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QUARTERLY

Up-regulation of spermidine/spermine N^1-acetyltransferase (SSAT) expression is a part of proliferative but not anabolic response of mouse kidney✪

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A differential expression pattern of spermidine/spermine N^1-acetyltransferase (SSAT), the enzyme critical to proper homeostasis of cellular polyamines, is reported in mouse kidney undergoing hyperplasia and hypertrophy. We have shown that SSAT activity and SSAT mRNA are significantly induced by antifolate CB 3717 and folate that evoke a drug-injury-dependent hyperplasia. In contrast, SSAT activity is down-regulated in the testosterone-induced hypertrophic kidney, while SSAT mRNA is positively controlled by this androgen. Catecholamine depletion evoked by reserpine drastically decreases the folate-induced activity of *S*-adenosylmethionine decarboxylase (AdoMetDC), which limits polyamine biosynthesis, but has no effect on SSAT activity augmented by CB 3717. Our results document that the increased SSAT expression solely accompanies the proliferative response of mouse kidney, and suggest the importance of post-transcriptional regulation to the control of SSAT activity in both hyperplastic and hypertrophic experimental models.

The polyamines spermidine and spermine, and their diamine precursor putrescine, are naturally occurring ubiquitous polycations required for cell growth, differentiation and cell

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Abbreviations: AdoMetDC, *S*-adenosylmethionine decarboxylase (EC 4.1.1.50); CB 3717, N^{10}-propargyl-5,8-dideazafolic acid; ODC, ornithine decarboxylase (EC 4.1.1.17); SSAT, spermidine/spermine N^1-acetyltransferase (EC 2.3.1.57).

death (Pegg, 1988; Igarashi & Kashiwagi, 2000; Thomas & Thomas, 2001). Among the many functional roles of polyamines in the cell (such as protein synthesis, membrane stability, chromatin structure or modulation of ion channels), recent reports reveal their role in the transduction of death signals in apoptosis (Stefanelli *et al.,* 2002) and in transcription, by regulation of the interactions of transcription factors with their response elements (Shah *et al.,* 2001). Furthermore, the recent discovery of a polyamine-responsive element in the SSAT gene and transacting proteins responding to polyamines, documents that polyamines can both directly and indirectly regulate gene expression (Wang *et al.,* 1998; 1999; 2002).

The intracellular polyamine pool appears to be precisely controlled by various homeostatic responses that typically include polyamine biosynthesis, catabolism, uptake, and excretion. The highly regulated polyamine metabolic pathway is finely controlled in both anabolic and catabolic directions. Three inducible enzymes, ODC, AdoMetDC (controlling polyamine biosynthesis), and SSAT are involved in this regulation. SSAT regulates the pathway governing the interconversion/degradation of cell polyamines (Seiler, 1987; Casero & Pegg, 1993; Xiao & Casero, 1995). It acetylates higher polyamines that are either excreted from the cell or acted upon by polyamine oxidase, leading to a back-conversion of spermine to spermidine and spermidine to putrescine. This important enzyme prevents the overaccumulation of polyamines by facilitating their excretion and degradation, thereby playing a key role in the regulation of intracellular polyamines. SSAT activity is normally very low in the cell. However, this highly inducible enzyme responds to many physiological and non-physiological stimuli including natural polyamines and their analogues. Therefore, SSAT has been considered a homeostatic mechanism preventing the accumulation of polyamines to high cytotoxic levels. A report on the physiological polyamine level maintained in transgenic mouse overexpressing ODC and AdoMetDC due to a compensatory, increased activity of SSAT points to the crucial role of this enzyme in polyamine homeostasis (Heljasvaara *et al.,* 1997).

