Transgenic mice over-expressing the human spermidine synthase gene

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We have generated a transgenic mouse line harbouring the functional (chromosome-1-derived) human spermidine synthase (EC 2.5.1.16) gene in their genome. The transgenic animals expressed the human gene-derived mRNA, as revealed by reverse-transcriptase/PCR analysis, in all tissues studied and displayed tissue spermidine synthase activity that was 2–6 times that in their syngenic littermates. The elevated spermidine synthase activity, however, had virtually no effect on tissue putrescine, spermidine or spermine levels. The view that the accumulation of spermidine and spermine is possibly controlled by *S*-adenosylmethionine decarboxylase was further supported by the finding that tissue spermidine and spermine contents also remained practically normal in hybrid transgenic mice over-expressing both human ornithine decarboxylase and spermidine synthase genes.

INTRODUCTION

The biosynthesis of the natural polyamines (putrescine, spermidine and spermine) in mammalian tissues involves the concerted action of four separate enzymes: ornithine decarboxylase (EC 4.1.1.17), *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22). The two decarboxylases are in all likelihood the rate-controlling enzymes of the biosynthetic pathway of the polyamines. As these decarboxylases are also strikingly inducible enzymes, with very short biological half-life, it is no wonder that ornithine and adenosylmethionine decarboxylases have been subjects of extensive investigations (Jänne et al., 1978; Pegg, 1986). The regulation of spermidine and spermine synthases is believed to occur at the level of the availability of their common substrate, decarboxylated adenosylmethionine (Jänne et al., 1978; Pegg, 1986). Although these propylamine transferases are not commonly considered as inducible enzymes, there are reports showing that spermidine synthase activity responds to mitogenic stimuli, such as lectin-induced lymphocyte activation (Korpela et al., 1981), hormone-induced tissue proliferation (Oka et al., 1977; Käpyaho et al., 1980) and compensatory growth of rodent liver (Hannonen et al., 1972).

We recently sequenced the cDNA encoding human spermidine synthase (Wahlfors et al., 1990) and subsequently isolated and sequenced a human chromosome-1-derived functional spermidine synthase gene (Myöhänen et al., 1991). With the aid of these genomic sequences we have now generated a transgenic mouse line over-expressing the human spermidine synthase gene. Analyses of tissue polyamine pools of the transgenic mice revealed no overt changes, emphasizing the important role of adenosylmethionine decarboxylase in the control of the accumulation of tissue spermidine and spermine. This view was further strengthened by the observation that tissue spermidine and spermine pools remained unaltered in tissues of members of a hybrid transgenic mouse line over-expressing both ornithine decarboxylase and spermidine synthase.

EXPERIMENTAL

Transgenic animals

The standard pronuclear microinjection technique (Hogan et al., 1986) was used to generate transgenic mice. Fertilized oocytes were obtained from superovulated BALB/c × DBA/2 females mated with males of the same strain. The transgene construct was human spermidine synthase gene (Myöhänen et al., 1991) containing all the exons (8) and intervening sequences (7) together with some 3000 nucleotides of the 5′ flanking and 500 nucleotides of the 3′ flanking region. Transgenic animals were identified by PCR using human specific primers recognizing intron sequences (Myöhänen et al., 1991). F1 or F2 offspring of a founder animal designated K43 were used in all experiments. Syngenic littermates served as controls. A hybrid transgenic mouse line was obtained by mating K43 mice with transgenic mice over-expressing the human ornithine decarboxylase gene (line K2; Halmekytö et al., 1991a,b).

Analytical methods

Total RNA was isolated by the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). Human-specific spermidine synthase mRNA was detected and quantified with the combined use of reverse transcriptase and PCR. The primers for polymerase chain reaction, 5′-CCTACTGCACCATCCCACCTAC-3′ and 5′-GTGCCTCGGCCCCTCCAGCCC-3′, recognized sequences in exons 6 (coding region) and 8 (non-coding region). The combined reverse transcriptase and PCR were carried out essentially as described by Halmekytö et al. (1991b).

