HORMONAL EFFECTS ON THE NEURAL RETINA: INDUCTION OF GLUTAMINE SYNTHETASE BY CYCLIC-3',5'-AMP.

Gerald J. Chader

Howe Laboratory, Harvard Medical School

Mass. Eye and Ear Infirm., Boston, Mass.

Received April 20, 1971

Summary: Glutamine synthetase activity of the chick embryo retina in tissue culture is induced by cyclic-3',5'-AMP and its dibutyryl derivative. De novo enzyme synthesis appears to be involved since the effect is inhibited by both Actinomycin D and cycloheximide.

In the neural retina of the chick embryo, glutamine synthetase (GS) activity can be induced by steroid hormones (1,2). The mechanism by which steroid increases the concentration of the synthetase enzyme has not yet been elucidated, although the induction has been considered to be specific for corticoids such as cortisol (3). The present report gives evidence that cyclic-3',5'-AMP (cAMP) and its dibutyryl derivative (BcAMP) also elevate the level of retinal GS through de novo synthesis of RNA and protein.

Materials and Methods: Retinas from 11 or 12 day old chick embryos (Cobb Farms, Littleton, Mass.) were maintained in tissue culture for 24 hours in 5 ml of Eagle's basal medium containing 5% commercially dialyzed fetal calf serum (Gibco, Grand Island, N.Y.). When appropriate, cyclic-3',5'-AMP, 6-N-2'-O-dibutyryl cyclic AMP, cortisol, cycloheximide(Sigma Chemical Co., St. Louis, Mo.) or Actinomycin D (a generous gift of Merck, Sharpe and Dohme) was added in a volume of 0.1 ml at the beginning of the culture period. Other conditions have been previously described (1,3). Protein analysis was according to Lowry et al.(4). Glutamine synthetase was determined colorimetrically at 500 nm by a modification of the method of Thorndike and Reif-Lehrer (5) utilizing a Bausch and Lomb Spectronic 100. Units

of enzyme activity are μmoles x 10^2 of γ-glutamyl hydroxamate formed per mg protein per minute at 37°. L-glutamic acid -γ-hydroxamate (Sigma Chem. Co.) was used as standard.

Results and Discussion: Comparative effects of cAMP and BcAMP on GS activity are shown in Table I. A 2 to 3 fold increase in activity was observed with 1.2 x 10^{-3}M cAMP. Inducing ability fell off rapidly at lower concentrations however, probably due to enzymatic breakdown of the cyclic nucleotide. BcAMP was considerably more effective than the natural nucleotide, in that a comparable 2 to 3 fold rise in GS activity was observed at 2 x 10^{-5}M. Maximal effects of BcAMP were observed at approximately 4 x 10^{-4}M and above. Levels of synthetase induced under these conditions were comparable to those induced with optimal concentrations of cortisol (see Table II).

The effects of Actinomycin D and cycloheximide on normal GS activity and on activity induced with BcAMP and cortisol are shown in Table II. Cycloheximide at 7 x 10^{-6} M somewhat decreased the normal basal level of the en-

Table I

addition	concentration (Molar)	enzyme activity
none	- -	2.26±0.13
cAMP	3.0 x 10^{-5}	2.33±0.28
	6.0 x 10^{-4}	4.14±0.49
	1.2 x 10^{-3}	5.46±0.30
BcAMP	2.0 x 10^{-5}	6.61±0.44
	4.0 x 10^{-4}	13.20±0.84
	0.8 x 10^{-3}	16.94±1.16

Comparison of the effects of cyclic AMP and dibutyryl cyclic AMP on glutamine synthetase activity. Retinal tissue culture period was 24 hours. Enzyme specific activity is expressed as μmoles x 10^2 glutamyl hydroxamate/mg/min at 37°. Blank value is 3 x 10^{-3} μmoles/mg/min. Values given are means (±S.D.) from 6 to 8 individual cultures.

