. Humana Press, Clifton, pp 33–39
Tabor CC, Tabor H (1984) Polyamines. Annu Rev Biochem 53: 749–790
Thomas T, Thomas TJ (1994) Estradiol control of ODC mRNA enzyme activity and polyamine levels in MCF-7 breast cancer cells: therapeutic implication. Breast Cancer Res Treat 29: 189–201
Wolvekamp MJ, Bernin RF (1994) Diamine oxidase: an overview of historical biochemistry and functional aspects. Dig Dis 12: 2–14

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