We have previously shown that homeostasis of intracellular polyamine pools was profoundly disturbed in female mouse kidney that underwent either hyperplasia or hypertrophy after mouse treatment with a quinazoline analogue of folic acid, CB 3717 or the steroid hormone, testosterone, respectively (Manteuffel-Cymborowska *et al.,* 1993). In mouse kidney CB 3717-induced injury of tubular epithelium was followed by regenerative hyperplasia. In contrast, an androgen-induced anabolic, non-proliferative response resulted in a hypertrophic kidney. However, in both cases, ODC activity and the ODC transcript level were greatly enhanced while modulation of AdoMetDC activity depended upon the kidney experimental model (Manteuffel-Cymborowska *et al.,* 1992; 1993).

In this paper we present the *in vivo* data on the differential modulation of SSAT expression in hyperplastic and hypertrophic mouse kidney. Moreover, we show that, in contrast to both polyamine biosynthetic enzymes, catecholamines are not prerequisite for induction of SSAT activity.

MATERIALS AND METHODS

Animal experimental model and drug administration. Swiss female mice (2.5–3 months old) were injected with testosterone (5 days, 125 mg/kg, s.c.) dissolved in soybean oil or for the period indicated with quinazoline antifolate CB 3717 or folate (300 mg/kg, i.p. or 250 mg/kg, i.p., respectively) dissolved in phosphate-buffered saline (PBS) adjusted with a few drops of 1 M NaOH to final pH 9–9.5. Control animals received either soybean oil or PBS, respectively. Anti-androgen casodex (40 mg/kg, s.c.) was injected 1 h be-

fore testosterone and reserpine (10 mg/kg, i.p.) was given 1 h before CB 3717 or folate. The experimental details are given in the legends to the appropriate figures. The mice were killed by cervical dislocation, the kidneys were removed, weighed, cut into several pieces and used immediately for enzyme determinations, or were frozen at –70°C and processed later for Northern blot analysis. Experiments on the animals were carried out in accordance with the Polish regulations, which closely match the Convention No. 123 of the European Council.

Enzyme activities and Northern blot analysis. The kidney pieces were immediately homogenized in appropriate buffers and processed for assays of SSAT (Stefanelli *et al.,* 1987) and AdoMetDC (Pegg & Pőső, 1983). The mean specific activities of SSAT and AdoMetDC in kidneys of control female mice (12–14 animals) were 0.47 ± 0.13 and 0.24 ± 0.05 nmol/h per mg protein, respectively. For Northern blot analysis total RNA was isolated, subjected to gel electrophoresis, and blotted on nylon membranes; the levels of the selected mRNA species were then estimated as described (Manteuffel-Cymborowska *et al.,* 1997). The probe used was an insert coding a fragment of SSAT cDNA in the *Bam*HI site of plasmid pETSSAT. The level of SSAT mRNA was quantified by densitometric scanning of the autoradiograms with a Molecular Dynamics Personal Laser Densitometer (Image Quant software) and corrected for total RNA loaded on the gels as determined by ethidium bromide staining of 28S RNA. Protein concentrations were estimated by the method of Lowry (Lowry *et al.,* 1951). Data were analysed statistically by the Mann-Whitney test.

Thymidine incorporation. To determine thymidine incorporation into acid-insoluble material, the mice were injected with [*methyl*-^{3}H]thymidine (i.p., 10 mg/kg; 0.67 MBq/25 g) 1 h prior to sacrifice. The kidneys were dissected and processed as described (Manteuffel-Cymborowska *et al.,* 1993).

Chemicals. All chemicals purchased from commercial sources were of analytical grade. Testosterone was obtained from Jelfa (Jelenia Góra, Poland). Rediprime (Random Primer Labeling), Hybond-N, Hyperfilm-MP, [α-^{32}P]dCTP (110 TBq/mmol), D,L-[1-^{14}C]ornithine dihydrochloride (2.15 GBq/mmol), *S*-adenosyl[*carboxy*-^{14}C]methionine (2.072 GBq/mmol) and [*methyl*-^{3}H]thymidine (2.96 TBq/mmol) were purchased from Amersham (Little Chalfont, Bucks., U.K.). [*Acetyl*-1-^{14}C]CoA (1.9 GBq/mmol) was from NEN (Boston, U.S.A.). CB 3717 and casodex were generously provided by Zeneca Pharmaceuticals (Alderley Park, Macclesfield, Cheshire, U.K.). The probe for SSAT was kindly given by Dr. R.A. Casero, Jr. (The Johns Hopkins Oncology Center Research Laboratories, Baltimore, MD, U.S.A.).

