Concurrent overexpression of ornithine decarboxylase and spermidine/spermine N^1-acetyltransferase further accelerates the catabolism of hepatic polyamines in transgenic mice

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We have generated a hybrid transgenic mouse line overexpressing both ornithine decarboxylase (ODC) and spermidine/spermine N^1-acetyltransferase (SSAT) under the control of the mouse metallothionein (MT) I promoter. In comparison with singly transgenic animals overexpressing SSAT, the doubly transgenic mice unexpectedly displayed much more striking signs of activated polyamine catabolism, as exemplified by a massive putrescine accumulation and an extreme reduction of hepatic spermidine and spermine pools. Interestingly, the profound depletion of the higher polyamines in the hybrid animals occurred in the presence of strikingly high ODC activity and tremendous putrescine accumulation. Polyamine catabolism in the doubly transgenic mice could be enhanced further by administration of zinc or the polyamine analogue N^1,N^{11}-diethylnorspermine. In tracer experiments with [^{14}C]spermidine we found that, in comparison with syngenic animals, both MT-ODC and MT-SSAT mice possessed an enhanced efflux mechanism for hepatic spermidine. In the MT-ODC animals this mechanism apparently operated in the absence of measurable SSAT activity. In the hybrid animals, spermidine efflux was stimulated further in comparison with the singly transgenic animals. In spite of a dramatic accumulation of putrescine and a profound reduction of the spermidine and spermine pools, only marginal changes were seen in the level of ODC antizyme. Even though the hybrid animals showed no liver or other organ-specific overt toxicity, except an early and permanent loss of hair, their life span was greatly reduced. These results can be understood from the perspective that catabolism is the overriding regulatory mechanism in the metabolism of the polyamines and that, even under conditions of severe depletion of spermidine and spermine, extremely high tissue pools of putrescine are not driven further to replenish the pools of the higher polyamines.

Key words: antizyme, N^1,N^{11}-diethylnorspermine, metallothionein promoter, putrescine.

INTRODUCTION

During the past few years we have generated a number of transgenic rodent lines with genetically altered polyamine metabolism. Experiments with transgenic animals displaying activated polyamine biosynthesis through an overexpression of ornithine decarboxylase (ODC) have revealed some interesting phenotypic changes. These include impaired spermatogenesis ultimately leading to male sterility [1,2], elevated resistance to seizure activity [3], improved tolerance to ischaemic insults [4] and enhanced papilloma formation in the two-stage skin carcinogenesis [5]. All these phenotypic changes are believed to be attributable to increased tissue putrescine pools, as an overexpression of ODC did not enhance the accumulation of the higher polyamines spermidine and spermine [6,7]. Even a combined overexpression of both ODC and *S*-adenosylmethionine decarboxylase (AdoMetDC) in hybrid transgenic mice did not result in an enhancement of the accumulation of the higher polyamines [8]. The most logical explanation for the unaltered tissue pools of spermidine and spermine would be an activation of polyamine catabolism. However, ODC-overexpressing animals displayed quite normal spermidine/spermine N^1-acetyltransferase (SSAT) activity [6], which is the rate-controlling enzyme in polyamine catabolism [9]. Some experimental evidence, derived from transgenic fibroblasts overexpressing both ODC and AdoMetDC, indicated that even in the presence of unaltered SSAT activity, the acetylation and excretion of the higher polyamines were enhanced [8].

In striking contrast to transgenic animals with activated polyamine biosynthesis, in which the tissue pools of the higher polyamines remained virtually unaltered, an activation of polyamine catabolism through overexpression of SSAT led to profound distortions of the tissue pools of all polyamines and to bizarre phenotypic changes. Overexpression of SSAT under its own promoter resulted in a massive accumulation of putrescine, the appearance of N^1-acetylspermidine and decreases in spermidine and/or spermine tissue pools [10]. The most conspicuous phenotypic change in these animals was an early and permanent loss of hair followed by extreme wrinkling of the skin [10]. Other phenotypic changes included a lack of subcutaneous fat and female sterility, apparently due to the lack of corpus luteum formation [10]. Overexpression of SSAT under the control of the mouse metallothionein (MT) promoter resulted in even more severe distortion of the higher polyamine pools in the liver,

Abbreviations used: ODC, ornithine decarboxylase; SSAT, spermidine/spermine N^1-acetyltransferase; MT, metallothionein; DENSPM, N^1,N^{11}-diethylnorspermine; AdoMetDC, *S*-adenosylmethionine decarboxylase.

