Glutamine Synthetase Induction in Chick Embryo Retina Monolayers

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Abstract

Induction of glutamine synthetase (GS) by cortisol has been shown to occur in monolayer cultures of cells obtained by enzymatic dissociation of retinas from 8- and 12-day-old chick embryos with papain (0.1%) or trypsin (0.25%). Although essentially single cells when plated, monolayers obtained by enzymatic dissociation show significant aggregation by 4–6 h. Monolayers prepared by mechanical dispersion (cells forced through successively smaller gage needles) are minimally inducible, perhaps owing to poor viability in such cultures.

Storage at 4°C for 24 h prior to treatment with cortisol significantly elevated both basal GS activity and inducibility in whole (but not in monolayer) retina cultures.

Index Entries: Retina, chick embryo; tissue dissociation; cell cultures; chick embryo; glutamine synthetase; induction, of glutamine synthetase; papain; trypsin; acetyltrypsin; mechanical dissociation.

Introduction

The enzyme glutamine synthetase (GS) has a characteristic developmental pattern in chick embryo retinas that coincides temporally with the onset of

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morphological changes indicative of retinal differentiation: the low basal level of GS rises sharply on days 16–17, continues to increase at a lesser rate for some days, and levels off soon after hatching (1–5). GS can be induced precociously by corticosteroids and structurally related compounds both in ovo and in vitro (6–9). This induction appears to occur only after a specific state of retinal differentiation has been reached (10, 11); it seems to be under transcriptional control and involves synthesis of new enzyme protein (12–14).

An interesting question in this system has been whether individual (possibly specific) cells of the retina are capable of glutamine synthetase induction in the absence of specific cell–cell contacts and interactions. Recent studies have localized GS in glial cells in brain (astrocytes) (15), and retina (Müller cells) of several species including chick (16–19). In investigating this question we had to consider that the methods used to dissociate the tissue might themselves adversely affect the mechanism of enzyme induction. Using three different methods of tissue dissociation, we have found that under appropriate experimental conditions, monolayer cultures of retinal cells can be induced appreciably for glutamine synthetase activity; decreased inducibility in cultures of dissociated cells reported by others (20, 21) could possibly result from experimental differences rather than the inherent inability of individual cells to respond to signals that elicit enhanced GS synthesis.

Methods and Materials

Whole retinas (WR), primary monolayer cultures (ML), and subcultured retinal cells were maintained with or without cortisol (0.1 μg/mL = 2.8 × 10⁻⁷ M) for 24, 48, or 72 h beginning either at the time the cultures were initiated or at various later times.

In some experiments retinas were induced for GS activity in ovo (as previously described) (22) prior to being utilized in dispersion and in vitro induction experiments.

Retinas from 12-day-old chick embryos were used except where otherwise specified. Whole retinas were cultured in 50-mL Erlenmeyer flasks, as previously described (14), with the exception that Eagle’s minimal essential medium (MEM) containing 10% dialyzed fetal calf serum (dFCS), 100 μg/mL of gentamycin, 100 μg/mL of kanamycin, and 0.5% glucose was used (“complete medium”). Monolayer cultures were prepared by use of proteolytic enzymes (papain, trypsin, or acetyltrypsin) or by mechanical dispersion. Thirty retinas (at a time), free of pigment epithelium, were excised aseptically and dissociated at room temperature by slow magnetic stirring in 10 mL of Ca²⁺-, Mg²⁺-free Hank’s balanced salt solution (HBSS), containing 0.1% DNase and either 0.1% papain or 0.25% trypsin or acetyltrypsin; this treatment was continued until a single cell suspension was