ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE AND ENZYME INDUCTION IN THE PERINATAL RAT

P. N. DI MARCO and I. T. OLIVER+

Department of Biochemistry, Monash University, Clayton, VIC 3168, and *+Department of Biochemistry, University of Western Australia, Nedlands, WA 6009, Australia*

Received 27 July 1978

1. Introduction

Adenosine 3',5'-cyclic monophosphate (cyclic AMP) is recognized to be the intracellular mediator of several hormones [1]. In the perinatal rat, cyclic AMP has been implicated in the control of synthesis of hepatic tyrosine aminotransferase and phosphopyruvate carboxylase [2–5]. Results from this laboratory suggest that the induction of the two enzymes is triggered by an increase in the intracellular concentration of cyclic AMP [5]. Increases in cyclic AMP concentration in the liver during the perinatal period have been demonstrated [5–7] which preceded or were coincident with the increases in the activities of the two enzymes [5,7–9]. In addition, it appears that a critical concentration of cylic AMP must be reached for effects on enzyme synthesis to occur [5].

In this report we present evidence of a positive significant correlation between the cyclic AMP concentration and the activity of tyrosine aminotransferase and phosphopyruvate carboxylase in the livers of newborn rats. The results support the hypothesis that cylic AMP is an important factor in the induction of the two enzymes in the perinatal rat in vivo.

2. Experimental

Progesterone was purchased from Oragon Laboratories Ltd., Crown House, Morden, England. All other chemicals and biochemicals were as in [5,8]. Perinatal rats of the Wistar albino strain of *Rattus norwegicus* of known gestation [8] were used. Gestation of this colony is 22 days. Gestation was prolonged by progesterone injection to the dam (2.5 mg progesterone in 0.1 ml peanut oil every 12 h) beginning on day 21 of gestation and continuing until the animals were killed. Newborn animals were delivered by uterine section after decapitation of the dam [8]. Cyclic AMP was determined in individual livers as in [5]. Tyrosine aminotransferase and phosphopyruvate carboxylase activities were determined as in [8] in portions of the same liver supernatant.

3. Results and discussion

The concentration of cyclic AMP and the activities of tyrosine aminotransferase and phosphopyruvate carboxylase were measured at 1 h intervals in the livers of newborn rats delivered surgically during the last 3 days of gestation and during artificially prolonged gestation. The results are reported in fig.1. Enzyme activity is expressed as a function of the hepatic cyclic AMP concentration in vivo. Regression analyses of the enzyme activity and the cyclic AMP concentration at 0–5 h after delivery on day 20–23 of gestation yielded positive significant correlations between the tyrosine aminotransferase activity and the cyclic AMP concentration (fig.1A) ($r = 0.85, p < 0.001$) and between the phosphopyruvate carboxylase activity and the cyclic AMP concentration (fig.1B) ($r = 0.88, p < 0.001$). In each case the relationship between enzyme activity and cyclic AMP concentration was exponential. The synthesis of the two enzymes both in the liver of neonatal rats in vivo [2–4,10] and in foetal liver

explants in organ culture [11,12] appears to be induced following the administration of cyclic AMP or the hormones for which it is the intracellular effector. The results presented here, therefore, support the hypothesis that cyclic AMP is an important factor in the induction of tyrosine aminotransferase and phosphopyruvate carboxylase activities in the perinatal rat in vivo.

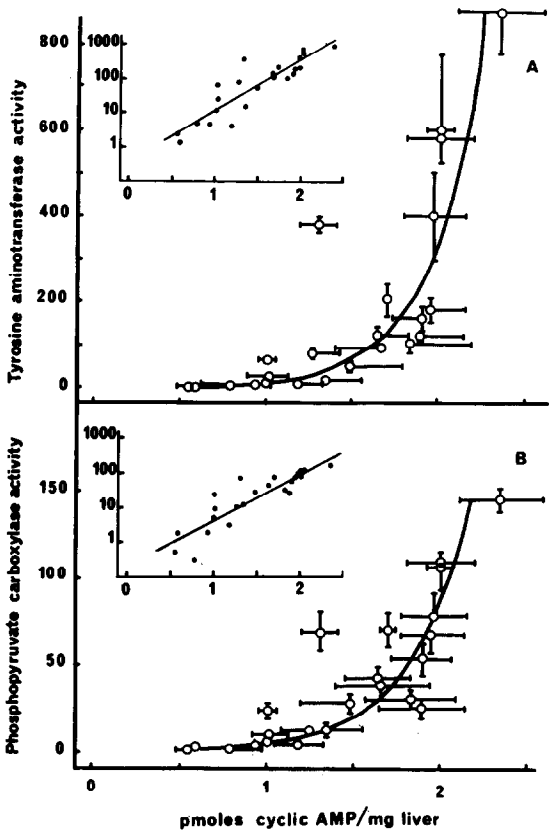

Fig.1. Correlation between enzyme activity and cyclic AMP concentration in the liver of perinatal rats. Foetal rats on days 20–23 of gestation were delivered by uterine section after decapitation of the dam and maintained in the humidicrib under a stream of carbogen (95% O_2:5% CO_2) at 37°C. The newborn rats were killed at hourly intervals up to 5 h after delivery and their livers were collected and treated as in section 2 for cyclic AMP or enzyme assay. (a) Correlation between tyrosine aminotransferase activity (μmol *p*-hydroxyphenylpyruvate formed at 37°C/h/g liver) and cyclic AMP concentration. (b) Correlation between phosphopyruvate carboxylase activity (μmol oxaloacetate formed at 37°C/h/g liver) and cyclic AMP concentration. Insets: The log of enzyme activity is plotted as a function of the cyclic AMP concentration. All lines were fitted by the least squares method. The concentration of cyclic AMP and the activities of the enzymes were determined in different liver samples. Each point is the mean ± SEM of at least 4 determinations.