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characterized by a striking reduction of the hepatic spermine pool [11]. In line with the earlier studies of the central nervous system with ODC-overexpressing mice, the very high putrescine content in the brain of the SSAT mice appeared to protect the animals from kainate-induced toxicity [12]. In transgenic rats overexpressing SSAT under the same MT promoter, induction of the transgene with zinc resulted in the development of acute oedematous pancreatitis [13]. Regardless of the promoter, these SSAT-overexpressing animals were extremely sensitive to the toxicity of polyamine analogues, such as N^1,N^{11}-diethylnorspermine (DENSPM), owing to a dramatic induction of SSAT in response to the drug [11,14].

In order to elucidate whether activated polyamine catabolism resulting in a depletion of spermidine and spermine can be compensated for through an overexpression of ODC, we generated a doubly transgenic mouse line overexpressing both SSAT and ODC. In striking contrast with any expectations, the activation of polyamine biosynthesis through overexpression of ODC in SSAT transgenic mice led to a further enhancement of polyamine catabolism. In comparison with SSAT-overexpressing mice, the doubly transgenic mice showed a 60 % reduction of the total hepatic spermidine and spermine pool and an expansion of the putrescine pool by a factor of 5. This all occurred in the presence of extremely high ODC activity in the hybrid mice. Tracer experiments with radioactive spermidine indicated that in all three transgenic lines the polyamine was less effectively retained by the liver than in syngenic animals, suggesting that an efficient efflux system for spermidine was operating in the transgenic animals. Interestingly, this occurred in the absence of measurable SSAT activity in the MT-ODC mice.

MATERIALS AND METHODS

Transgenic mice and materials

The transgenic mouse line overexpressing ODC under control of the mouse MT promoter (MT-ODC; UKU48) has been described in [15]. The transgenic mouse line overexpressing SSAT similarly under control of the MT promoter (MT-SSAT; UKU181) was described in [11]. The hybrid transgenic mouse line was obtained by mating UKU48 female mice with UKU181 males.

Zinc was administered intraperitoneally (20 mg/kg) as zinc sulphate ($ZnSO_4 \cdot 7H_2O$) dissolved in distilled water. DENSPM was synthesized using a modification of the method described in [16], dissolved in physiological saline and injected intraperitoneally (125 mg/kg). In the tracer experiments [^{14}C]spermidine (specific radioactivity, 115 mCi/mmol; Amersham) was used. Each animal received 1 μCi of the tracer as an intraperitoneal injection.

Analytical methods

Polyamines and their acetylated derivatives were determined with the aid of HPLC as described by Hyvönen et al. [17]. The activities of ODC [18], AdoMetDC [19] and SSAT [20] were assayed by the published methods.

Antizyme ELISA was carried out essentially as described previously [21]. In brief, liver homogenates were further diluted 25-fold in 20 mM phosphate buffer, pH 7.4, containing 0.5 M NaCl, 2 mM EDTA, 0.5 mM PMSF, 3 % BSA and 0.05 % Tween 20, and 100 μl samples were applied to microtitre plates. Mouse antizyme protein recombinantly expressed in *Escherichia coli* [21] was used as a standard.

For statistical analyses the two-tailed Student's *t* test was used when applicable. For multiple comparisons ANOVA with the *post hoc* method of Bonferroni (Statview 4.0; Abacus Concept, Berkeley, CA, U.S.A.) was used.

RESULTS

Enzyme activities and polyamine pools in syngenic and transgenic animals

As shown in Table 1, overexpression of ODC resulted in approx. 10-fold expansion of the hepatic putrescine pool, with marginal and insignificant changes in the concentrations of spermidine and spermine. Even though SSAT activity was only moderately enhanced in the SSAT-overexpressing mice, these animals showed all the signs of activated polyamine catabolism, i.e. more than 40-fold increase in the hepatic putrescine pool (in comparison with syngenic animals), appearance of N^1-acetylspermidine and an almost 85 % reduction in spermine content while spermidine remained unaltered (Table 1). The most dramatic changes in hepatic polyamine metabolism were, however, seen in the doubly transgenic mice overexpressing both ODC and SSAT. ODC activity was increased by a factor of 25 and the pool of putrescine by a factor of 20 in comparison with MT-ODC mice. Whereas there was only a small increase in SSAT activity in the hybrid animals, the pools of spermidine and spermine were further depleted profoundly. In fact, hepatic spermidine was decreased by 75 % and spermine by 93 % as compared with syngenic animals (Table 1). Interestingly, the massive putrescine pools in the doubly transgenic animals were not converted into spermidine and spermine to replenish their depletion. This block was apparently not attributable to any decrease in AdoMetDC activity, which was actually higher in the hybrid animals than in the syngenic animals (Table 1). Similarly, an analysis of spermidine synthase activity indicated that, if anything, the MT-SSAT and the doubly transgenic animals displayed somewhat higher (15–40 %) spermidine synthase activity than that found in the syngenic animals (results not shown).

