GLUTAMINE SYNTHETASE LOCALIZATION IN CORTISOL-INDUCED CHICK EMBRYO RETINAS

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ABSTRACT

We report here for the first time, in chick retina, Muller cell localization of glutamine synthetase (GS) activity by an immunohistochemical technique, in agreement with previous reports of glial localization of this enzyme in rat brain and retina. Age-dependent changes in the endogenous enzyme activity as well as cortisol-induced changes in GS activity, both in ovo and in vitro, measured biochemically, reflect the changes observed by staining.

KEY WORDS retina - glutamine synthetase - localization - induction - chick embryo

The enzyme glutamine synthetase (GS) catalyzes the conversion of glutamate to glutamine, metabolites which are central to a large number of biochemical pathways, including formation of gamma-aminobutyric acid, an inhibitory neurotransmitter. GS activity (often measured by its gamma-glutamyl transferase [GT] activity) occurs in the central nervous system (including brain and retina) as well as in other tissues in a variety of species (18). Nowhere is the activity of the GS higher, on a per milligram protein basis, than in the mature retina (1). In the chick embryo, a dramatic increase in the activity of the enzyme occurs in the latter stages of development of the retina. Such an increase in retinal GS activity can be prematurely induced, both in ovo and in vitro, by 11-β-hydroxyglucocorticoids (2, 6, 10), and thus is useful as a model for the study of gene expression and differentiation.

To date, GS has not been localized in chick retina nor has the site (i.e., cell type) of the cortisol effect been determined. In the rat, Norenberg and co-workers have recently found the enzyme localized in glial cells both in brain (astrocytes) (5, 7, 8) and in retina (Muller cells) (13, 14). In chick brain, Piddington (9) has reported that GS activity appears to be predominantly in a fraction rich in small neurons. In an attempt to understand the role of GS in the retina, we have studied its localization in this tissue immunohistochemically. We report here Muller cell localization of the enzyme in chick embryo retina, for both endogenous and induced enzyme. This is the first report of localization of premature, steroid-induced enzyme and is in agreement with the location of endogenous enzyme in this tissue.

MATERIALS AND METHODS

The experiments consisted of determination, by biochemical as well as immunohistochemical techniques, of both endogenous and induced GS activity in chick embryo retina. GS induction was carried out with the use of overnight treatment with cortisol. With the exceptions mentioned below, retinas were from 12-d-old embryos.

In vitro experiments: Retinas were excised sterilely from the embryo and cultured in 5 ml of minimal essential medium (MEM) containing 10% fetal calf serum (FCS), 100 μg/ml gentamycin, 100 μg/ml kanamycin and, when appropriate, 0.1 μg/ml (2.6 × 10⁻⁷ M) cortisol; control cultures received no steroid. Conditions of culture and harvest were as previously described (11, 12).

In ovo experiments: Embryonated eggs were injected (onto the egg shell membrane) with 100 μg of cortisol in 100 μl of water.
95% ethanol (1:1 vol/vol), as previously described (2), and returned to the incubator for induction for 20-24 h. Controls received only the vehicle.

Retinas from uninjected 7-, 14-, and 20-d-old embryos were used for comparison of endogenous GS activity. In most cases, biochemical and morphological experiments were run in parallel. In some experiments, retinas were paired, i.e., the retina from one eye was used for immunohistochemical localization of GS, and the retina from the other eye of the same embryo was used for assay of GS activity. Details of the assay method have been described elsewhere (12, 17).

For the immunohistochemical demonstration of GS, retinas were fixed for 1 h at room temperature with a mixture of 2% paraformaldehyde and 15% saturated picric acid in 0.1 M sodium phosphate buffer, pH 7.3 (16). Retinas were then washed overnight in phosphate-buffered saline containing 10% sucrose, quickly frozen, and stored at −68°C before staining with the indirect peroxidase-labeled antibody method as previously described (14), except that the antibody was diluted 1:240 before use. Control sections were treated in the same manner as experimental sections, except that GS-adsorbed IgG fraction of rabbit antiserum and nonimmune rabbit IgG were substituted for the anti-GS IgG. Additionally, serial sections were counterstained with hematoxylin to aid in cellular identification.

Embryonated chicken eggs were obtained from Spafas, Inc. (Norwich, Conn.). MEM, FCS, gentamycin, and kanamycin were obtained from Grand Island Biological Co. (Grand Island, N. Y.). Cortisol was obtained from Nutritional Biochemicals (Cleveland, Ohio).