The activities of ornithine decarboxylase (Jänne and Williams-Ashman, 1971a) and adenosylmethionine decarboxylase (Jänne and Williams-Ashman, 1971b) were assayed by published methods. The activities of spermidine and spermine synthases were determined by the method of Raina et al. (1983). The activity of spermidine/spermine acetyltransferase was assayed by the method of Matsui et al. (1981).

The concentrations of the polyamines were determined by h.p.l.c. as described in detail by Hyvönen et al. (1992).

The two-tailed *t* test was used for statistical analyses.

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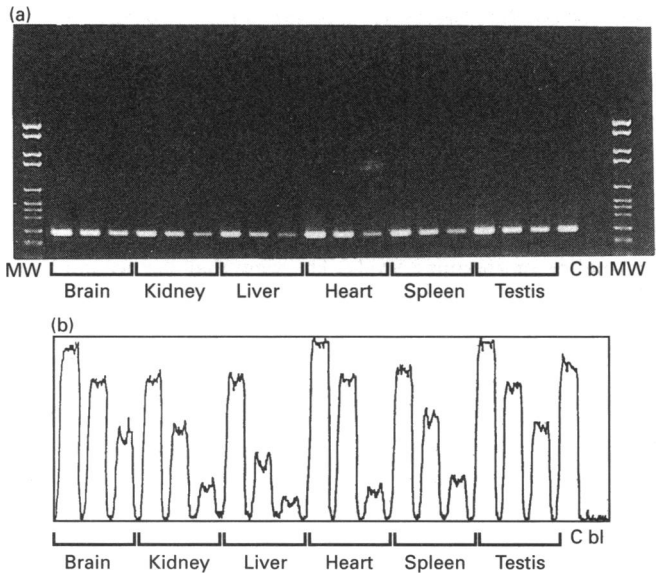

Figure 1 Detection of human spermidine synthase mRNA in tissues of transgenic male mouse by combined reverse-transcriptase/PCR assay

(**a**) Total RNA was isolated from the tissues and used as the template for reverse transcriptase. The cDNA was amplified by using human mRNA specific primers. The dilutions used for each tissue were: undiluted (left); 1:5 (middle); 1:15 (right). Abbreviations: bl, blank (no cDNA synthesis); C, control (total RNA from human Sultan myeloma cells); mw, molecular-size markers (pBR328 digested with *Bgl*I and *Hinf*I). (**b**) Scanning image of the PCR signals from (**a**).

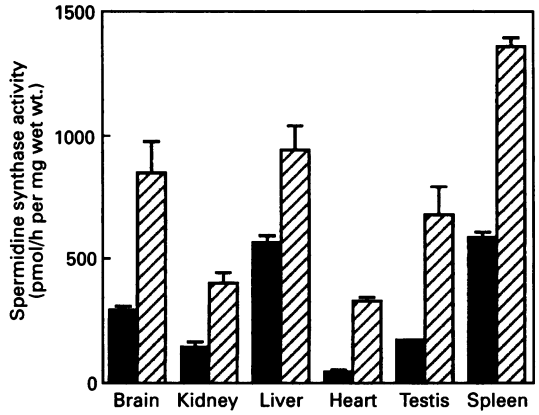

Figure 2 Spermidine synthase activity in tissues of non-transgenic (black bars) and transgenic (hatched bars) male mice

Values are means ± S.D. of three animals.

Chemicals

L-[1-^{14}C]Ornithine (sp. radioactivity 55 Ci/mol) and [1-^{14}C]acetyl-CoA (sp. radioactivity 50 Ci/mol) were purchased from Amersham International (Amersham, Bucks., U.K.). Radioactive *S*-adenosylmethionine was prepared by the method of Pegg and Williams-Ashman (1968) and decarboxylated adenosylmethionine by the method of Pösö et al. (1976). Deoxyribonucleoside triphosphates were obtained from Pharmacia LKB (Uppsala, Sweden).