SSAT induction by zinc

A single injection of zinc expectedly enhanced ODC activity by a factor of 16 and putrescine accumulation by a factor of 8 in MT-ODC animals, yet even under these conditions putrescine apparently was not further converted into spermidine and spermine to any appreciable extent (Table 1). In the MT-SSAT animals, zinc significantly, yet only modestly, enhanced SSAT activity, whereas the higher-polyamine pools, especially that of spermidine, were markedly decreased compared with untreated animals (Table 1). In striking contrast with MT-SSAT mice, zinc dramatically enhanced SSAT activity (nearly 90-fold) in the doubly transgenic mice (Table 1). This was accompanied by a further rise in ODC activity and putrescine accumulation and, most notably, by a profound depletion of spermidine and spermine. In fact, after zinc the hepatic pools of spermidine were reduced to 13 % and the spermine pool to 7 % of that found in the untreated syngenic animals (Table 1).

SSAT induction by DENSPM

As indicated by the results of the experiment depicted in Table 2, the changes in polyamine metabolism in untreated MT-ODC, MT-SSAT and doubly transgenic mice were practically identical with those described in Table 1. Interestingly, the polyamine analogue DENSPM, which is supposed to be an indirect sup-

Table 1 Liver polyamine metabolism in MT-ODC/MT-SSAT transgenic mice with 4 h of $ZnSO_4$ treatment

Data are means ± S.E.M. where $n = 3$–6; where $n = 2$, both values are shown. Animals were 13-week-old female mice. Enzyme activities are expressed as pmol/10 min per mg of tissue (SSAT), pmol/30 min per mg of tissue (ODC) and pmol/h per mg of tissue (AdoMetDC). Ac-Spermidine, N^1-acetylspermidine; Tg (−), non-transgenic control animals. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ refer to the statistical significance of difference between non-treated and treated animals.

	Enzyme activity			Polyamine pool (pmol/mg of tissue)			
Animal and treatment	SSAT	ODC	AdoMetDC	Putrescine	Ac-Spermidine	Spermidine	Spermine
Untreated							
Tg (−)	0 ± 0	0 ± 0	18 ± 1	24 ± 6	< 7	1292 ± 272	1039 ± 145
MT-ODC	0 ± 0	98 ± 27	13; 11	278 ± 85	< 7	1414 ± 78	938 ± 56
MT-SSAT	16 ± 3	127 ± 22	54; 50	973 ± 86	228 ± 14	1079 ± 62	165 ± 10
MT-ODC/MT-SSAT	45 ± 11	2519 ± 393	35 ± 7	5835 ± 415	174 ± 9	313 ± 40	73 ± 13
Zinc-treated							
Tg (−)	0 ± 0	1 ± 1	15; 16	34 ± 6	< 7	1480 ± 65	1223 ± 98
MT-ODC	1 ± 0	1866 ± 517*	11; 12	2533 ± 382**	< 7	1485 ± 40	965 ± 57
MT-SSAT	40 ± 6*	61 ± 16*	11; 8	1322 ± 64*	402 ± 76	276 ± 38***	113 ± 16*
MT-ODC/MT-SSAT	3918 ± 1395*	9268 ± 1818**	35 ± 7	7484 ± 239**	253 ± 22*	166 ± 9**	72 ± 7

Table 2 Liver polyamine metabolism in MT-ODC/MT-SSAT transgenic mice with 2 days of DENSPM treatment

Data are means ± S.E.M. where $n = 4$–8. Animals were 9-week-old male mice. Enzyme activities, statistical significances and abbreviations are as expressed in Table 1.