RESULTS

Fig. 1 shows the localization of endogenous GS in a retina from a 20-d-old embryo; at this age, enzyme activity is approaching the adult value. Note that GS activity is localized specifically in areas of apparent Muller cell distribution. The Muller cells are the only retinal cells that extend from the inner limiting membrane to beyond the outer limiting membrane, with polygonal nuclei located in the inner nuclear layer. These cells pass through the inner plexiform layer as narrow parallel columns widening into end-feet that correspond to funnel-shaped deposits of GS reaction product in the nerve fiber and ganglion cell layers. Note also, distinct beads of reaction product passing through the outer plexiform layer and outer nuclear layer, terminating along the outer limiting membrane, and following the known morphology of Muller cells. In retinas from 7-d-old embryos (not shown), no stain could be detected; even in retinas from 14-d embryos (not shown), only trace amounts could be identified. Fig. 2 shows retinas from 12-d embryos which have (Fig. 2a) and have
FIGURE 2  Retinas from 12-d-old embryos treated in culture (a) with cortisol and (b) without cortisol. The cortisol-treated retina shows a dramatic increase in immunoreactive GS in areas corresponding to Muller cell perikarya (arrows) and their processes. At the posterior margin of the outer plexiform layer (OPL) the stain has a globular configuration. No counterstain. Bar, 20 μm.

FIGURE 3  Retinas from 12-d-old embryos treated in ovo with (a) cortisol and (b) vehicle. The pattern is similar to that for retinas treated in culture (Fig. 2). No counterstain. Bar, 20 μm.
not (Fig. 2b) been induced for GS in culture by cortisol; Fig. 3 shows the corresponding results for an in ovo experiment; the retina in Fig. 3a is from an embryo (12 d) injected with cortisol 24 h before excision; the retina in Fig. 3b derives from an embryo (12 d) injected with only vehicle. Note the dramatic increase in staining, in areas corresponding to Muller cell localization, in the cortisol-induced retina compared to that in the uninduced retina. In the induced retina, from the 12-d embryo, the amount of reaction product, though obviously less than the endogenous level observed in the retina from the 20-d embryo (Fig. 1), is clearly well developed compared to the uninduced retina from a 12-d embryo, which contains only trace amounts of immunoreactive GS. Sections treated with nonimmune sera, or adsorbed immune sera, contained no reaction product.

Endogenous and induced levels of GS, measured biochemically (by GT activity), were in good agreement with the results of the immunohistochemical studies. In a large number of experiments with cultured retinas from 12-d embryos, the GS activity ratio for 24-h cortisol-treated, as compared to untreated retinas, ranged between 7 and 9. In a representative experiment, the GS specific activity (µg glutamohydroxamic acid formed per h per µg protein) was 1.5 ± 0.56 for the cortisol-treated and 0.13 ± 0.06 for the untreated control retinas. (Each value is the average for four retinas assayed independently). The values for GS specific activity in a representative in ovo experiment were 0.64 ± 0.26 (average from six retinas) for the cortisol-treated, and 0.08 ± 0.001 (average of four retinas) for the controls not given cortisol. In this experiment, the actual specific activity of GS in the retina contralateral to the one shown in Fig. 3a was 0.86, and that for the retina contralateral to the one shown in Fig. 3b was 0.08. By comparison, endogenous GS specific activity in freshly excised retinas from 20-d-old embryos is in the range of 3.5 ± 1.1.

DISCUSSION

The results of this study indicate that GS is localized in regions corresponding to the location of Muller cells (glia) in chick embryo retina, as has previously been shown in rat brain (5, 7, 8) and rat retina (13, 14). Our results indicate that the GS prematurely induced by steroid in the retina is derived from the same cells as the endogenous enzyme and is in all likelihood identical to it. This is the first report of localization of the induced enzyme in retinal tissue.

In the rat brain, a recent fine-structure study has shown the GS to be in glia, and more specifically, to be localized in astrocytes in both white and gray matter, and within these cells, to be frequently associated with the endoplasmic reticulum (8). Astroglial processes, containing GS, were found surrounding synaptic endings of neuronal cells, a location in keeping with the currently suspected neurotransmitter role of glutamate, the substrate of GS. According to a current hypothesis (4), glutamate released from nerve endings into the synaptic cleft is subsequently taken up by glia, reconverted to glutamine by GS, and in that neuro-inactive form, recycled back to the neuron to be used as a transmitter-precursor. Although the fine-structure localization of GS in chick retina has not yet been determined, the glial localization of the enzyme is consistent with such a mechanism in this tissue as well.

On the basis of bulk fractionation studies of chick brain, Piddington (9) had suggested a small-neuronal localization of GS in that tissue; however, in those experiments, problems related to enzyme diffusion (3), cellular homogeneity, purity of cell fractions, and precise cellular identification (especially the small neuronal fraction in which GS was found) did not allow for definitive conclusions about GS localization. Light microscope immunohistochemical studies in chick brain have shown a glial localization of GS (M. D. Norenberg, unpublished results). The correspondence between the immunohistochemical and biochemical results in GS-induced and uninduced retina substantiates the specificity of the immunohistochemical method used to visualize GS in this study; moreover, the results of these experiments indicate that the induced and the endogenous enzyme are the same. Lastly, that the staining for GS coincides with the location of the Muller cells is also in agreement with recent reports of Sarthy and Lam (15) that there is appreciable GS activity in isolated, hand-picked Muller cells from turtle retina.

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RAPID COMMUNICATIONS