RESULTS AND DISCUSSION

CB 3717 and folate up-regulate SSAT expression

Antifolate CB 3717 and folate, when applied at high doses, induce renal damage owing to their precipitation in renal tubules at physiological pH (Newell *et al.,* 1986; Mauer, 1986). The consequence of this acute renal injury is regeneration of the tubule epithelium leading to kidney hyperplasia. As shown previously in the CB 3717-induced hyperplastic kidney a proliferative response was correlated with the induction of the activities of two decarboxylases, ODC and AdoMetDC, controling polyamine biosynthesis (Manteuffel-Cymborowska *et al.,* 1993).

The activity of SSAT, the enzyme limiting polyamine interconversion and degradation, is also significantly increased by CB 3717 (Figs. 1 and 2) and folate (not shown). This is consisted with a several-fold increase in SSAT product, N^{1}-acetylspermidine and a significant decrease in its substrate, spermine, in

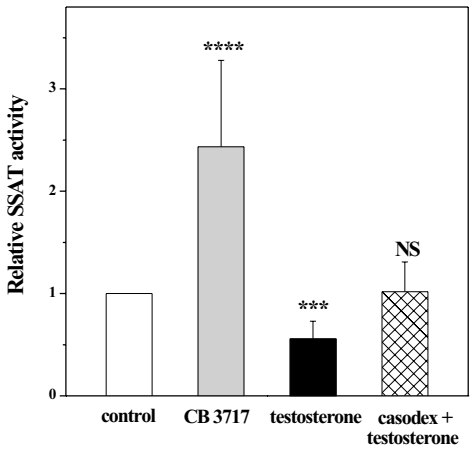

Figure 1. Antifolate CB 3717 but not testosterone induces SSAT activity.

CB 3717 was injected for one day, testosterone for five days, and casodex 1 h before testosterone administration. Relative values (means ± S.D.) of SSAT activity are given; each experimental group consisted of ten to twenty mice. ****P < 0.0001; ***P < 0.001; NS, non-significant as compared with control.

kidneys undergoing regenerative hyperplasia, but not hypertrophy (Manteuffel-Cymborowska *et al.,* 1993), where SSAT induction does not occur (shown below). Moreover, our findings extend and confirm an earlier report

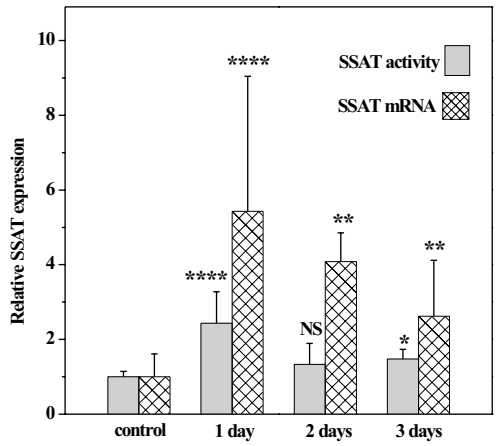

Figure 2. Time-course of induction of SSAT activity and SSAT mRNA level by CB 3717.

The results are means ± S.D.; each experimental group consisted of three to twenty mice. ****P < 0.0001, **P < 0.01, *P < 0.05; NS, non-significant as compared with an appropriate control.

on the effect of folate on SSAT activity (Matsui & Pegg, 1982).