	Enzyme activity			Polyamine pool (pmol/mg of tissue)			
Animal and treatment	SSAT	ODC	AdoMetDC	Putrescine	Ac-Spermidine	Spermidine	Spermine
Untreated							
Tg (−)	0 ± 0	0 ± 0	32 ± 4	23 ± 1	< 7	1239 ± 87	1041 ± 34
MT-ODC	0 ± 0	49 ± 6	25 ± 1	150 ± 22	< 7	1379 ± 51	982 ± 20
MT-SSAT	4 ± 1	37 ± 8	55 ± 6	604 ± 87	125 ± 49	937 ± 43	145 ± 18
MT-ODC/MT-SSAT	33 ± 11	2552 ± 508	62 ± 11	5561 ± 583	199 ± 19	420 ± 100	54 ± 16
DENSPM-treated							
Tg (−)	0 ± 0	4 ± 3	26 ± 2	51 ± 11	< 7	779 ± 42**	900 ± 36*
MT-ODC	0 ± 0	102 ± 11**	39 ± 5*	363 ± 35***	< 7	1182 ± 66*	816 ± 26***
MT-SSAT	168655 ± 50848*	65 ± 8**	44 ± 8	302 ± 41*	120 ± 5	40 ± 10***	25 ± 7**
MT-ODC/MT-SSAT	49350 ± 14053*	2199 ± 202	57 ± 4	3964 ± 309*	169 ± 17	77 ± 15**	13 ± 8*

pressor of ODC, significantly induced ODC activity in both MT-ODC and MT-SSAT animals (Table 2). In the MT-SSAT mice, administration of DENSPM immensely (more than 40000-fold) enhanced SSAT activity and led to a virtually complete disappearance of hepatic spermidine and spermine (Table 2). Likewise, DENSPM induced SSAT in the doubly transgenic mice, yet the induction was not as striking as seen in the MT-SSAT animals.

Role of ODC antizyme

We subsequently measured the level of hepatic ODC antizyme in the wild-type and singly and doubly transgenic mice. As shown in Table 3, antizyme contents were only marginally and not significantly different between the animal groups.

Table 3 The level of hepatic ODC antizyme

Data are means ± S.E.M. ($n = 4$); 12-week-old male mice were used. Tg (−), non-transgenic control animals.

Animal	Amount of antizyme (pg/mg of protein)
Tg (−)	42 ± 2
MT-ODC	50 ± 4
MT-SSAT	38 ± 3
MT-ODC/MT-SSAT	38 ± 2

Hepatic retention of radioactive spermidine in syngenic and transgenic mice

In attempts to elucidate the mechanism of the additive catabolic effect seen in the hybrid mice, we injected radioactive spermidine into the animals and measured its hepatic retention over a period of 4 h. As seen in Figure 1, the syngenic animals continued to accumulate the tracer over the whole period of observation. However, the transgenic mice, although initially accumulating the label as well as or better than the syngenic mice, either halted accumulation after 15 min or started to lose the radioactivity (hybrid mice). This phenomenon can be understood from the point of view that the transgenic mice possessed an effective efflux system for the polyamine. In fact, 4 h after the injection of the tracer the singly transgenic mice had retained less than half of the radioactivity found in syngenic mice and the doubly transgenic animals approx. 20 % of that value (Figure 1). Interestingly, the loss of radioactivity in the hybrid mice was roughly the sum of the losses in the singly transgenic animals. It is also remarkable that in the MT-ODC animals this enhanced spermidine efflux occurred in the absence of SSAT activity. Table 4

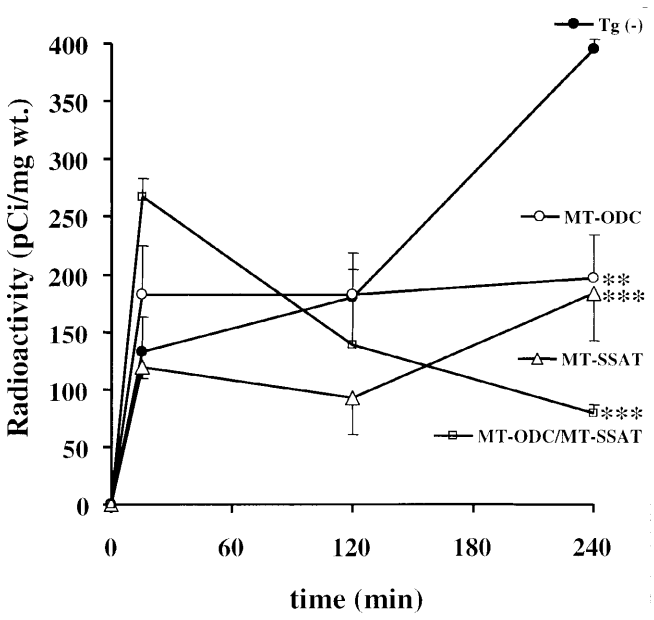

Figure 1 Uptake and retention of radioactive spermidine in the livers of syngenic and transgenic animals

Animals received 1 μCi of [^{14}C]spermidine as an intraperitoneal injection and three animals in each group were killed at the time points indicated. Means ± S.E.M. are shown; $^{**}P < 0.01$ and $^{***}P < 0.001$ in comparison with the syngenic group. Tg (−), non-transgenic control animals.

