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Effects of transient expression of spermidine/spermine N^1-acetyltransferase in COS cells

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Abstract Mammalian spermidine/spermine N^1-acetyltransferase (SSAT) was transiently expressed in COS cells. As compared to COS cells transfected with control vector alone, cells transfected with the expression vector containing SSAT cDNA contained lower concentrations of spermidine and spermine. The putrescine content, on the other hand, was markedly increased in the COS cells expressing large amounts of SSAT. These changes in polyamine content were most likely caused by an interconversion of spermine and spermidine into putrescine. The SSAT-induced changes in cellular polyamine content resulted in a compensatory increase in the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, i.e. the enzymes catalyzing the rate-limiting steps in polyamine biosynthesis. This is the first demonstration that a primary increase in SSAT activity will induce an interconversion-like change in the polyamine levels and the physiological role of SSAT is most likely to protect cells against too high concentrations of spermidine and spermine.

Key words: Polyamine; Spermidine/spermine N^1-acetyltransferase; Ornithine decarboxylase; *S*-Adenosylmethionine decarboxylase; Transient expression

1. Introduction

The polyamines putrescine, spermidine and spermine are essential cell constituents important for growth and differentiation [1–3]. An enzyme catalyzing the acetylation of the nitrogen of the aminopropyl groups of spermidine and spermine has been isolated and characterized [4–9]. This enzyme, spermidine/spermine N^1-acetyltransferase (SSAT), has a very short biological half-life [8,10] and is induced by a variety of toxic agents as well as by various polyamines and polyamine analogues [5,7,10–15]. The physiological role of SSAT may be to protect the cells against too high levels of polyamines, which can have deleterious effects on the cell function. The acetylation of the nitrogen reduces the tendency of the amino group to be charged at a physiological pH. Furthermore, the N^1-acetyl-derivatives of spermidine and spermine are good substrates for a flavin-dependent polyamine oxidase [16,17] and SSAT is considered to catalyze the rate-limiting step in a catabolic pathway in which spermidine and spermine are first acetylated and then oxidized to putrescine and spermidine, respectively [9].

Most studies on the biological effects of SSAT are based on the induction of the enzyme by various toxic agents or polyamines [9]. However, the interpretation of the results may be hampered by the fact that these inducers often damage the cell and/or affect other enzymes involved in polyamine metabolism. In the present study, we have instead used COS cells transiently expressing high levels of SSAT to investigate the role of this enzyme in polyamine metabolism.

2. Materials and methods

2.1. Materials

(Acetyl-1-[^{14}C])-Acetyl-CoA (56 Ci/mol) was obtained from Amersham International (Amersham, UK). The eukaryotic expression vector pSVL, containing the SV 40 late promoter and origin of replication, was purchased from Pharmacia, Uppsala, Sweden. The cDNA encoding human SSAT (AP3/F7) was a kind gift from Dr. Robert Casero [18,19]. The full-length cDNA was subcloned into the cloning site of pSVL using *Eco*RI/*Xho*I and *Eco*RI/*Bam*HI linkers.

2.2. Cell culture and transfection

COS cells were grown in Dulbecco's MEM containing 10% fetal calf serum, nonessential amino acids and antibiotics (penicillin 50 units/ml and streptomycin 50 μg/ml). The cells were seeded at density of 5,000/cm^2 and grown for 3 days before being used for transfection. The cells were washed and resuspended in growth medium at a density of 7–12 × 10^6 cells/ml. DNA (15 μg) was then added to the cells (0.8 ml) which were pulsed with 300 V at 250 μF. After 5 min of recovery at room temperature the cells were resuspended in growth medium and seeded at a density of 25,000 cells/cm^2. The cells were analyzed 2 days after the pulse.

2.3. Determination of enzyme activities

Cells were sonicated in 0.1 M Tris-HCl (pH 7.5) containing EDTA (0.1 mM) and dithiothreitol (2.5 mM). The extract was then centrifuged for 20 min at 20,000 × *g* at 4°C. SSAT, *S*-adenosylmethionine decarboxylase and ornithine decarboxylase activities were determined in aliquots of the supernatant essentially as described earlier [20,21].

2.4. Miscellaneous methods

Cellular polyamines were quantitated as previously described [22] using an amino acid analyzer (Biotronik LC 5001). Protein concentrations were determined by the method of Bradford [23].

3. Results and discussion

COS cells transfected with the expression vector pSVL containing SSAT cDNA (pSVL-SSAT) exhibited high SSAT activity two days after transfection as compared to the control COS cells transfected with the vector alone (pSVL) (Fig. 1). The COS cells transfected with pSVL-SSAT contained more than 10-fold higher SSAT activity than the control cells. The assay used for measuring SSAT activity does not discriminate between the specific SSAT and other enzymes capable of acetylating spermidine, and it has been demonstrated that often less than 10% of the apparent SSAT activity in non-induced cells/tissues is caused by the specific enzyme SSAT [8]. Thus, assuming that