RESULTS

Using the functional human spermidine synthase gene, we generated two transgenic founder animals. Analysis of spermidine synthase activity in tissues of their offspring indicated that only one of the founders expressed the gene. A combined reverse-transcriptase/PCR analysis of tissues of the transgenic mice revealed the accumulation of human-specific spermidine synthase mRNA in all of the tissues studied as depicted in Figure 1(a). Figure 2 shows that the transgenic animals exhibited a much higher tissue spermidine synthase activity than did their non-transgenic littermates. However, if one compares the amounts of the serially diluted (left, undiluted; middle, 1:5 dilution; right, 1:15 dilution) cDNAs in Figure 1(a) and the enzyme activities (or the apparent contribution of the transgene to the enzyme activities) in Figure 2, it is obvious that very little, if any, correlation existed between the two variables. This is exemplified by the fact that highest transgene-derived spermidine synthase activity was found in spleen, yet scanning the corresponding cDNA signals in Figure 1(b) revealed that the amount of spleen

Table 1 Enzyme activities involved in the metabolism of the polyamines in tissues of transgenic and non-transgenic mice

Results are means ± S.D. from three animals in the group. tg−, non-transgenic; tg+, transgenic. Activities are expressed as pmol/h per mg tissue wet wt. (n.d., not determined). Significance of the differences: $^{**}P < 0.01$; $^{***}P < 0.001$.

Tissue		Ornithine decarboxylase	Adenosylmethionine decarboxylase	Spermidine synthase	Spermine synthase	Spermidine/spermine acetyltransferase
Testis	tg−	12 ± 3	41 ± 4	174 ± 4	72 ± 9	104 ± 13
	tg+	20 ± 9	49 ± 8	680 ± 110***	82 ± 15	108 ± 14
Brain	tg−	0.1 ± 0.1	45 ± 6	299 ± 15	154 ± 15	63 ± 3
	tg+	0.2 ± 0.2	44 ± 6	847 ± 127**	148 ± 24	64 ± 8
Spleen	tg−	3 ± 1	n.d.	588 ± 24	185 ± 10	86 ± 13
	tg+	3 ± 1	n.d.	1358 ± 34***	197 ± 3	100 ± 14
Heart	tg−	2 ± 1	7 ± 2	50 ± 6	85 ± 23	0.7 ± 1
	tg+	1 ± 1	10 ± 3	336 ± 8***	90 ± 16	3 ± 2
Kidney	tg−	30 ± 14	n.d.	151 ± 21	124 ± 15	70 ± 5
	tg+	27 ± 12	n.d.	403 ± 45***	156 ± 14	76 ± 13
Liver	tg−	0.2 ± 0.2	n.d.	566 ± 25	71 ± 19	47 ± 22
	tg+	0.3 ± 0.5	n.d.	943 ± 100**	77 ± 21	32 ± 5

Table 2 Concentrations of putrescine, spermidine and spermine in tissues of transgenic and non-transgenic mice

K43, transgenic mouse line over-expressing human spermidine synthase gene; K2/K43, hybrid transgenic mouse line over-expressing both ornithine decarboxylase (K2) and spermidine synthase (K43) genes. Results are means ± S.D. from four (non-transgenic and K2/K43) or five (K43) animals in the group. Significance of differences: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Tissue	Line	Concn. (nmol/g wet wt.)			Spermidine/spermine
		Putrescine	Spermidine	Spermine	
Brain	Non-transgenic	29 ± 1	287 ± 61	401 ± 39	0.71 ± 0.12
	K43	< 20	254 ± 94	340 ± 56	0.73 ± 0.14
	K2/K43	118 ± 37*	356 ± 106	350 ± 108	1.02 ± 0.08
Heart	Non-transgenic	20 ± 8	207 ± 50	380 ± 35	0.55 ± 0.16
	K43	< 20	207 ± 79	398 ± 48	0.52 ± 0.16
	K2/K43	59 ± 28*	289 ± 135	399 ± 114	0.70 ± 0.15
Liver	Non-transgenic	28 ± 9	1051 ± 217	614 ± 47	1.71 ± 0.35
	K43	< 20	1154 ± 249	601 ± 62	1.91 ± 0.25
	K2/K43	44 ± 19	1296 ± 395	477 ± 103	2.69 ± 0.34**
Kidney	Non-transgenic	41 ± 22	441 ± 77	866 ± 97	0.52 ± 0.12
	K43	37 ± 14	393 ± 122	848 ± 156	0.46 ± 0.07
	K2/K43	48 ± 26	555 ± 127	699 ± 111	0.79 ± 0.06**
Spleen	Non-transgenic	96 ± 38	1121 ± 210	865 ± 134	1.31 ± 0.28
	K43	77 ± 32	1473 ± 177*	726 ± 73	2.06 ± 0.43*
	K2/K43	100 ± 49	1229 ± 381	553 ± 60*	2.19 ± 0.43*
Testis	Non-transgenic	22 ± 9	430 ± 135	681 ± 92	0.62 ± 0.14
	K43	25 ± 19	432 ± 79	575 ± 35*	0.75 ± 0.11
	K2/K43	536 ± 21***	684 ± 125*	438 ± 62**	1.56 ± 0.08***