Table 4 The distribution of radioactivity in hepatic tissue 15 min after injection of radioactive spermidine

Data are means ± S.E.M. where $n = 3$ in each group. Tg (−), non-transgenic control animals.

	Distribution of radioactivity (%)	
	N^1-Acetylspermidine	Spermidine + spermine
Tg (−)	< 1	99 ± 0
MT-ODC	2 ± 0	98 ± 1
MT-SSAT	2 ± 1	95 ± 1
MT-ODC/MT-SSAT	29 ± 6	70 ± 6

indicates that while most of the spermidine-derived radioactivity was in the higher polyamine fraction in the syngenic and singly transgenic animals, at 15 min a substantial portion was already converted into acetyl-spermidine in the hybrid mice. Similar measurements at later time points indicated that spermidine was effectively converted into acetyl-spermidine and putrescine in MT-SSAT and hybrid mice whereas acetyl-spermidine really never accumulated in syngenic and MT-ODC animals. This can be understood on the terms that in the MT-ODC animals spermidine efflux occurred without prior acetylation.

Shortened life span of the hybrid mice

As described previously, one of the most prominent phenotypic changes associated with SSAT overexpression is permanent loss of hair [10]. In transgenic animals overexpressing SSAT under its own promoter the loss of hair occurred at the age of about 4 weeks [10] whereas in MT-SSAT animals this occurred later, at 13–14 weeks [11]. MT-ODC mice showed normal hair growth.

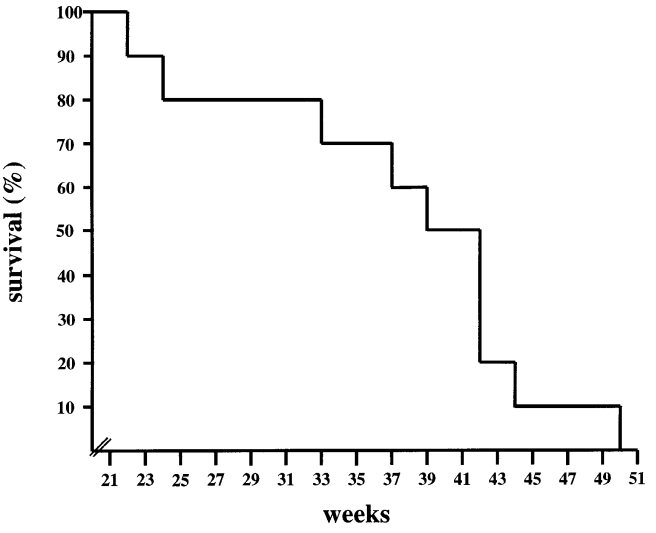

Figure 2 Long-term survival of the doubly transgenic mice (MT-ODC/MT-SSAT; $n = 10$)

The doubly transgenic mice used in this study likewise permanently lost their hair at the age of 8–9 weeks, i.e. earlier than MT-SSAT animals. The doubly transgenic mice also showed more severe histopathological changes in their skin than MT-SSAT animals. None of the untreated transgenic lines showed overt organ-specific histopathological changes; however, as depicted in Figure 2, the life span of the doubly transgenic mice was remarkably short. The median life span of the doubly transgenic animals was only 39 weeks whereas, according to our earlier experience, about 50 % of non-transgenic or ODC-overexpressing mice of the same BALBc × DBA/2 background were still alive after 2 years [22].

DISCUSSION

A forced expression of ODC in transgenic rodents leads to a marked over-accumulation of putrescine in most tissues, yet it appears obvious that this diamine is not metabolized further into higher polyamines under these conditions [6–8]. A distortion of the homoeostasis of the higher polyamines is thus not easily achievable through an activation of polyamine biosynthesis in whole animals. On the other hand, an activation of polyamine catabolism through the overexpression of SSAT in transgenic rodents resulted in profound alterations of tissue polyamine pools as illustrated by an accumulation of unphysiological amounts of putrescine, appearance of N^1-acetylspermidine and decreases in tissue spermidine and/or spermine pools [10,11,23]. The activation of polyamine catabolism in transgenic rodents brings about interesting phenotypic changes not readily explainable by distorted polyamine metabolism. These include permanent loss of hair, female infertility and a lack of subcutaneous fat deposits [10]. However, the finding that overexpression of SSAT rendered the animals extremely sensitive to the toxic actions of polyamine analogues, such as DENSPM, is directly attributable to an immense stimulation of SSAT activity and subsequent profound depletion of spermidine and spermine pools in response to the drug [11,14]. In the liver, the toxicity of the analogue seemed to be targeted to mitochondria [11]. Similarly, transgenic rats harbouring the SSAT transgene under

