Induction of tyrosine aminotransferase *in utero* by anti-insulin agents

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The hepatic enzyme tyrosine aminotransferase, normally expressed in very low amounts until shortly after birth, is prematurely induced in foetal rats made diabetic by the administration of streptozotocin *in utero*. Similarly, the enzyme is precociously induced in foetuses if the circulating insulin concentration is artificially decreased by the administration of anti-insulin serum. These observations support the proposal that the natural decrease in plasma insulin, known to occur at birth, is a major contributor to the postnatal induction of tyrosine aminotransferase.

INTRODUCTION

The hepatic enzyme tyrosine aminotransferase (EC 2.6.1.5) is in low concentration in foetal animals, but rapidly accumulates within a few hours of birth (Sereni *et al.*, 1959) or after premature delivery (Holt & Oliver, 1968). Adrenalectomy at birth diminishes the postnatal increase in the enzyme (Sereni *et al.*, 1959), implicating glucocorticoids in the postnatal acquisition of the enzyme. However, although the administration of glucocorticoids to postnatal rats causes a marked increase in the enzyme activity, this fails to induce tyrosine aminotransferase precociously when administered to foetal rats *in utero* (Franz & Knox, 1967; Greengard, 1969; Cake *et al.*, 1973). This occurs despite the presence of a functional steroid-receptor system in foetal hepatocytes (Cake *et al.*, 1981). Other investigators, however, have established that glucocorticoids can induce tyrosine aminotransferase in foetal-rat liver, provided that the tissue is first removed from the uterine environment and cultured as an explant (Wicks, 1968; Ruiz-Bravo & Ernest, 1985) or as a monolayer of cells (Yeoh *et al.*, 1979). On the basis of this, it has been suggested that an inhibitory component is present in the uterine environment which limits expression of the enzyme (Ho *et al.*, 1981; Perry *et al.*, 1983).

We previously demonstrated that low concentrations of insulin markedly decrease the extent of induction of tyrosine aminotransferase in primary cultures of foetal-rat hepatocytes (Ho *et al.*, 1981). We proposed that the high concentrations of insulin, known to be present in the plasma of foetal rats (Cohen & Turner, 1972; Girard *et al.*, 1977), antagonize the action of glucocorticoids by acting as a repressor of the tyrosine aminotransferase system *in utero*. In support of this is the observation that insulin administration to prematurely delivered rats markedly inhibits the postnatal development of tyrosine aminotransferase (Holt & Oliver, 1969). However, until now, no direct demonstration of tyrosine aminotransferase repression by insulin *in utero* has been made. In the present study I have shown that, if the foetal plasma insulin is artificially decreased by the administration of either streptozotocin or anti-insulin serum, tyrosine aminotransferase is precociously induced.

MATERIALS AND METHODS

Chemicals

L-[3,5-³H]Tyrosine was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. L-Tyrosine and sodium diethyldithiocarbamate were from Merck, Darmstadt, Germany. Pyridoxal 5'-phosphate, 2-oxoglutarate and crystalline bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. 2,4-Dinitrophenylhydrazine was supplied by Ajax Chemical Co., Sydney, N.S.W., Australia, and Coomassie Brilliant Blue G250 by Eastman Kodak Co., Rochester, NY, U.S.A. CM-Sephadex C-50 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Guinea-pig anti-(pig insulin) antiserum (0.24 unit of insulin bound/ml) was purchased from Miles-Yeda, Rehovot, Israel, and rat insulin was from Novo Research Institute, DK-2880 Bagsvaerd, Denmark. Streptozotocin (U9889) was given by the Upjohn Co., Kalamazoo, MI, U.S.A.

Methods

Rats of the Wistar albino strain of *Rattus norvegicus* were used. These inbred animals have a 22-day gestation period and were supplied by the Animal Resource Centre, Murdoch, Western Australia. Male and female rats were caged overnight, and conception was considered to occur on the following morning when a vaginal smear detected the presence of sperm. This was designated day 0 of pregnancy and is accurate to within ± 8 h.

Pregnant dams of 20 days gestation were anaesthetized with diethyl ether and their uteri were exposed by partial laparotomy. In one series of experiments, foetuses were injected intraperitoneally through the uterine wall with freshly prepared streptozotocin (750 μ g/foetus) in 25 μ l of 0.01 M-citrate-buffered saline, pH 4.5, or with an equivalent volume of vehicle, starting at opposite ends of the uterus to minimize antibiotic transfer to the controls. In another series, some of the foetuses were injected with 40 μ l of guinea-pig anti-(pig insulin) antiserum. In a preliminary experiment it was shown that this dose was required for optimal enzyme induction (results not shown). The dams were killed by cervical dislocation at the times indicated, and the foetuses were removed and

killed in the same manner. Foetal livers were removed, rinsed in ice-cold saline and homogenized in 2 vol. of ice-cold 0.05 M-potassium phosphate buffer, pH 6.5.

