Transgenic mice expressing the human ornithine decarboxylase gene under the control of mouse metallothionein I promoter

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We have generated a transgenic mouse line harbouring the human ornithine decarboxylase gene under the control of mouse metallothionein I promoter. Even in the absence of an exposure to heavy metals, ornithine decarboxylase was over-expressed in heart, testis, brain, and especially in liver, of the transgenic animals. An exposure of the transgenic mice to zinc further enhanced the enzyme activity to a level which in liver represented up to 8000-fold increase in comparison with non-transgenic animals. The striking stimulation of liver ornithine decarboxylase activity upon treatment of the transgenic mice with zinc was accompanied by a nearly 150-fold increase in the hepatic

putrescine content as compared with similarly treated non-transgenic animals. Even though the liver putrescine concentration reached that of spermidine and spermine in the transgenic animals, the contents of the higher polyamines only transiently increased upon zinc administration and then returned to the basal level. These findings once again indicate that mammalian cells possess extremely powerful regulatory machinery to prevent an over-accumulation of spermidine and spermine in non-dividing cells, and that very high tissue putrescine concentrations can be tolerated, at least for periods of a few days, with seemingly no phenotypic changes.

INTRODUCTION

Over the past few years we have generated a number of transgenic mouse lines over-expressing the human ornithine decarboxylase gene [1] and the spermidine synthase gene [2]. Even though these animals displayed in particular tissues an ornithine decarboxylase activity that could be more than 100 times higher than in the corresponding tissues of their syngenic littermates, surprisingly few phenotypic changes were associated with the over-expression of the enzyme. In most tissues, the over-expression of ornithine decarboxylase was accompanied by grossly elevated putrescine contents, with no changes in spermidine and spermine concentrations [1,3]. Testicular tissue appeared to be an exception, as small but significant rises in spermidine concentrations were associated with the enhanced ornithine decarboxylase activity in transgenic male animals [1,4]. Whether the increase in testicular spermidine concentration was related to the observed toxic effects on spermatogenesis in transgenic males [1,4] is not known for certainty. The tissue polyamine pattern in transgenic mice over-expressing the spermidine synthase gene likewise remained unaltered [2]. The polyamine pools in hybrid transgenic mice over-producing both ornithine decarboxylase and spermidine synthase very much resembled those found in transgenic animals with ornithine decarboxylase over-expression, i.e. marked tissue putrescine accumulation as the sole change [2].

In the present study we have operationally fused human ornithine decarboxylase gene to metallothionein I promoter, which is widely used to achieve liver-specific transgene expression [5,6], in order to generate transgenic mice with heavy-metal-inducible expression of ornithine decarboxylase. As expected, the mouse metallothionein promoter directed the expression especially into liver, where a striking over-expression of the gene was found even in the absence of inducing heavy metals. A single intraperitoneal injection of ZnSO_4 further enhanced the enzyme activity and resulted in a massive accumulation of putrescine in

liver. However, the diamine was not converted into higher polyamines to any appreciable extent.

EXPERIMENTAL

The gene construct

Mouse metallothionein I promoter [5] was amplified from mouse genomic DNA by using 5'-ATGAGTCGACGGTTTAACTCC-TGGTTCTGG-3' and 5'-CGAGAGCGCTTGAAGCTGGAGCTACGG-3' as the primers for PCR. The amplified promoter (950 nucleotides) was subsequently cloned into pUC19 vector (New England Biolabs, Beverly, MA, U.S.A.) cleaved with *Sma*I restriction endonuclease and sequenced with the aid of an automated DNA sequencer (A.L.F.; Pharmacia, Uppsala, Sweden). Human ornithine decarboxylase gene [7] was cloned into pBluescript vector 11 KS (Stratagene, La Jolla, CA, U.S.A.) where its promoter and part of the first non-coding exon were replaced by mouse metallothionein I promoter. To retain a single *Eco*47III cleavage site in exon I, the ornithine decarboxylase gene was first cleaved with *Cla*I at the exon VI, followed by the fusion of the metallothionein promoter with the exon I at the *Eco*47III site and finally linking the rest of the ornithine decarboxylase gene back to the construct. The fusion gene construct is outlined in Figure 1.

Transgenic animals

The standard pronuclear microinjection technique [8] was used to generate transgenic mice. Fertilized oocytes were obtained from superovulated BALB/c $\times$ DBA/2 females mated with males of the same strain. Transgenic animals were identified by PCR. Female and male F1 offspring of the founder animal designated UKU48 were used in the experiments. The mice were fed on a standard pellet diet and had water *ad libitum*.