the control of the MT promoter responded to zinc administration by a dramatic induction of pancreatic SSAT, near-complete depletion of pancreatic spermidine and spermine pools and development of acute pancreatitis [13].

The present results obtained with the doubly transgenic mouse line overexpressing both ODC and SSAT brought about new and interesting information on the homoeostasis of the polyamines. Forced expression of ODC in SSAT-overexpressing transgenic mice led, entirely unexpectedly, not to the replenishment of the depleted pools of higher polyamines but instead to a further enhancement of their catabolism. The latter was exemplified by the fact that in the doubly transgenic animals the combined hepatic pool of spermidine and spermine was further reduced by 60 % from that in SSAT-overexpressing mice and this occurred in the presence of extremely high putrescine concentrations. It thus appears that under these conditions putrescine does not serve as a natural precursor for the synthesis of spermidine. Obviously, this could be related partly to the fact that a tremendous excess of putrescine just swamps the capacity of AdoMetDC to provide decarboxylated *S*-adenosylmethionine for the synthesis of spermidine. This does not, however, explain why overexpression of ODC further enhances SSAT-dependent catabolism of the higher polyamines. The labelling experiments with radioactive spermidine indicated distinctly that both the singly and doubly transgenic animals were not able to retain exogenous spermidine in their livers to the extent seen in the syngenic animals. In the case of SSAT overexpression this is easy to understand as acetylated spermidine is expected to be excreted rapidly. However, even though ODC-overexpressing animals were in this sense quantitatively fully comparable with the SSAT-overexpressing mice, they did not show any enhanced SSAT activity. A similar polyamine 'exclusion' system appears to be operating in the ODC transgenics, as in the hybrid animals the inability to retain spermidine in the liver was, in fact, the sum of the inabilities found in singly transgenic animals. Although inhibition of polyamine uptake cannot be excluded as the reason for the impaired hepatic retention of spermidine in the transgenic animals, the fact that the initial uptake (15 min) of the polyamine in the transgenic animals was faster than or similar to that in the syngenic animals would suggest that after a certain lag period an efflux component was activated in the transgenic animals. This was seen in the doubly transgenic animals as a loss of the already accumulated radioactivity and in the singly transgenic animals as a steady-state condition where influx and efflux rates were similar and, unlike in the syngenic animals, no net accumulation of the tracer occurred. It is therefore highly likely that any 'nascent' spermidine that has been taken up or formed through biosynthesis is similarly excluded in the transgenic animals. We recently showed that in SSAT-overexpressing cultured cells it is impossible to replenish depleted spermidine and spermine pools by the addition of the polyamines to the culture medium, as the exogenous polyamines are immediately and quantitatively acetylated [24]. The present results likewise strongly suggest that the major aim of the machinery regulating polyamine metabolism is to prevent an over-accumulation of the higher polyamines.

Our present results do not apparently assign any central role to ODC antizyme in the regulation of polyamine homoeostasis under present experimental conditions. ODC antizyme is a regulatory protein that binds to ODC and is induced by an excess of the polyamines [25]. Antizyme not only inhibits ODC activity but also facilitates its degradation by 26 S proteasome [26] and inhibits polyamine uptake [27]. In spite of the very high ODC activity and striking over-accumulation of putrescine, the antizyme level only increased insignificantly in the doubly transgenic mice (Table 3). This is in striking contrast with a report describing a cell line over-producing stable ODC [28]. Under these conditions, the amount of antizyme was increased by a factor of more than 50 in comparison with the parental cell line [28]. However, the doubly transgenic mice had profoundly reduced hepatic pools of spermidine and spermine, a condition that is supposed to inhibit the expression of the antizyme [29]. In any event, an antizyme-based regulatory system is not able to correct the highly distorted polyamine homoeostasis under these conditions. Moreover, the present results likewise indicate that a constitutive activation of polyamine catabolism in transgenic mice considerably shortens the life span of the animals.