The time course analysis reveals that the highest induction of SSAT activity occurs one day after antifolate treatment (Fig. 2), similarly to the induction of ODC and AdoMetDC (Manteuffel-Cymborowska *et al.,* 1993). However, the enhancement of SSAT activity is distinctly lower than that of ODC, being comparable with the induction of AdoMetDC by CB 3717 (Manteuffel-Cymborowska *et al.,* 1993) or folate (shown below, Fig. 5).

Northern analysis shows that SSAT mRNA expression is also up-regulated both in the antifolate- and folate-induced hyperplastic kidneys (Figs. 2 and 3) with the folate induction of the SSAT mRNA equal to 3.93 ± 0.31, (n = 3). The pattern of increase in SSAT mRNA by CB 3717 strictly parallels that of SSAT activity, although it is higher at each examined time interval. This is in contrast with ODC expression increase in the CB 3717-induced hyperplastic kidney or testosterone-induced hypertrophic kidney where the enzyme activity is many fold higher than that of mRNA (Manteuffel-Cymborowska *et al.,* 1997; Dudkowska *et al.,* 1999; 2001). A higher up-regulation of the SSAT mRNA than of SSAT activity, although rare, is not an exception. A similar observation was also found in proliferating rat thymocytes (Desiderio *et al.,* 1995), in the COS-7 cells (Parry *et al.,* 1995) and in hepatocarcinoma cells (Desiderio *et al.,* 1998). These data indicate that, under the above experimental conditions, the increased transcription of the SSAT gene and/or mRNA stability are not sufficient for the up-regulation of SSAT expression and point to the critical role of post-transcriptional regulation for the control of SSAT activity.

Previously, we have documented that CB 3717 or folate induce the expression of the hepatocyte growth factor (HGF)/c-Met signalling pathway in the injured kidney (Dudkowska *et al.,* 2001). HGF induction of SSAT expression documented *in vitro* in hepatocarcinoma cells (Desiderio *et al.,* 1998)

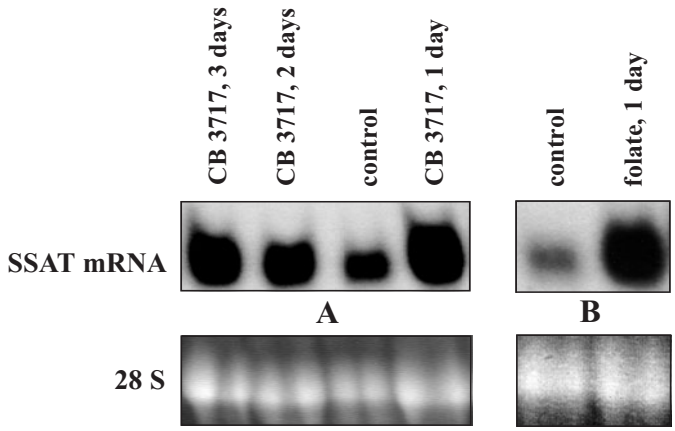

Figure 3. SSAT mRNA level is induced by CB 3717 and folate as demonstrated by Northern blot analysis.

Each lane represents SSAT mRNA (upper panel) and 28S rRNA (lower panel) from an individual mouse. The relative numerical values for A are given in Fig. 2 and for B in the text. 28S rRNA ethidium bromide stained bands indicating the amount of RNA blotted onto the filter are shown below.

seems to support the suggestion that up-regulated HGF/c-Met signalling can be involved in the induction of renal SSAT activity and SSAT mRNA *in vivo*.