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Figure 1 Mouse metallothionein-human ornithine decarboxylase fusion gene used for the generation of transgenic mice

The open boxes represent the non-coding and the filled boxes coding exons of the ornithine decarboxylase gene. The cross-hatched box is the MT-I promoter region. MT-I, mouse metallothionein.

Analytical methods

The activity of ornithine decarboxylase was assayed by the method described in [9]. The concentrations of the polyamines were determined by h.p.l.c. essentially as described in [10].

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

Chemicals

Restriction endonucleases and other DNA-modifying enzymes, T4 DNA ligase, T4 DNA polymerase and Taq polymerase, were from Promega (Promega Corp., Madison, WI, U.S.A.), Boehringer Mannheim (Mannheim, Germany) or New England Biolabs. L-[1-¹⁴C]Ornithine (sp. radioactivity 55 Ci/mol) was purchased from Amersham International (Amersham, Bucks., U.K.).

RESULTS

The expression of the fusion gene in selected tissues of transgenic mice is shown in Table 1. With the exception of kidney and spleen, the transgene was over-expressed even without a prior exposure to the inducing zinc salt. An intraperitoneal injection of ZnSO₄ stimulated ornithine decarboxylase 2–15-fold in the tissues of the transgenic animals. With the possible exception of kidney, the endogenous ornithine decarboxylase was rather insensitive to the zinc salt (Table 1).

Table 1 Ornithine decarboxylase activities in tissues of non-transgenic and transgenic mice with or without zinc treatment

Results are means ± S.D. from three animals in the group. Activities were measured 4 h after an intraperitoneal administration of ZnSO₄ (20 mg/kg) and expressed as pmol/h per mg tissue wet wt. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, for significance of the differences between non-transgenic and transgenic animals.

Tissue	ZnSO ₄	Non-transgenic	Transgenic
Liver	—	0	108 ± 57***
	+	0.08 ± 0.11	654 ± 260**
Kidney	—	295 ± 139	244 ± 187
	+	767 ± 451	813 ± 339
Heart	—	0.68 ± 0.09	12.1 ± 2.1***
	+	0.57 ± 0.21	64.3 ± 11.6***
Spleen	—	7.9 ± 5.20	10.4 ± 6.6
	+	3.08 ± 1.81	158 ± 93*
Testis	—	5.90 ± 1.38	64.7 ± 13.8**
	+	6.74 ± 3.98	130 ± 23***
Brain	—	0.192 ± 0.017	4.62 ± 0.66***
	+	0.443 ± 0.627	15.2 ± 9.8*

Table 2 Tissue polyamines in non-transgenic and transgenic animals with or without zinc treatment

Results are means ± S.D. from three animals in the group. Polyamines were measured 4 h after an intraperitoneal administration of ZnSO₄ (20 mg/kg) and expressed as pmol/mg tissue wet wt. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, for significance of the differences between non-transgenic and transgenic animals.

Tissue	ZnSO ₄	Putrescine	Spermidine	Spermine
Liver	—	9.6 ± 8.7	988 ± 232	1174 ± 215
	+	9.3 ± 8.6	1003 ± 229	1160 ± 83
	—	257 ± 134*	1308 ± 217	1383 ± 170
	+	1345 ± 583*	1471 ± 181*	1685 ± 93**
Kidney	—	70 ± 9	552 ± 193	1197 ± 388
	+	75 ± 16	595 ± 69	1149 ± 94
	—	106 ± 10*	875 ± 71	1167 ± 67
	+	160 ± 102	701 ± 67	979 ± 49*
Heart	—	2.5 ± 2.9	186 ± 60	322 ± 15
	+	6.2 ± 4.1	152 ± 31	329 ± 25
	—	24 ± 1***	170 ± 11	293 ± 7
	+	57 ± 5***	159 ± 21	329 ± 7
Spleen	—	183 ± 95	1272 ± 170	798 ± 170
	+	199 ± 83	1212 ± 186	767 ± 249
	—	228 ± 116	1381 ± 44	845 ± 15
	+	478 ± 231	1363 ± 212	724 ± 132
Testis	—	52 ± 5	674 ± 60	1105 ± 130
	+	45 ± 4	643 ± 2	940 ± 34
	—	213 ± 27***	767 ± 15	910 ± 36
	+	238 ± 67**	857 ± 129	879 ± 16
Brain	—	3.5 ± 1.6	567 ± 47	388 ± 5
	+	6.0 ± 7.6	528 ± 58	417 ± 19
	—	78 ± 4***	603 ± 23	412 ± 28
	+	94 ± 13**	554 ± 38	463 ± 14

The concentrations of the polyamines in the same tissues of non-transgenic and transgenic animals without or with zinc treatment are depicted in Table 2. In all tissues studied, with the possible exception of spleen, the concentration of putrescine was increased in transgenic animals, even without the heavy-metal induction. An exposure to zinc further enhanced the accumulation of putrescine in transgenic animals (Table 2). The increase in putrescine content was most dramatic in liver of transgenic animals treated with zinc, as the pool of the diamine expanded nearly 150-fold in comparison with similarly treated non-

Figure 2 Effect of ZnSO₄ on liver polyamines in non-transgenic (A) and transgenic (B) animals

Results are means $\pm$ S.D. from three animals in the group. The animals received a single intraperitoneal injection of ZnSO₄ at zero time. Symbols: □, putrescine; ▲, spermidine; ●, spermine.