For determination of serum insulin concentrations, blood was collected from the umbilical vein and allowed to clot. Serum was collected by centrifugation and immediately stored at -20°C until assayed. The insulin concentration was determined with an insulin radio-immunoassay kit from Wellcome Research Laboratories, Beckenham, U.K., with a rat insulin standard. Tyrosine aminotransferase activity was first chromatographically separated from aspartate aminotransferase and assayed by the radiochemical assay described previously (Yeoh *et al.*, 1979). Protein concentration was determined by the dye-binding method of Bradford (1976), incorporating the modification of Bearden (1978) and with bovine serum albumin as a standard. Statistical analyses were performed by Student's paired *t* test.

RESULTS AND DISCUSSION

Although glucocorticoids have been clearly implicated in the postnatal increase in tyrosine aminotransferase activity (Sereni *et al.*, 1959), the extent to which they contribute to the normal development of the enzyme has been questioned (Perry *et al.*, 1983; Ruiz-Bravo & Ernest, 1985). Expression of the enzyme is limited in the liver of foetal rats despite the exceptionally high concentrations of free corticosterone in circulation during late gestation (Cohen, 1973; Martin *et al.*, 1977) and, furthermore, exogenously administered glucocorticoids are unable to elicit premature increases in catalytic (Cake *et al.*, 1973) or mRNA activity (Perry *et al.*, 1983; Ruiz-Bravo & Ernest, 1985). However, on the basis of studies with cultured foetal hepatocytes (Ho *et al.*, 1981) we proposed that the tyrosine aminotransferase gene is refractory to stimulation by glucocorticoids *in utero* because the high circulating concentration of insulin acts as a repressor, thereby antagonizing the action of the steroids.

In order to test this proposal, the effect of artificially lowering the circulating insulin concentration on the expression *in utero* of tyrosine aminotransferase has been examined. Portha *et al.* (1974) have shown that streptozotocin, a specific β -cell-cytotoxic antibiotic, is able to elicit a diabetogenic response in foetal rats. We have shown that a 24 h exposure to streptozotocin, after administration *in utero* on day 20 of gestation, results in the premature appearance of hepatic tyrosine aminotransferase: 164.6 ± 33.4 (6) nmol/h per mg of protein in streptozotocin-treated foetuses, compared with 13.9 ± 3.0 (6) nmol/h per mg of protein in their control littermates. This 11.8-fold enhancement ($P < 0.01$) of enzyme activity was concomitant with a 62% decrease in the circulating concentration of immunoreactive insulin (36.1 ± 7.6 $\mu\text{units/ml}$ in streptozotocin-treated foetuses compared with 96.2 ± 26.2 $\mu\text{units/ml}$ in their control littermates). These results contradict the previous report by Ruiz-Bravo & Ernest (1985) that decreasing serum insulin by administration of streptozotocin is insufficient to induce tyrosine aminotransferase precociously. However, those authors used a lower dose of streptozotocin and a different administration regimen, and it is apparent from their data that the treatment resulted in only a 32% decrease in serum insulin. It is possible that

Table 1. Effect of anti-insulin serum and pre-immune serum on hepatic tyrosine aminotransferase activity in foetal rats

Pregnant dams of 20 days gestation were anaesthetized and their uteri exposed by partial laparotomy. Half the litter were injected with either 40 μl of guinea-pig anti-(pig insulin) antiserum or pre-immune serum (test), as indicated; the remainder served as uninjected littermate controls (control). After 15 h exposure, cytosol was prepared from individual foetal livers and assayed for tyrosine aminotransferase. For comparison, foetal rats of 21 days gestation were assayed in the same manner but without being subjected to prior surgical intervention. In these animals the tyrosine aminotransferase activity was 15.4 ± 7.9 (3) nmol of 4-hydroxyphenylpyruvate/h per mg of protein. The values represent means $\pm$ S.E.M. obtained from three or four separate litters as indicated in parentheses.

Condition	Activity (nmol/h per mg of protein)	
	Control	Test
Anti-insulin serum	46.0 ± 10.0 (3)	610.2 ± 88.4 (3)
Pre-immune serum	34.2 ± 24.3 (4)	25.9 ± 17.2 (4)

this decrease was not enough to result in the premature appearance of the enzyme.