Testosterone differentially modulates SSAT activity and SSAT mRNA

In mouse kidney, which is a testosterone responsive organ, androgens evoke a profound, nonproliferative response producing a marked hypertrophy of the proximal tubules and of the whole organ. In testosterone-evoked hypertrophic kidney, ODC and AdoMetDC show a different response — ODC expression is dramatically induced while that of AdoMetDC remains unaffected (Manteuffel-Cymborowska *et al.,* 1992; 1993). Thus, the lack of AdoMetDC induction differentiates the anabolic, testosterone-evoked response from the proliferative one, induced in kidney by antifolate or folate. The differences between the two murine kidney experimental models also apply to SSAT expression. Unlike CB 3717 or folate, testosterone does not induce, but significantly decreases, SSAT activity (Fig. 1). An over 40% reduction in SSAT activity evoked by testosterone is completely abolished by casodex, an anti-androgen (Fig. 1), sugesting the involvement of the androgen receptor in SSAT regulation. In agreement with this finding is slightly lower SSAT activity in kidneys of male mice than in females correlated with the level of endogenous testosterone in both sexes (not shown).

The effect of testosterone on SSAT activity appeared to be tissue specific. In contrast to kidney, no statistically significant difference in SSAT activity was found in the liver of control and testosterone-treated mice, respec-

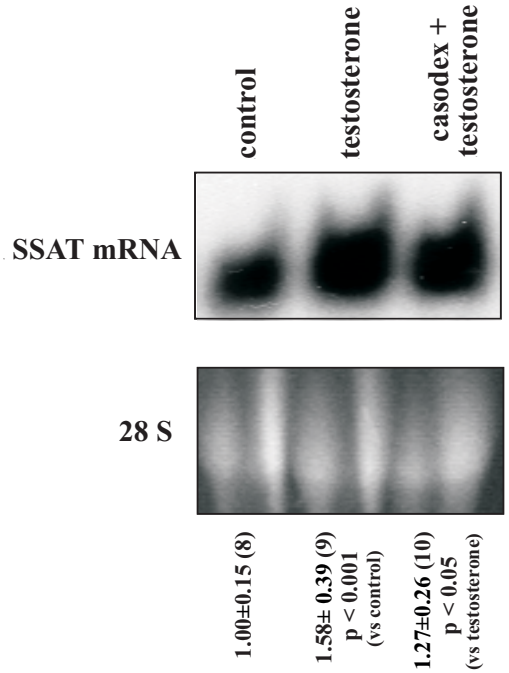

Figure 4. Testosterone induces SSAT mRNA expression, the effect being partially prevented by anti-androgen — casodex (Northern blot analysis).

Testosterone was injected for five days, casodex 1 h before testosterone administration. Each lane represents SSAT mRNA (upper panel) and 28S rRNA (lower panel) from an individual mouse. 28S rRNA ethidium bromide stained bands indicating the amount of RNA blotted onto the filter are shown below. Relative values (means ± S.D.) of SSAT mRNA compared with control, the number of mice in each experimental group (in parentheses), and the *P* values are shown beneath the appropriate lane.

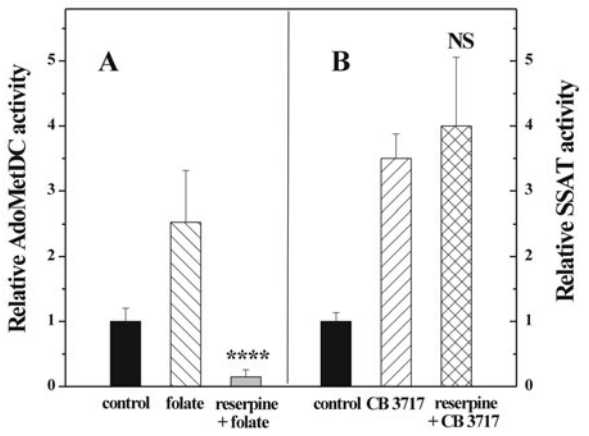

Figure 5. Reserpine down-regulates folate-induced AdoMetDC activity (A) but not CB 3717-induced SSAT activity (B).