spermidine synthase mRNA was less than one-fifth of that found in brain.

We also determined the activities of the enzymes involved in the biosynthesis (ornithine decarboxylase, adenosylmethionine decarboxylase and spermine synthase) and in the catabolism (spermidine/spermine acetyltransferase) of the polyamines in tissues of transgenic and non-transgenic animals. As shown in Table 1, there were no differences between syngenic and transgenic animals in any of the tissues studied.

Table 2 lists putrescine, spermidine and spermine concentrations in selected tissues of control mice, of transgenic mice harbouring the human spermidine synthase gene (line K43), and of hybrid transgenic mice obtained by mating the transgenic mouse line K43 with the transgenic mouse line K2 that over-expresses the human ornithine decarboxylase gene (Halmekytö et al., 1991a,b). The over-expression of the spermidine synthase gene (line K43) only appeared to have a marginal effect on the tissue polyamine pools (Table 2). In the hybrid transgenic mice (line K2/K43), the testicular concentration of spermidine was significantly increased and that of spermine significantly decreased. The changes, however, were not dramatic and can be occasionally found in testis of animals of the K2 line (Halmekytö et al., 1991c). The over-expression of both spermidine synthase and ornithine decarboxylase distinctly increased the molar ratio of spermidine to spermine in most tissues.

DISCUSSION

The propylamine transferases, spermidine and spermine synthases, are commonly considered to be metabolically stable enzymes that are expressed constitutively and display modest inducibility (Jänne et al., 1978; Pegg, 1986). In most mammalian tissues these enzymes appear to occur in large excess in comparison with the two decarboxylases, and their activity seems to be limited by the availability of their common substrate, decarboxylated adenosylmethionine. There are, however, reports indicating that under certain anabolic conditions spermidine synthase, but not spermine synthase, can be substantially induced. The best example of the latter conditions is the activation of peripheral human lymphocytes in response to an exposure to lectins. Spermidine synthase activity was increased more than 10-fold after a phytohaemagglutinin exposure of 48 h (Korpela et al., 1981). The extent of this stimulation was fully comparable with the changes found in the activities of ornithine and adenosylmethionine decarboxylases, and was believed to contribute to the swift accumulation of spermidine during lymphocyte activation (Korpela et al., 1981). Our present results with transgenic mice over-expressing spermidine synthase seem to indicate that grossly elevated tissue activity of the latter enzyme had only a marginal effect on tissue spermidine and spermine concentrations, as did the combined over-expression of ornithine decarboxylase and spermidine synthase in the transgenic hybrid mice (Table 2). This finding may indicate that adenosylmethionine decarboxylase plays a critical role in the regulation of the tissue pools of the higher polyamines spermidine and spermine. This straightforward view is, however, complicated by the observation indicating that it is not the content of the enzyme itself, but probably the ambient concentration of putrescine, that regulates the rate of adenosylmethionine decarboxylation (Hölttä and Jänne, 1972). Our earlier tissue polyamine analyses in transgenic animals over-expressing ornithine decarboxylase revealed that, in spite of a massive putrescine accumulation achieved by inhibiting the utilization of L-ornithine by ornithine transaminase, the diamine was not apparently converted into the higher polyamines (Halmekytö et al., 1993). Remarkably, this was not due to depressed adenosylmethionine decarboxylase activity (Halmekytö et al., 1993). These observations are, in fact, in line with the recently expressed views suggesting that the major function of the polyamine homoeostatic system is to prevent the accumulation of toxic levels of spermidine and spermine in quiescent cells (Davis et al., 1992).

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