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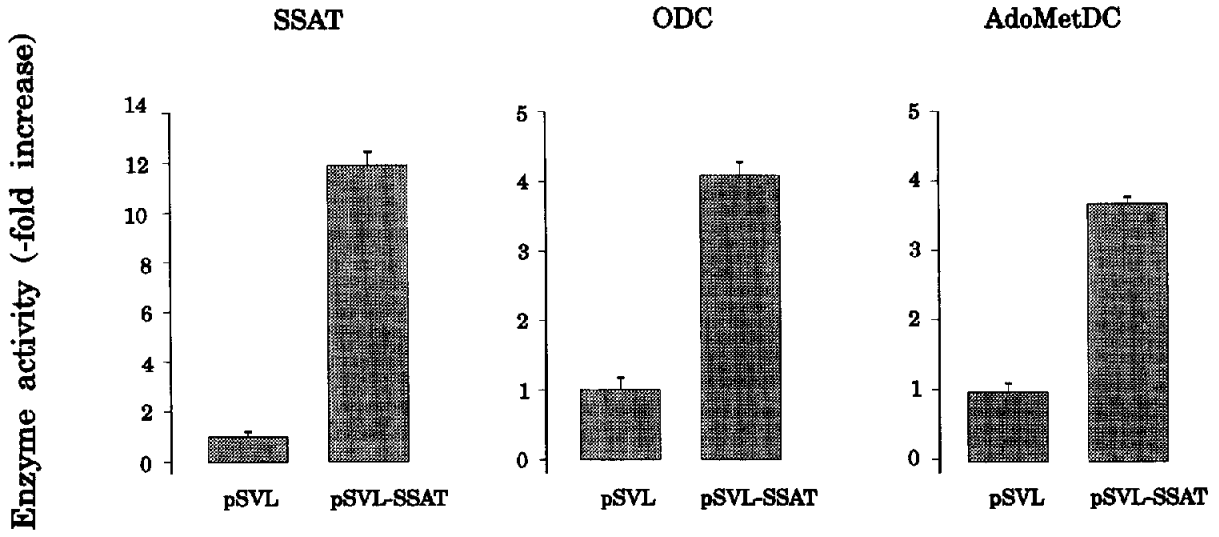

Fig. 1. Spermidine/spermine N^1-acetyltransferase (SSAT), ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) activities in COS cells transfected with pSVL or pSVL-SSAT. The cells were grown for 48 h before analyzed for SSAT, ODC and AdoMetDC activities. Values are mean ± S.D., $n = 4$.

this is also true for the control COS cells, the actual increase in specific SSAT activity would be more than 100-fold.

As shown in Table 1, the concentrations of the individual polyamines putrescine, spermidine and spermine were markedly changed in the COS cells expressing a high SSAT activity. As compared to the COS cells transfected with pSVL alone, the cells transfected with pSVL-SSAT contained lower concentrations of spermidine and spermine (Table 1). The putrescine concentration, on the other hand, was much higher in the cells having a high SSAT activity than in the control COS cells (Table 1). These changes in polyamine content are consistent with the notion that SSAT is catalyzing the regulatory step in the polyamine interconversion pathway, in which spermine and spermidine are degraded to spermidine and putrescine, respectively [9]. The finding of measurable amounts of N^1-acetylspermidine in the COS cells after transfection with pSVL-SSAT (Table 1) supports the assumption that the changes in polyamine content are due to an interconversion of the polyamines. N^8-acetylspermidine was not found in the cells, neither in the control cells nor in the pSVL-SSAT transfected cells (Table 1).

The rate-controlling enzymes in polyamine synthesis are ornithine decarboxylase and *S*-adenosylmethionine decarboxylase [2,3]. As shown in Fig. 1, the activities of both of these enzymes were significantly increased in the COS cells transfected with pSVL-SSAT. The reason for this is most likely that the SSAT-induced decrease in spermidine and spermine content reduced the negative feedback that these polyamines exert on the expression of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase. A similar phenomenon is seen when cells are depleted of their spermidine/spermine content using specific inhibitors of polyamine synthesis [2,3].

Even though electroporation has been demonstrated to be one of the better techniques for transfection it is known that usually only a fraction of the cells are successfully transfected. Thus, the changes in enzyme activities as well as in polyamine content caused by the expression of SSAT in the COS cells are most likely much larger than the values shown in the present study.

In conclusion, this is the first demonstration that a primary increase in SSAT activity will induce an interconversion-like change in the polyamine levels. In contrast to earlier studies we have used a technique to induce high SSAT activity which does not by itself affect ornithine decarboxylase and/or *S*-adenosylmethionine decarboxylase, or in some way may directly interfere with the cellular pools of polyamines. Thus, since SSAT is strongly induced by polyamines, or polyamine analogues, it is highly likely that the physiological role of SSAT is to protect the cells against too high concentrations of spermidine and spermine.

Table 1
Polyamine content in COS cells transfected with pSVL or pSVL-SSAT

Vector	Putrescine	Spermidine	Spermine	N^1-Acetylspermidine	N^8-Acetylspermidine
pSVL	0.49 ± 0.07	3.03 ± 0.27	2.77 ± 0.36	N.D.	N.D.
pSVL-SSAT	3.15 ± 0.34	1.75 ± 0.17	1.98 ± 0.22	0.16 ± 0.01	N.D.

The COS cells were transfected with pSVL or pSVL-SSAT and then seeded at a density of 25,000 cells/cm^2. Cells were collected for polyamine analysis 2 days after seeding. The polyamine concentrations are given in nmol/10^6 cells. Mean ± S.E.M., $n = 4$. N.D., not detectable.

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