Folate and CB 3717 were injected for one day, reserpine 1 h before folate or CB 3717. Relative values (means ±S.D.) of AdoMetDC and SSAT activity are given; each experimental group consisted of four to fourteen mice. ****$P < 0.0001$; NS, non-significant as compared with folate (A) and CB 3717 (B).

tively (0.48 ± 0.11, n = 11 and 0.52 ± 0.12, n=17 nmol/h per mg protein).

To our knowledge, there is no data on the direct relationship between SSAT activity and androgens. The only available report on the increase in SSAT activity after the administration of gossypol, an inhibitor of testosterone biosynthesis, and its decrease following testosterone application in canine prostate (Chang *et al.,* 1997) are in line with our findings.

The response of SSAT mRNA level to testosterone is different from that of SSAT activity. Thus, in contrast to the decreased SSAT activity observed in hypertrophic kidney, SSAT mRNA is induced by testosterone (Fig. 4). This induction, although not very high, is statistically significant. It is decreased by casodex to a level significantly different from the level induced by testosterone, but indistinguishable (NS) from the control (Fig. 4).

The results on the effect of testosterone on SSAT expression resemble the greater effect of antifolate CB 3717 on SSAT mRNA than on SSAT activity (Fig. 2), and stress the importance of post-transcriptional regulation of SSAT expression.

Reserpine impairs folate-induced AdoMetDC activity but not CB 3717-induced SSAT activity

As shown previously, catecholamines are required both for androgen- and antifolate-induced renal ODC expression (Manteuffel-Cymborowska *et al.,* 1997; Dudkowska *et al.,* 1999). The spectacular decrease in ODC activity and its transcript level in catecholamine-depleted kidneys points to cross talk between the examined signalling pathways.

The activity of AdoMetDC, which also limits polyamine biosynthesis, is 2.5-fold increased by folate ($P < 0.0001$, Fig. 5A), in agreement with previously reported induction of this enzyme by antifolate (Manteuffel-Cymborowska *et al.,* 1993). To evaluate the role of catecholamines in AdoMetDC induction by folate we have applied in our experiments reserpine, a drug known to produce catecholamine depletion. Reserpine administered prior to folate not only prevents the increase in AdoMetDC activity, but also reduces this activity below the control level (Fig. 5A). It appears, therefore, that AdoMetDC expression is catecholamine-dependent similarly to the ODC expression in mouse kidney (Manteuffel-Cymborowska *et al.,* 1997; Dudkowska *et al.,* 1999) or rat liver. In the latter, depletion of catecholamines or blocking of adrenoreceptors prevented stimulation of ODC activity by glucorticoid and laparatomy (Corti *et al.,* 1985; Astancolle *et al.,* 1991). Moreover, agonists of catecholamine receptors induced ODC expression in mouse kidney (Dudkowska *et al.,* 1999) and in cells cultured *in vitro* (Juráni *et al.,* 1996), and also increased SSAT activity in rat parotid glands (Nilsson & Rosengren, 1993).

At variance with the catecholamine control of AdoMetDC activity, the SSAT activity, which increases several-fold in the antifolate-induced hyperplastic kidney, is not in-

fluenced by reserpine (Fig. 5B). This result is rather unexpected. Reserpine-evoked catecholamine depletion attenuates the expression of both ODC and AdoMetDC and also decreases antifolate-induced kidney hyperplasia (Dudkowska *et al.,* 1999). Under these conditions, a decrease rather than an increase in the intracellular polyamine content could be expected since the induced SSAT activity would lead to further depletion of intracellular polyamines.

The findings presented here provide evidence that induction of SSAT expression is part of the proliferative response evoked in the CB 3717- or folate-injured hyperplastic kidney but not of the androgen-induced anabolic response in hypertrophic kidney. It is not clear why SSAT activity, known to respond to the polyamine pool, is not decreased under the conditions of catecholamine depletion. However, compensatory mechanisms such as increased polyamine uptake and/or decreased excretions of these polycations, preserving to some extent polyamine homeostasis, could provide an explanation. At present the question remains unanswered and deserves further studies.

