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ACTIVATION OF TYROSINE AMINOTRANSFERASE EXPRESSION IN FETAL LIVER BY 5-AZACYTIDINE

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SUMMARY: Rat fetuses of 20 days gestational age were treated in utero with the inhibitor of DNA methylation, 5-azacytidine. The liver enzyme tyrosine aminotransferase, normally expressed at very low levels until several hours after birth, was increased by the drug in the fetal livers after a lag period of about 9 hours, reaching a level 70-fold above control levels 18 hours after treatment. The high levels attained after 5-azacytidine treatment are comparable to those of glucocorticoid-treated adult livers, and were not further increased by administration of hydrocortisone to dams carrying treated fetuses. Cytidine and two other analogs, cytosine arabinoside and 6-azacytidine, were essentially without effect.

As rat fetuses approach term their livers are experiencing rapid change, with hematopoietic elements undergoing involution while hepatocytes are increasing in number and size as well as undergoing differentiation-associated activation of expression of liver-specific functions (1). Tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) is one of a number of liver enzymes whose expression is markedly activated during the perinatal period (2, 3). Expression of this enzyme is largely suppressed prior to birth, both the enzyme and its translatable mRNA being only barely detectable until release from the intrauterine environment triggers a rapid increase in these functional gene products (4). We report here that treatment of fetuses in utero with the inhibitor of DNA methylation, 5-azacytidine, brings about a rapid precocious activation of tyrosine aminotransferase expression in the fetal livers.

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MATERIALS AND METHODS

Sprague-Dawley rats from Charles River Laboratories were bred and their pregnancies timed as described before (4). 5-azacytidine (Sigma) was dissolved in sterile 0.15 M NaCl at the appropriate concentrations and injections of 100 µl were made intraperitoneally to individual 20 day-old fetuses of anesthetized, laparotomized dams. The abdomen was then sutured closed. At the appropriate times following injection, the dams were again anesthetized and the fetuses taken and killed by decapitation. Their livers were homogenized, the homogenates centrifuged at 12,000 x g for 10 min, and aliquots of supernatant fractions assayed for tyrosine aminotransferase specific activity (5); the enzyme unit is the amount catalyzing formation of 1 nmol product/min. Controls were age matched fetuses, either untreated or administered similar concentrations of cytidine or cytosine arabinoside (Sigma), or of 6-azacytidine (PL Biochemicals) in the same fashion as 5-azacytidine was given.

RESULTS AND DISCUSSION

Activation of aminotransferase expression in fetal liver by 5-azacytidine was strongly dose-dependent (Fig. 1). At the lowest concentrations tested (100-200 µg/fetus) there was little effect on aminotransferase activity. At the optimal dose of 400 µg/fetus enzyme activity increased about 70-fold in 18 hours. Under these conditions survival was always greater than 50 percent, but this figure increased to unacceptable levels and induction was reduced in surviving fetuses if the dose was increased to 500 µg. The time course of this activation following an

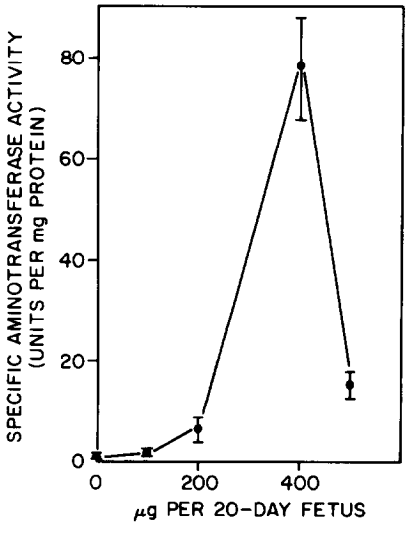

Fig. 1. Response of tyrosine aminotransferase in livers of fetuses treated in utero with increasing doses of 5-azacytidine. The measurements were made 18 hours after treatment; data are the mean ± S.E. for 2 to 3 individual determinations made on pooled livers of 3 to 4 fetuses in each group.

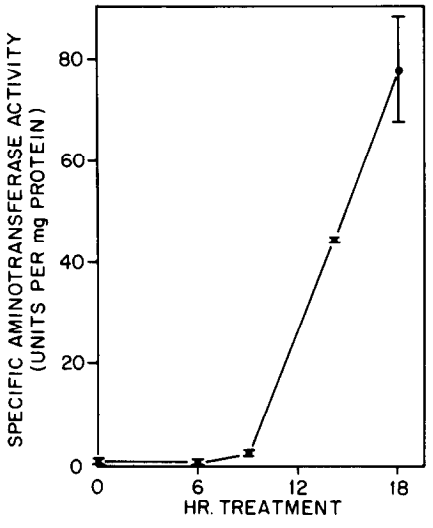

Fig. 2. Time course of activation of tyrosine aminotransferase expression in fetal livers after treatment with 5-azacytidine. Fetuses were treated in utero with 400 µg 5-azacytidine each. Data are the mean ± S.E. for a minimum of 2 or 3 individual determinations made on pooled livers of 2 to 3 litters (24 to 36 fetuses) in each group.

optimal dose of 5-azacytidine is interesting in that there is a lag time of about 9 hours before an appreciable increase in enzyme activity is detected (Fig. 2), followed by an abrupt and rapid increase that is not unlike the transition normally seen in the hours immediately following birth. Eighteen hours after treatment, aminotransferase levels are very high (78 units/mg protein), nearly five-fold above the steady-state level in adult livers (about 16 units/mg protein) which lack the hematopoietic cells contributing protein but not enzyme activity to the measurements made here. Toxicity of the drug became increasingly apparent at treatment times greater than 18 hours, so we have not extended these experiments beyond that time.

