REGULATION OF GLUTAMINE SYNTHETASE IN THE EMBRYONIC NEURAL RETINA IN ORGAN CULTURE

H. Weissman & S. Ben-Or

Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received September 7, 1970

Summary - Glutamine synthetase (GS) activity in the embryonic neural retina is correlated with cellular differentiation. Its activity can be induced precociously by hydrocortisone (HC). This effect, which is initially dependent on RNA and protein synthesis, is released from the dependency on RNA synthesis after several hours in culture. GS continues to be synthetized on stable m-RNA molecules. In this work an increase in GS activity was obtained in the absence of HC by adding actinomycin D(AD) to the culture. The AD effect is dependent on an initial period of RNA synthesis, on protein synthesis and on a factor(s), yet unknown, which is(are) present in the "non-inducing" fetal bovine serum. A possible role played by HC in the regulation of GS in the developing neural retina is suggested.

Glutamine synthetase (GS) activity in the neural retina has a characteristic developmental pattern which is correlated with other aspects of retinal differentiation (1.2). The sharp rise in its activity occurring during the final stages of embryonic differentiation can be precociously induced by hydrocortisone (HC) (3) and related corticosteroids (4,5). This effect is dependent on RNA and protein synthesis (6). However, after the first hours in culture the increase of GS is released from its dependency on RNA synthesis and the enzyme continues to be synthetized on stable templates (7,8).

In this work, the effect of inhibition of RNA synthesis on GS activity in the absence of HC (control "non-induced" retina) has been investigated. It was found that GS can be induced in the absence of the steroid by arrest of transcription under certain experimental conditions. The results suggest that a factor(s), yet unknown, present in the "non inducing" medium play a role in the regulation of GS activity in the neural retina of the chick embryo.

The neural retina, explanted from the 12 day chick embryo, was immediately transferred to the organ culture conditions. The medium consisted of Eagle's medium, (without glutamine), 5000 units/ml of both penicillin and streptomycin to which "non-inducing" fetal bovine serum (FS) (Microbiological Associates, lot No. 73055, 14413) was added at different concentrations, as specified in the results. When induction was obtained by the steroid hormone, a freshly prepared solution of HC-Na-succinate (Organon) was used. RNA synthesis was inhibited by 10 µg/ml actinomycin D(AD) (donated by Merck) and protein synthesis was inhibited by 10 µg/ml cycloheximide. GS was determined by the transferase activity, according to the method of Kagan et al (8) on 15,000g supernatant prepared from the frozen retina after a very tight homogenization (Potter-Elvehjem glass tissue grinder). Protein was determined by the method of Lowry (10). Enzyme specific activity is expressed as E_{540}/mg protein.

The results presented in fig.1 demonstrate the 15 fold increase in GS specific activity which develops during 24 hrs. in culture with HC. GS activity in the control culture increases only slightly during this period. The dependency of this increase on protein synthesis is also presented in fig.1.

While investigating the effect of inhibition of RNA synthesis by AD(10 µg/ml) on this system, it was found that if AD was added

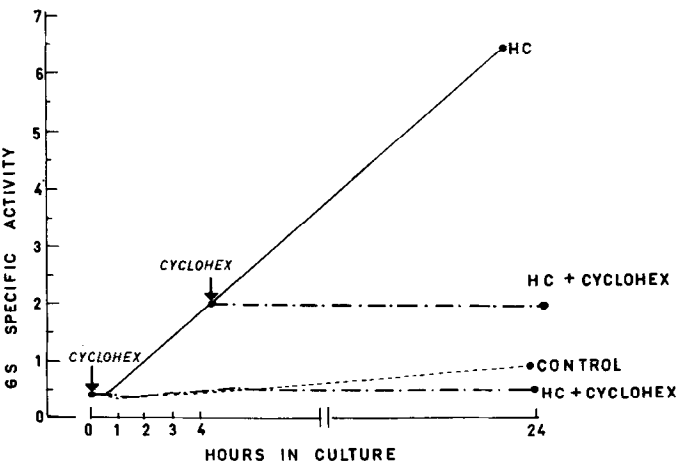

Figure 1. Induction of glutamine synthetase (GS) in the 12 day embryonic retina by hydrocortisone (HC) 1 µg/ml. Culture medium contained 20% fetal serum. Cycloheximide, 10 µg/ml, added at the beginning of the culture or several hours thereafter, inhibits the increase of GS activity. The points are the average of very close values obtained in 3 different experiments.

to the controls at the onset of culture and GS determined 24 hrs. thereafter, a slight decrease in GS activity occurred. But if AD was added to the culture after 5 hrs. of incubation, an increase in GS activity, significantly higher than GS level in the control uninhibited retina, was obtained. However, this increased value was far below the levels obtained by HC.