Table II

addition	enzyme activity
none	1.61± 0.12
cycloheximide	0.96± 0.22
Actinomycin D	1.50± 0.09
BcAMP	8.27± 0.54
cortisol	17.16± 1.30
BcAMP + cyclohex.	1.03± 0.16
BcAMP + Act. D	1.29± 0.44
cortisol + cyclohex.	1.80± 0.39
cortisol + Act. D	1.80± 0.28

Effect of antibiotics on induction of glutamine synthetase by dibutyryl cyclic AMP and cortisol. Concentrations used were: BcAMP, 1×10^{-4}M; cortisol, 3×10^{-8}M; cycloheximide, 7.0×10^{-6}M; Actinomycin D, 1.6×10^{-7}M. Other conditions as in Table I.

zyme, whereas no such effect was observed with Actinomycin D (1.6×10^{-7}M). In contrast, the increased enzyme activity elicited by hormone was abolished by both antibiotics. This indicates that the effect of the cyclic nucleotide as well as that of cortisol is expressed through new synthesis of RNA and protein.

The induction of GS in the retina has been used as a convenient model for studying control mechanisms in differentiation (6-9) and also for investigating the action of corticoids (3, 10, 11). The retinal system is somewhat different however from the others used for studying these general phenomena (12,13), since the retina is neural and is an integral part of the central nervous system (CNS). The presence of cAMP in the CNS has now been well established (14), and it could be expected therefore that cAMP

might play a regulatory role in the CNS (15) and perhaps a specific one in the retina (16). The present study further demonstrates this by showing the similar effect of cAMP and cortisol on the induction of GS in the retina. It is not known at present if the cyclic nucleotide acts as a hormonal "second messenger" (17) in the retina, or by some other, as yet unknown, mechanism.

Acknowledgements: I thank Miss R. Nierdra and Mrs. L. Saunders for excellent technical assistance. This work was supported by General Research Support Grant FR-05485-08 of the USPHS.

References

1) Reif-Lehrer, L. and Amos, H., Biochem. J. 106 425 (1968).

2) Moscona, A.A., Moscona,M.H. and Saenz, N., Proc. Nat. Acad. Sci. 61 160 (1968).

3) Reif-Lehrer, L. and Chader, G.J., Biochim. Biophys. Acta 192 310 (1969).

4) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J. Biol. Chem. 193 265 (1951).

5) Thorndike, J. and Reif-Lehrer, L., Enzymol. clin. biol. (in the press).

6) Reif-Lehrer, L., Biochem. Biophys. Res. Comm. 41 260 (1970).

7) Horisberger, M. and Amos, H., Biochem. J. 117 347 (1970).

8) Moscona, A.A., Moscona, M.H. and Jones, R.E., Biochem. Biophys. Res. Comm. 39 943 (1970).

9) Weissman, H. and Ben-Or, S., Biochim. Biophys. Res. Comm. 41 260 (1970).

10) Moscona, A.A. and Piddington, R., Science 158 496 (1967).

11) Reif-Lehrer, L., Biochim. Biophys. Acta 170 263 (1968).

12) Tomkins, G.M., Gelehrter, T.D., Granner, D., Martin, D., Samuels, H.H., and Thompson, E.B., Science 166 1474 (1969).

13) O'Malley, B.W. and Kohler, P., Proc. Nat. Acad. Sci. 58 2359 (1967)

14) "The Role of Cyclic AMP in the Nervous System", Neurosci. Res. Program Bulletin 8 #3 (1970).

15) McAfee, D.A., Schorderet, M. and Greengard, P., Science 171 1158 (1971).

16) Bitensky, M.W., Gorman, R.E. and Miller, W.H., Proc. Nat. Acad. Sci. 68 561 1971.

17) Sutherland, E.W., Øye, I. and Butcher, R.W., Rec. Prog. Hor. Res. 21 623 (1965).