The results presented in fig.1 suggest that the activities of tyrosine aminotransferase and phosphopyruvate carboxylase develop when the total liver concentration of cyclic AMP increases to 1 pmol/mg tissue or more. This occurs in utero during the last day of gestation and within 1–2 h of surgical delivery of preterm foetuses [5]. However, once this concentration is reached, large increases in enzyme activity are associated with relatively small changes in cyclic AMP concentration (fig.1). These observations confirm that a critical concentration of cyclic AMP must be reached for the cyclic nucleotide to have its effects.

According to the concept in [13], cyclic AMP-mediated effects of hormones in mammalian tissues come about by an activation of cyclic AMP-dependent protein kinases which mediate the phosphorylation of specific proteins, thereby modifying their biological activities. In the activation process, cyclic AMP promotes the dissociation of the inactive enzyme yielding the catalytically-active free subunit, while the cyclic nucleotide is bound to the regulatory subunit [14,15]. The activation of protein kinase is the only well-documented mechanism of action of cyclic AMP in mammalian cells [16]. This mechanism of action of cyclic AMP has also been implicated in the control of tyrosine aminotransferase and phosphopyruvate carboxylase synthesis in rat liver [17,18]. In addition, a direct effect of cyclic AMP on the release of tyrosine aminotransferase from polysomes has been proposed [19,20], which does not involve protein kinase but requires a microsomal extract which has been identified as a protein which binds cyclic AMP with high affinity [21].

Studies on the interaction of cyclic AMP with the receptor protein in the activation of protein kinase in the rat liver in vivo [22] have shown that an increase in the total tissue concentration of cyclic AMP from 0.7–1.2 pmol/mg liver is required for half-maximal activation of protein kinase as well as half-maximal binding of cyclic AMP to the receptor protein. The results presented in fig.1 suggest that the induction of tyrosine aminotransferase and phosphopyruvate carboxylase in the perinatal rat liver in vivo is asso-

ciated with similar increases in the hepatic cyclic AMP concentration. These observations suggest that the binding of cyclic AMP to the receptor protein or the activation of protein kinase may be an important step in the physiological mechanism for enzyme induction by cyclic AMP in the perinatal rat.

Acknowledgements

We wish to thank Mr Malcolm McGrath for excellent technical assistance. This work was supported by grants from the Australian Research Grants Committee and the University of Western Australia.

References

[1] Robison, G. S., Butcher, R. W. and Sutherland, E. W. (1971) Cyclic AMP, Academic Press, New York.
[2] Yeung, D. and Oliver, I. T. (1968) Biochemistry 7, 3231–3239.
[3] Greengard, O. and Dewey, H. K. (1967) J. Biol. Chem. 242, 2986–2991.
[4] Holt, P. G. and Oliver, I. T. (1969) Biochemistry 8, 1429–1437.
[5] Di Marco, P. N. and Oliver, I. T. (1978) Eur. J. Biochem. in press.
[6] Blazquez, E., Sugase, T., Blazquez, M. and Foa, P. P. (1974) J. Lab. Clin. Med. 83, 957–967.
[7] Butcher, F. R. and Potter, V. R. (1972) Cancer Res. 32, 2141–2147.
[8] Di Marco, P. N., Ghisalberti, A. V., Pearce, P. H. and Oliver, I. T. (1976) Biol. Neonate 30, 205–215.
[9] Ghisalberti, A. V., Pearce, P. H., Di Marco, P. N. and Oliver, I. T. (1975) Proc. Endocr. Soc. Aus. 18, 65.
[10] Greengard, O. (1969) Biochem. J. 115, 19–24.
[11] Wicks, W. D. (1969) J. Biol. Chem. 244, 3941–3950.
[12] Wicks, W. D. (1971) J. Biol. Chem. 246, 217–223.
[13] Kuo, J. F. and Greengard, P. (1969) Proc. Natl. Acad. Sci. USA 64, 1349–1355.
[14] Kumon, A., Yamamura, H. and Nishizuka, Y. (1970) Biochem. Biophys. Res. Commun. 41, 1290–1297.
[15] Gill, G. N. and Garran, L. D. (1970) Biochem. Biophys. Res. Commun. 39, 335–343.
[16] Jungman, R. A. and Russel, D. H. (1977) Life Sci. 20, 1787–1798.
[17] Wagner, K., Roper, M. D., Leichtling, B. H., Wimalsena, J. and Wicks, W. D. (1975) J. Biol. Chem. 250, 231–239.
[18] Wicks, W. D., Koontz, J. and Wagner, K. (1975) J. Cyclic Nucleot. Res. 1, 49–58.
[19] Chuah, C. C. and Oliver, I. T. (1971) Biochemistry 10, 2990–3001.
[20] Chuah, C. C. and Oliver, I. T. (1972) Biochemistry 11, 2547–2553.
[21] Donovan, G. and Oliver, I. T. (1972) Biochemistry 11, 3904–3909.
[22] Schwoch, F. and Hilz, H. (1977) Eur. J. Biochem. 76, 269–276.