Table 3 Effects of heavy metals and dexamethasone on liver ornithine decarboxylase activity in non-transgenic and transgenic mice

Results are means $\pm$ S.D. from three animals in the group. Activities were measured 4 h after an intraperitoneal injection of ZnSO₄ (20 mg/kg), CoCl₂ (150 mmol/kg) or dexamethasone (5 mg/kg) and expressed as pmol/h per mg tissue wet wt. * $P < 0.05$, ** $P < 0.01$, for significance of the differences induced by the treatments within non-transgenic and transgenic groups.

Mice	Treatment	Ornithine decarboxylase activity
Non-transgenic	None	0.97 $\pm$ 0.64
	ZnSO ₄	5.2 $\pm$ 3.6
	CoCl ₂	5.8 $\pm$ 2.8*
	Dexamethasone	1.5 $\pm$ 0.6
Transgenic	None	50 $\pm$ 38
	ZnSO ₄	838 $\pm$ 257**
	CoCl ₂	96 $\pm$ 64
	Dexamethasone	37 $\pm$ 5

transgenic animals (Table 2). In fact, the concentration of putrescine reached that of spermidine and spermine. In the liver of transgenic animals treated with zinc, the concentrations of spermidine and spermine were also significantly elevated in comparison with syngenic animals (without or with the treatment). In the rest of the tissues, the concentrations of the higher polyamines remained unaltered in the transgenic animals (Table 2).

The time course of liver putrescine accumulation in response to ZnSO₄ in non-transgenic and transgenic mice is depicted in Figure 2. In non-transgenic animals (Figure 2A), the heavy metal caused negligible changes in the concentration of any of the polyamines. Except the time point 4 h, where the levels of spermidine and spermine were significantly increased, the massive hepatic putrescine accumulation in transgenic animals was not accompanied by changes in the higher polyamines at any further time points studied (Figure 2B).

Table 3 indicates that CoCl₂, but not zinc or the glucocorticoid dexamethasone, significantly stimulated the endogenous hepatic ornithine decarboxylase activity. In transgenic animals, only the

treatment with zinc resulted in a significant stimulation of liver ornithine decarboxylase (Table 3).

DISCUSSION

Mouse metallothionein I promoter is a regulatory sequence widely used to achieve a liver-specific expression of a transgene [5]. Mouse metallothionein promoter operationally fused with ovine growth-hormone gene has been used to induce hepatocellular cancer in transgenic mice [11]. Although the expression of metallothionein I gene is supposed to be restricted to the liver (and to a lesser extent to kidney), there exist several examples indicating that some transgenes under the control of metallothionein I promoter are expressed ectopically, either constitutively or inducibly, in tissues such as testis and brain [12,13]. As likewise indicated by the present results, the expression of the metallothionein-ornithine decarboxylase fusion gene was not restricted to liver, but a constitutive transgene expression was found in testis, brain and heart muscle and an inducible expression also in spleen. The very high constitutive expression in the liver may be related to dietary uptake of heavy metals in amounts sufficient to induce the promoter. In addition to the extremely high zinc-induced ornithine decarboxylase activity, liver is an exception from the rest of the tissues, as also the hepatic accumulation of putrescine was most strikingly enhanced in the transgenic animals (Table 2). This is in line with previous studies suggesting that the concentration of ornithine is the limiting factor for putrescine formation in most of the tissues [14]. Due to the active urea cycle, the concentration of ornithine appears to be much higher in liver than in other tissues [14], hence providing more saturating levels of substrate for putrescine generation.

Although intact human ornithine decarboxylase transgene is, among other tissues, also over-expressed in liver of transgenic animals [1,3], the expression rate was indeed modest in comparison with that of the present fusion gene, as illustrated by the up to 8000-fold increase in the hepatic enzyme activity as compared with non-transgenic mice.

The present transgenic mice can conceivably serve as useful experimental tools for the elucidation of possible consequences of a long-term exposure of the liver cells to extremely high concentrations of putrescine. It would also be of interest to know whether a continuous induction of ornithine decarboxylase can

create a metabolic imbalance of the distribution of ornithine between putrescine formation and the urea cycle.

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