One must be cautious in suggesting a mechanism by which streptozotocin is able to enhance the activity of tyrosine aminotransferase. Mencher *et al.* (1979) have shown that streptozotocin-induced hypoinsulinaemia does not result in any significant alteration in the hepatic concentration of cyclic AMP. This is an important observation, in this context, as it has been shown that the cyclic nucleotide elicits a premature appearance of the enzyme (Greengard, 1969; Ruiz-Bravo & Ernest, 1982) when administered to foetal rats. Hoshino *et al.* (1980) have shown that high concentrations of streptozotocin increase the extent of tyrosine aminotransferase induction by dexamethasone in cultured adult rat liver cells and proposed that its effect is via a lower cellular NAD content.

An alternative mechanism is suggested here which might be applicable in the circumstances described. The responsiveness of the hepatic tyrosine aminotransferase system to glucocorticoids is acutely sensitive to the concentration of circulating insulin (Ho *et al.*, 1981) and would be expected to increase dramatically if the concentration of insulin is diminished. Thus we propose that the administration of streptozotocin, by lowering the plasma insulin concentration through its diabetogenic action, enables the high concentrations of free corticosterone in circulation (Cohen, 1973; Martin *et al.*, 1977) to induce tyrosine aminotransferase precociously. Further support for this suggestion comes from the data presented in Table 1. If the insulin concentrations in near-term foetal rats are artificially lowered by the administration of guinea-pig anti-insulin serum, the activity of tyrosine aminotransferase is markedly enhanced over that of littermate controls (13.3-fold; $P < 0.02$). In contrast, if the animals are injected with an equivalent volume of pre-immune guinea-pig serum there is no significant change in the activity of the enzyme ($P > 0.7$). Although the activity in the control animals

was higher than in foetal rats of untreated dams, this would appear to be simply the result of the trauma of surgery, as it is equally apparent in rats treated with pre-immune serum.

These results represent the first direct demonstration *in utero* that the hepatic enzyme tyrosine aminotransferase is repressed by the high concentrations of insulin that prevail in the uterine environment. Furthermore they support our previous proposal (Ho *et al.*, 1981) that the natural decrease in plasma insulin which occurs at birth is a major contributor to the postnatal induction of tyrosine aminotransferase.

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REFERENCES

- Bearden, J. C. (1978) *Biochim. Biophys. Acta* **533**, 525–529
Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
Cake, M. H., Ghisalberti, A. V. & Oliver, I. T. (1973) *Biochem. Biophys. Res. Commun.* **54**, 983–990
Cake, M. H., Yeoh, G. C. T. & Oliver, I. T. (1981) *Biochem. J.* **198**, 301–307
Cohen, A. (1973) *Horm. Metab. Res.* **5**, 66
Cohen, N. M. & Turner, R. C. (1972) *Biol. Neonate* **21**, 107–111
Franz, J. M. & Knox, W. E. (1967) *Biochemistry* **6**, 3463–3471
Girard, J. R., Ferre, P., Kervran, A., Pegorier, J. P. & Assan, R. (1977) in *Glucagon: Its Role in Physiology and Clinical Medicine* (Foa, P. P., Bajaj, J. S. & Foa, N. L., eds.), pp. 563–581, Springer-Verlag, New York
Greengard, P. (1969) *Biochem. J.* **115**, 19–24
Ho, K. K. W., Cake, M. H., Yeoh, G. C. T. & Oliver, I. T. (1981) *Eur. J. Biochem.* **118**, 137–142
Holt, P. G. & Oliver, I. T. (1968) *Biochem. J.* **108**, 333–338
Holt, P. G. & Oliver, I. T. (1969) *Biochemistry* **8**, 1429–1437
Hoshino, J., Strudinger, G. & Kroger, H. (1980) *Chem.-Biol. Interact.* **32**, 267–279
Martin, C. E., Cake, M. H., Hartmann, P. E. & Cook, I. F. (1977) *Acta Endocrinol. (Copenhagen)* **84**, 167–171
Mencher, D., Shouval, D. & Reshef, L. (1979) *Eur. J. Biochem.* **102**, 489–495
Perry, S. T., Rothrock, R., Isham, K. R., Lee, K.-L. & Kenney, F. T. (1983) *J. Cell. Biochem.* **21**, 47–61
Portha, B., Levacher, C., Picon, L. & Rosselin, G. (1974) *Diabetes* **23**, 889–895
Ruiz-Bravo, N. & Ernest, M. J. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 365–368
Ruiz-Bravo, N. & Ernest, M. J. (1985) *Endocrinology (Baltimore)* **116**, 2489–2496
Sereni, F., Kenney, F. T. & Kretchmer, N. (1959) *J. Biol. Chem.* **234**, 609–612
Wicks, W. D. (1968) *J. Biol. Chem.* **243**, 900–906
Yeoh, G. C. T., Bennett, F. A. & Oliver, I. T. (1979) *Biochem. J.* **180**, 153–160

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