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REFERENCES

1 Halmekytö, M., Hyttinen, J.-M., Sinervirta, R., Utriainen, M., Myöhänen, S., Voipio, H.-M., Wahlfors, J., Syrjänen, S., Syrjänen, K., Alhonen, L. and Jänne, J. (1991) Transgenic mice aberrantly expressing human ornithine decarboxylase gene. J. Biol. Chem. **266**, 19746–19751

2 Hakovirta, H., Keiski, A., Toppari, J., Halmekytö, M., Alhonen, L., Jänne, J. and Parvinen, M. (1993) Polyamines and regulation of spermatogenesis: selective stimulation of late spermatogonia in transgenic mice overexpressing the human ornithine decarboxylae gene. Mol. Endocrinol. **7**, 1430–1436

3 Halonen, T., Sivenius, J., Miettinen, R., Halmekytö, M., Kauppinen, R., Sinervirta, R., Alakuijala, L., Alhonen, L., MacDonald, E. et al. (1993) Elevated seizure threshold and impaired spatial learning in transgenic mice with putrescine overproduction in the brain. Eur. J. Neurosci. **5**, 1233–1239

4 Lukkarinen, J. A., Kauppinen, R. A., Gröhn, O. H. J., Oja, J. M. E., Sinervirta, R., Alhonen, L. I. and Jänne, J. (1998) Neuroprotective role of ornithine decarboxylase activation in transient cerebral focal ischemia: a study using ornithine decarboxylase-overexpressing transgenic rats. Eur. J. Neurosci. **10**, 2046–2055

5 Halmekytö, M., Syrjänen, K., Jänne, J. and Alhonen, L. (1992) Enhanced papilloma formation in response to skin tumor promotion in transgenic mice overexpressing the human ornithine decarboxylase gene. Biochem. Biophys. Res. Commun. **187**, 493–497

6 Halmekytö, M., Alhonen, L., Wahlfors, J., Sinervirta, R., Eloranta, T. and Jänne, J. (1991) Characterization of a transgenic mouse line over-expressing the human ornithine decarboxylase gene. Biochem. J. **278**, 895–898

7 Halmekytö, M., Alhonen, L., Alakuijala, L. and Jänne, J. (1993) Transgenic mice over-producing putrescine in their tissues do not convert the diamine into higher polyamines. Biochem. J. **291**, 505–508

8 Heljasvaara, R., Veress, I., Halmekytö, M., Alhonen, L., Jänne, J., Laajala, P. and Pajunen, A. (1997) Transgenic mice over-expressing ornithine and S-adenosylmethionine decarboxylases maintain a physiological polyamine homeostasis in their tissues. Biochem. J. **323**, 457–462

9 Casero, R. A. and Pegg, A. E. (1993) Spermidine/spermine N1-acetyltransferase – the turning point in polyamine metabolism. FASEB J. **7**, 653–661

10 Pietilä, M., Alhonen, L., Halmekytö, M., Kanter, P., Jänne, J. and Porter, C. W. (1997) Activation of polyamine catabolism profoundly alters tissue polyamine pools and affects hair growth and female fertility in transgenic mice overexpressing spermidine/spermine N^1-acetyltransferase. J. Biol. Chem. **272**, 18746–18751

11 Suppola, S., Pietilä, M., Parkkinen, J. J., Korhonen, V. P., Alhonen, L., Halmekytö, M., Porter, C. W. and Jänne, J. (1999) Overexpression of spermidine/spermine N1-acetyltransferase under the control of mouse metallothionein I promoter in transgenic mice: evidence for a striking post-transcriptional regulation of transgene expression by a polyamine analogue. Biochem. J. **338**, 311–316

12 Kaasinen, K., Koistinaho, J., Alhonen, L. and Jänne, J. (2000) Overexpression of spermidine/spermine N^1-acetyltransferase in transgenic mice protects the animals from kainate-induced toxicity. Eur. J. Neurosci. **12**, 540–548

13 Alhonen, L., Parkkinen, J. J., Keinänen, T., Sinervirta, R., Herzig, K. H. and Jänne, J. (2000) Activation of polyamine catabolism in transgenic rats induces acute pancreatitis. Proc. Natl. Acad. Sci. U.S.A. **97**, 8290–8295

14 Alhonen, L., Pietilä, M., Halmekytö, M., Kramer, D. L., Jänne, J. and Porter, C. W. (1999) Transgenic mice with activated polyamine catabolism due to overexpression of spermidine/spermine N^1-acetyltransferase show enhanced sensitivity to the polyamine analog, N^1,N^{11}-diethylnorspermine. Mol. Pharmacol. **55**, 693–698