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REFERENCES

Astancolle S, Davalli P, Corti A. (1991) Blockade of α- and β-adrenergic receptors can prevent stimulation of liver ornithine decarboxylase activity by glucocorticoid or laparatomy. *Biochem Biophys Res Commun.*; **174**: 915–21.

Casero RA, Jr, Pegg AE. (1993) Spermidine/spermine N^1-acetyltransferase – the turning point in polyamine metabolism. *FASEB J.*; **7**: 653–61.

Chang WY, Sugimoto Y, Shidaifat F, Kulp SK, Canatan H, Lin YC. (1997) Gossypol induces spermidine/spermine N^1-acetyltransferase in canine prostate epithelial cells. *Biochem Biophys Res Commun.*; **231**: 383–8.

Corti A, Astancolle S, Davalli P. (1985) Response of hepatic ornithine decarboxylase and polyamine concentration to surgical stress in the rat: evidence for a permissive effect of catecholamines on glucocorticoid action. *Biochem Biophys Res Commun.*; **129**: 885–91.

Desiderio MA, Grassilli E, Bellesia E, Salomoni P, Franceschi C. (1995) Involvement of ornithine decarboxylase and polyamines in glucocorticoid-induced apoptosis of rat thymocytes. *Cell Growth Differ.*; **6**: 505–13.

Desiderio MA, Pogliaghi G, Dansi P. (1998) Regulation of spermidine/spermine N^1-acetyltransferase expression by cytokines and polyamines in human hepatocarcinoma cells (HepG2). *J Cell Physiol.*; **174**: 125–34.

Dudkowska M, Manteuffel-Cymborowska M, Chmurzyńska W, Grzelakowska-Sztabert B. (1999) Catecholamines participate in the induction of ornithine decarboxylase gene expression in normal and hyperplastic mouse kidney. *Biochim Biophys Acta.*; **1454**: 71–9.

Dudkowska M, Stachurska A, Chmurzyńska W, Grzelakowska-Sztabert B, Manteuffel-Cymborowska M. (2001) Cross-talk between steroid-receptor-mediated and cell-membrane-receptor-mediated signalling pathways results in the *in vivo* modulation of c-Met and ornithine decarboxylase gene expression in mouse kidney. *Biochem J.*; **353**: 317–23.

Heljasvaara R, Veress I, Halmekytő M, Alhonen L, Jänne J, Laajala P, Pajunen A. (1997) Transgenic mice overexpressing ornithine and *S*-adenosylmethionine decarboxylases maintain a physiological polyamine homoeostasis in their tissues. *Biochem J.*; **323**: 457–62.

Igarashi K, Kashiwagi K. (2000) Polyamines: Mysterious modulators of cellular functions. *Biochem Biophys Res Commun.*; **271**: 559–64.

Juráni M, Vaneková M, Vyboh P, Lamosová D. (1996) Enhanced ornithine decarboxylase activity of chick muscle cells in culture by β-adrenergic agonists. *Methods Find Exp Clin Pharmacol.*; **18**: 13–7.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem.*; **193**: 265–75.

Manteuffel-Cymborowska M, Chmurzyńska W, Grzelakowska-Sztabert, B. (1992) Tissue-specific effects of testosterone on *S*-adenosylmethionine formation and utilization in the mouse. *Biochim Biophys Acta.*; **1116**: 166–72.

Manteuffel-Cymborowska M, Chmurzyńska W, Grzelakowska-Sztabert B. (1993) Polyamines in testosterone-induced hypertrophic and antifolate-induced hyperplastic mouse kidney Differential effect of α-difluoromethylornithine. *Biochim Biophys Acta.*; **1182**: 133–41.

Manteuffel-Cymborowska M, Pęska M, Chmurzyńska W, Grzelakowska-Sztabert B. (1997) Catecholamines are required for androgen-induced ODC expression but not for hypertrophy of mouse kidney. *Biochim Biophys Acta.*; **1356**: 292–8.