Since previous, unpublished data indicated that the induction of GS by HC is facilitated by increasing the serum concentration in the culture medium, (HC, being kept at 1 µg/ml), the effect of FS concentration on the activity of GS in the absence of the steroid was investigated.

The results summarized in fig.2 show that different FS concentrations (1-20%) have hardly any effect on GS activity in the control, uninhibited retina. However, the AD-treated retina

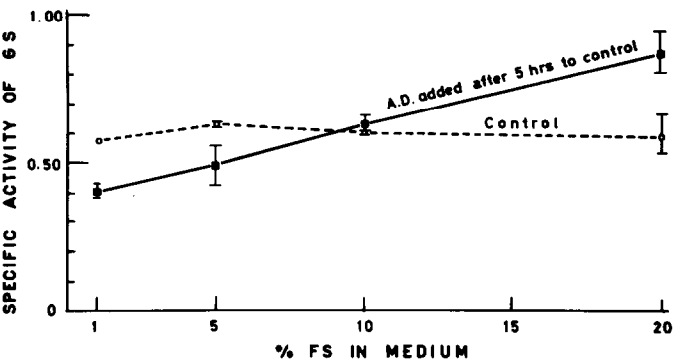

Figure 2. The effect of fetal bovine serum (FS) concentration on GS activity in control and AD-treated retinas, in the absence of HC. AD, 10 µg/ml was added to the culture after 5 hours of incubation and GS was determined after additional 20 hrs. The points are the average values from 3 experiments. The vertical lines represent the range of response.

---

is very sensitive to them; very low serum concentrations cause a decrease in GS activity, while increased concentration induces it. The increase in GS thereby obtained is linearly related to serum concentration. This "AD-dependent" increase of GS is also dependent on protein synthesis since as shown in fig 3, it is inhibited by cycloheximide.

A more detailed analysis of the effect of time in culture, prior to transcription arrest by AD, in control and HC-treated cultures has been performed. The results summarized in figs.4 & 5

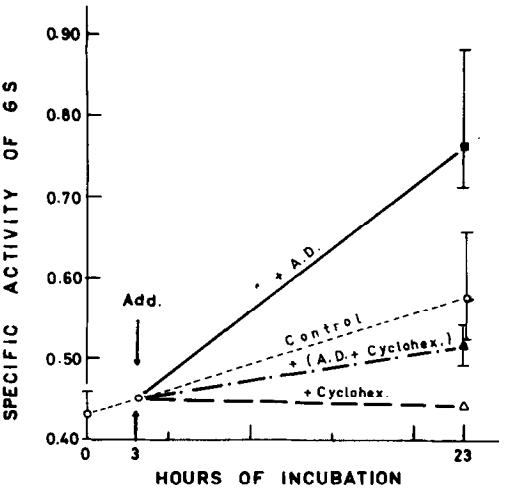

Figure 3. The dependency of the "AD effect" on protein synthesis. AD (10 µg/ml), cycloheximide (10 µg/ml) or both were added to the control cultures after 3 hrs. of incubation as indicated by arrows. GS was determined 20 hrs. later. Medium contained 20% FS. The points represent the average value from 2-3 experiments. The lines indicate the range of response.

show, that an initial period of RNA synthesis is required for the increase in GS to occur, in the two experimental set-ups. But while the rate of increase of GS, induced by HC, is directly proportional to the period of transcriptional activity, the period of RNA synthesis required for the inductive phenomemon in the "controls" is limited to the first 2-3 hours. Allowing further transcription to go on in the latter, does not significantly increase GS activity.

In summary, the results of these experiments indicate that the increase of GS activity, induced by FS in the absence of HC, is dependent on: a) an initial period of RNA synthesis, b) conditions which permit protein synthesis to take place, c) a factor(s) which is (are) present in the FS and d) cellular changes that result from AD inhibition.

These results might be interpreted according to Kenney et al (11) who showed that AD effectively blocks enzyme turnover, thereby causing "superinduction". However, experiments in progress indicate that this is not the case here. Another explanation, suggested by Tomkins in another experimental system (12), is that AD replaces the requirement for the steroid hormone, which is otherwise needed

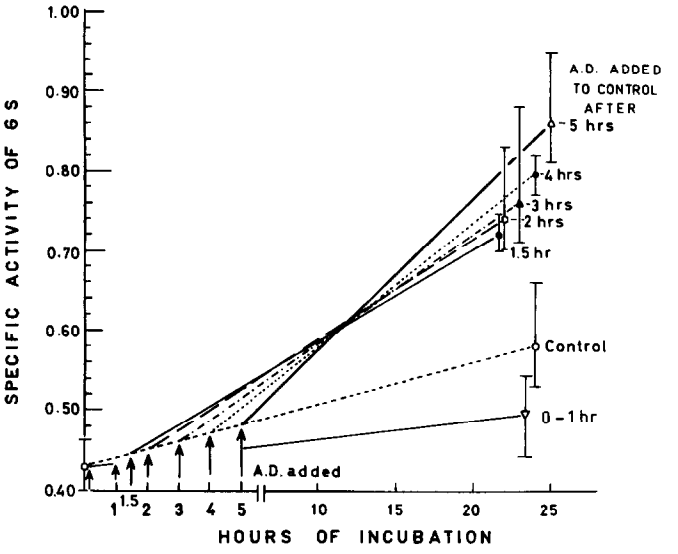

Figure 4.