15 Alhonen, L., Heikkinen, S., Sinervirta, R., Halmekytö, M., Alakuijala, P. and Jänne, J. (1996) Transgenic mice expressing the human ornithine decarboxylase gene under the control of mouse metallothionein I promoter. Biochem. J. **314**, 405–408

16 Rehse, K., Puchert, E. and Leissring, S. (1990) Alkyl- und Arylalkyderivate von Putrescin, Spermidin und Spermin. Arch. Pharmacol. (Weinheim) **323**, 287–294
17 Hyvönen, T., Keinänen, T. A., Khomutov, A. R., Khomutov, R. M. and Eloranta, T. O. (1992) Monitoring of the uptake and metabolism of aminooxy analogues of polyamines in cultured cells by high-performance liquid chromatography. J. Chromatogr. **574**, 17–21
18 Jänne, J. and Williams-Ashman, H. G. (1971) On the purification of L-ornithine decarboxylase from rat prostate and effects of thiol compounds on the enzyme. J. Biol. Chem. **246**, 1725–1732
19 Jänne, J. and Williams-Ashman, H. G. (1971) Dissociation of putrescine-activated decarboxylation of S-adenosyl-L-methionine from the enzymic synthesis of spermidine and spermine by purified prostatic enzyme preparations. Biochem. Biophys. Res. Commun. **42**, 222–229
20 Bernacki, R. J., Oberman, E. J., Seweryniak, K. E., Atwood, A., Bergeron, R. J. and Porter, C. W. (1995) Preclinical antitumor efficacy of the polyamine analogue N^1,N^{11}-diethylnorspermine administered by multiple injections or continuous infusion. Clin. Cancer Res. **1**, 847–857
21 Kankare, K., Uusi-Oukari, M. and Jänne, O. A. (1997) Structure, organization and expression of the mouse ornithine decarboxylase antizyme gene. Biochem. J. **324**, 807–813
22 Alhonen, L., Halmekytö, M., Kosma, V.-M., Wahlfors, J., Kauppinen, R. and Jänne, J. (1995) Life-long over-expression of ornithine decarboxylase (ODC) gene in transgenic mice does not lead to generally enhanced tumorigenesis or neuronal degeneration. Int. J. Cancer **63**, 402–404
23 Alhonen, L., Karppinen, A., Uusi-Oukari, M., Vujcic, S., Korhonen, V.-P., Halmekytö, M., Kramer, D. L., Hines, R., Jänne, J. and Porter, C. W. (1998) Correlation of polyamine and growth responses to N^1,N^{11}-diethylnorspermine in primary fetal fibroblasts derived from transgenic mice overexpressing spermidine/spermine N^1-acetyltransferase. J. Biol. Chem. **273**, 1964–1969
24 Vujcic, S., Halmekytö, M., Diegelman, P., Gan, G., Kramer, D. L., Jänne, J. and Porter, C. W. (2000) Effects of conditional overexpression of spermidine/spermine N1-acetyltransferase on polyamine pool dynamics, cell growth, and sensitivity to polyamine analogs. J. Biol. Chem. **275**, 38319–38328
25 Heller, J. S., Fong, W. F. and Canellakis, E. S. (1976) Induction of a protein inhibitor to ornithine decarboxylase by the end products of its reaction. Proc. Natl. Acad. Sci. U.S.A. **73**, 1858–1862
26 Hayashi, S., Murakami, Y. and Matsufuji, S. (1996) Ornithine decarboxylase antizyme: a novel type of regulatory protein. Trends Biochem. Sci. **21**, 27–30
27 Mitchell, J. L. A., Judd, G. G., Bareyal-Leyser, A. and Ling, S. Y. (1994) Feedback repression of polyamine transport is mediated by antizyme in mammalian tissue-culture cells. Biochem. J. **299**, 19–22
28 Mitchell, J. L., Choe, C. Y., Judd, G. G., Daghfal, D. J., Kurzeja, R. J. and Leyser, A. (1996) Overproduction of stable ornithine decarboxylase and antizyme in the difluoromethylornithine-resistant cell line DH23b. Biochem. J. **317**, 811–816
29 Nilsson, J., Koskiniemi, S., Persson, K., Grahn, B. and Holm, I. (1997) Polyamines regulate both transcription and translation of the gene encoding ornithine decarboxylase antizyme in mouse. Eur. J. Biochem. **50**, 223–231

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