Matsui I, Pegg AE. (1982) Induction of spermidine N^1-acetyltransferase in rat kidney by treatment with folic acid. *FEBS Lett.*; **139**: 205–8.

Mauer AM. (1986) Effects of pharmacologic doses of folates on the kidney. In *Folates and pterins*. Blakley RL, Whitehead VM, eds, vol 3 pp. 325–38. Wiley-Interscience, New York.

Newell DR, Alison DL, Calvert AH, Harrap KR, Jarman M, Jones TR, Manteuffel-Cymborowska M, O'Connor P. (1986) Pharmacokinetics of the thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (CB3717) in the mouse. *Cancer Treat Rep.*; **70**: 971–9.

Nilsson BO, Rosengren E. (1993) Effects of receptor agonists on polyamine concentrations and spermidine/spermine N^1-acetyltransferase activity in rat parotid gland. *Experientia.*; **49**: 881–4.

Parry L, Balaña Fouce R, Pegg AE. (1995) Post-transcriptional regulation of the content of spermidine/spermine N^1-acetyltransferase by N^1N^{12}-bis(ethyl)spermine. *Biochem J.*; **305**: 451–8.

Pegg AE. (1988) Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.*; **48**: 759–74.

Pegg AE, Pőső H. (1983) *S*-adenosylmethionine decarboxylase (rat liver). *Methods Enzymol.*; **94**: 234–5.

Seiler N. (1987) Functions of polyamine acetylation. *Can J Physiol Pharmacol.*; **65**: 2024–35.

Shah N, Thomas TJ, Lewis JS, Klinge CM, Shirahata A, Gelinas C, Thomas T. (2001) Regulation of estrogenic and nuclear factor B functions by polyamines and their role in polyamine analog-induced apoptosis of breast cancer cells. *Oncogene.*; **20**: 1715–29.

Stefanelli C, Flamigini F, Carati D, Rossoni C, Caldarera CM. (1987) Effects of dexamethasone on spermidine N^1-acetyltransferase and ornithine decarboxylase activities in rat spleen. *Biochim Biophys Acta.*; **930**: 79–86.

Stefanelli C, Tantini B, Fattori M, Stanic I, Pignatti C, Clo C, Guarnieri C, Caldarera CM, Mackintosh CA, Pegg AE, Flamigni F. (2002) Caspase activation in etoposide-treated fibroblasts is correlated to ERK phosphorylation and both events are blocked by polyamine depletion. *FEBS Lett.*; **527**: 223–8.

Thomas T, Thomas TJ. (2001) Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. *Cell Mol Life Sci.*; **58**: 244–58.

Wang Y, Xiao L, Thiagalingam A, Nelkin BD, Casero RA, Jr. (1998) The identification of a cis-element and a trans-acting factor involved in the response to polyamines and polyamine analogues in the regulation of the human spermidine/spermine N^1-acetyltransferase gene transcription. *J Biol Chem.*; **273**: 34623–30.

Wang Y, Devereux W, Stewart TM, Casero RA, Jr. (1999) Cloning and characterization of human polyamine-modulated factor-1 a transcriptional cofactor that regulates the transcription of the spermidine/spermine N^1-acetyltransferase gene. *J Biol Chem*.; **274**: 22095–101.

Wang Y, Devereux W, Stewart TM, Casero RA Jr. (2002) Polyamine-modulated factor 1 binds to the human homologue of the 7a subunit of the *Arabidopsis*. COP9 signalosome: implications in gene expression. *Biochem J*.; **366**: 79–86.

Xiao L, Casero RA Jr. (1995) Regulation of spermidine/spermine N^1-acetyltransferase. In *Polyamines: regulation and molecular interaction*. Casero RA Jr, ed, pp. 77–99. RG Landes Company.