In normal development the postnatal activation of tyrosine aminotransferase expression is accompanied by acquisition of the capacity to respond to induction by glucocorticoids (2, 4). In an effort to determine if the precocious activation by 5-azacytidine also confers this capacity, hydrocortisone (5 mg/100 g) was given either intraperitoneally or intravenously to dams carrying treated fetuses, 13 hours after the drug and

5 hours before the fetuses were taken for analysis. The hormone did not further increase enzyme levels at 18 hours (specific activity = 54.8 ± 3.7). However, it is difficult to interpret this result, since the enzyme levels reached following treatment with 5-azacytidine alone are comparable to those of a glucocorticoid-treated adult. Plasma glucocorticoid levels are high in late term fetuses (6), and induction by the endogenous steroids might be expected if expression of the gene was made sensitive.

It is now well established that the 5-azacytidine is an effective inhibitor of methylation of cytosine bases in DNA (reviewed in refs. 7, 8). A number of investigations have further established that hypomethylation of DNA following 5-azacytidine treatment is directly associated with activation of expression of specific genes and of induction of phenotypic differentiation in a variety of cultured cells (e.g. refs. 9-15). To approach the question of whether hypomethylation of DNA may be implicated in the *in vivo* activation of tyrosine aminotransferase expression observed here, we treated rat fetuses with unmodified cytidine and with the analogs cytosine arabinoside and 6-azacytidine, none of which affect methylation of DNA. Results of these control experiments show that these agents are not capable of inducing the dramatic increase in expression effected by 5-azacytidine (Table I). The 6-aza analog did elicit a

TABLE I

EFFECTS OF CYTIDINE AND ITS ANALOGS ON TYROSINE AMINOTRANSFERASE EXPRESSION IN LIVERS OF FETAL RATS

Treatment	Aminotransferase activity* (units/mg protein)
None	0.78 ± 0.11
Cytidine	0.52 ± 0.04
Cytosine arabinoside	1.06 ± 0.06
6-azacytidine	2.56 ± 1.96
5-azacytidine	78.61 ± 10.33

*Data are the mean ± S.E. for a minimum of 3 or 4 individual determintions made on pooled livers of 3 to 4 fetuses in each group. Doses of cytidine and its analogs were 400 µg/fetus; all measurements were made 18 hours after treatment.

slight increase in aminotransferase activity in some fetuses but this effect was quite inconsistent; cytidine and cytosine arabinoside were virtually without effect. These data support the suggestion that precocious activation of tyrosine aminotransferase expression following treatment with 5-azacytidine is a consequence of altered patterns of methylation in DNA.

Hepatic differentiation appears to be influenced by 5-azacytidine in a more profound fashion than simply the activation of expression of this single enzyme. Thus we have observed that the gross morphology of the fetal livers is markedly altered 14 or 18 hours after treatment with the drug, with loss of much of the normally preponderant hemoglobin and a shift toward the color and texture of the fully differentiated adult liver. More precise biochemical and morphological definitions of these phenomena are currently in progress.

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REFERENCES

1. Greengard, O., Federman, M. and Knox, W. E. (1972) J. Cell. Biol. 52, 261-272.
2. Sereni, F., Kenney, F. T. and Kretchmer, N. (1959) J. Biol. Chem. 234, 609-612.
3. Greengard, O. (1964) Science 163, 891-895.
4. Perry, S. T., Rothrock, R., Isham, K. R., Lee, K.-L. and Kenney, F. T. (1983) J. Cell. Biochem., in press.
5. Kenney, F. T. (1959) J. Biol. Chem. 234, 2707-2711.
6. Holt, P. G. and Oliver, I. T. (1968) Biochem. J. 108, 339-341.
7. Razin, A. and Riggs, A. D. (1980) Science 210, 604-610.
8. Felsenfeld, G. and McGhee, J. (1982) Nature 296, 602-603.
9. Compere, S. J. and Palmiter, R. D. (1981) Cell 25, 233-239.
10. Groudine, M., Eisenman, R. and Weintraub, H. (1981) Nature 292, 311-317.
11. Christy, B. and Scangos, G. (1982) Proc. Natl. Acad. Sci. USA 79, 6299-6303.
12. Taylor, S. M. and Jones, P. A. (1979) Cell 17, 771-779.
13. Jones, P. A. and Taylor, S. M. (1980) Cell 20, 85-93.
14. Sager, R. and Kovak, P. (1982) Proc. Natl. Acad. Sci. USA 79, 480-484.
15. Creusot, F., Acs, G. and Christman, J. K. (1982) J. Biol. Chem. 257, 2041-2048.