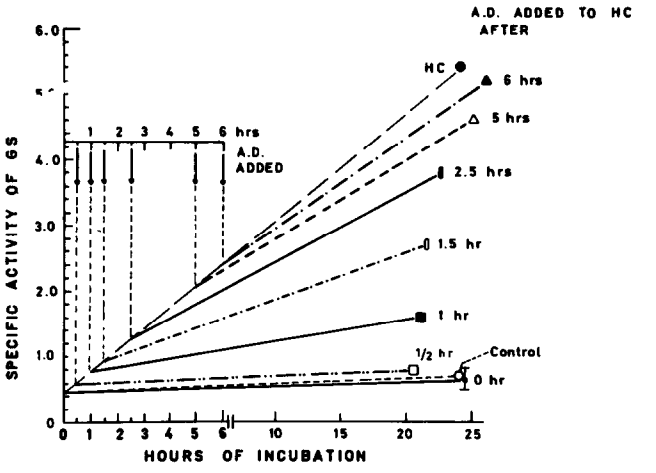

Figure 5.

Figure 4.& 5. The effect of transcription arrest by AD (10 μg/ml) on GS activity in control (fig.4) and HC-treated retina (fig.5). AD was added at zero time and after different incubation periods, as indicated by the arrows. GS was generally determined 20 hrs. after the addition of AD. Medium contained 20% FS. While the values obtained in the HC-treated retinas were very close, the values of the "controls" varied greatly, as represented by the vertical lines. The points are the average from 4-5 experiments.

---

to antagonize a labile inhibitor. Regulation of GS synthesis by a labile, post-transcriptional inhibitor, has also been suggested by Moscona et al (7), as an interpretation of results obtained under

different experimental conditions. This tempting suggestion waits, however, for more direct experimental proof. The results here presented suggest another possible explanation which relates the "AD effect" to changes in permeability properties of the cell membrane As has been shown, the unfavourable culture conditions (low serum conc.) have no apparent effect on the uninhibited controls, (during the 24 hrs. of these experiments) but cause a decrease in GS in the AD-treated retinas. On the other hand, the AD-treated retina responds to the improved culture conditions (increased serum concentration), by a proportional increase in GS, while the controls remain indifferent. (Fig.2). It is therefore suggested that unspecific changes in the permeability properties of the cell boundaries occur after treatment with AD, and that molecules present in the medium (other than the steroid), which have a regulative effect on GS, are thereby able to penetrate. Accordingly, it is suggested, that the role played by HC is to introduce specific changes in the cell membrane which facilitate the transport of certain required molecules present in the medium.

These suggestions are supported by results obtained from experiments in progress, which demonstrate that the synthesis of a membrane protein which might play a role in GS regulation is induced at the early stages of culture in FS.

References

1. Rudnick, D., J. Exptl. Zool. 142, 643 (1959).
2. Piddington, R. an d Moscona, A.A., J. Cell. Biol. 27, 247 (1965).
3. Moscona, A.A. and Piddington, R., Biochim. Biophys. Acta. 121, 409 (1966)
4. Moscona, A.A. and Piddington, R., Science 158, 496 (1967)
5. Reif. Lehrer, L., Biochim. Biophys. Acta 170, 263 (1968)
6. Kirk, D.L., Proc. Natl. Acad. Sci.U.S. 54, 1345 (1965).
7. Moscona, A.A., Moscona M.H. and Saenz N., Proc. Natl. Acad. Sci.U.A. 61, 160 (1968)
8. Alescio, T. and Moscona, A.A., Biochem. Biophys. Res. Commun. 34 (2), 176 (1969)
9. Kagan, H.M., Manning, L.R. and Meister, A., Biochem. 4, 1063 (1965).
10. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J. Biol. Chem. 193, 265 (1951).
11. Kenney, F.T., Reel, J.R., Hager, C.B., and Wittliff, T.L. In: Regulatory Mechanisms for Protein Synthesis in Mammalian Cells (A. San Pietro, M.R. Lamborg and F.T. Kenney, eds) Academic Press, New York, 1968, p. 119.
12. Tomkins, G.M. In: Regulatory Mechanisms for Protein Synthesis in Mammalian Cells (A. San Pietro, M.R. Lamborg and F.T. Kenney, eds.) Academic Press, New York, 1968, p. 269.