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MODULATION OF CORTISOL RECEPTORS IN EMBRYONIC RETINA CELLS BY CHANGES IN CELL-CELL CONTACTS: CORRELATIONS WITH INDUCTION OF GLUTAMINE SYNTHETASE

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SUMMARY

Cortisol induces glutamine synthetase (GS) in neural retina tissue of chick embryos. GS induction represents a characteristic feature of embryonic retina differentiation. However, if the tissue is dissociated into single cells, the dispersed cells are not inducible for GS. We report that cell dispersion results in a rapid and marked reduction in the level of cortisol-binding cytoplasmic receptors. This reduction persists if the cells are maintained in a dispersed state. However, if the cells are reaggregated and they reconstruct tissue-like contacts and architecture, the level of cortisol receptors increases, and so does inducibility for GS. The results indicate that, in the embryonic neural retina histotypic cell contacts and interactions are involved in regulating the level of cortisol receptors. We propose that cell contact-dependent signals from the cell surface may modulate levels of cytoplasmic cortisol receptors necessary for GS induction.

Induction of glutamine synthetase (L-glutamate; ammonia ligase, EC.6.3.1.2.), referred to as GS, is a differentiation-marker in the embryonic neural retina and has been studied in detail in the retina of chick embryos (1, 2). GS can be readily induced in organ cultures of isolated retina tissue from 10-day chick embryos by cortisol and other closely related 11-β-hydroxycorticosteroids (3, 4). The induction involves differential gene expression elicited by the hormone (5-7) and results in rapid synthesis and accumulation of the enzyme (2, 6). As in other target tissues (8-10), also in the retina cortisol binds to cytoplasmic receptors to form complexes which are translocated into the nucleus and bind to chromatin (11-14). GS is induced and localized specifically in Müller cells, which are the glia elements of the retina (15).

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While GS is rapidly inducible in cultures of isolated whole retina tissue, it is not inducible in dissociated and dispersed retina cells plated as monolayer cultures, or maintained as a single-cell suspension (15, 16). However, if the dispersed cells are reaggregated so that they reconstruct retina-like tissue architecture (17), they regain inducibility for GS (15, 16). This suggested that specific contacts and interactions between Müller cells and neurons, such as exist within retina tissue, regulate responsiveness to the hormonal induction of GS (2). Among the candidate-mechanisms for such regulation are the cortisol receptors. In this report we present data which show that dispersion of retina cells results in marked reduction of cortisol-binding receptors, and that cell reaggregation reverses this reduction.

MATERIALS AND METHODS

Cultures of retina tissue: Retinas were dissected aseptically from 10-day chick embryos and the intact tissue was cultured in Erlenmeyer flasks (4 retinas per flask) in Medium 199 (with Hanks' salts) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin mixture. The flasks were gassed with 5% CO_2-95% air mixture and incubated at 37°C on a rotary shaker (70 rpm).

Monolayer cultures: Suspensions of dissociated retina cells were prepared by the standard trypsinization procedure (19, 20), with the following modifications. After treatment with trypsin the tissue was washed with Tyrode's solution that contained soybean trypsin inhibitor (300 µg/ml) and Trasylol (20 KIU/ml), then rinsed in Medium 199 with soybean trypsin inhibitor, Trasylol and DNase (50 µg/ml) and dispersed into a single cell suspension in this solution. The cells were plated in Falcon culture dishes with Medium 199 (150×10^6 cell/dish). 10% FBS was added 2 hours later. Cultures were maintained at 37°C in a humidified incubator in a 5% CO_2-95% air atmosphere.

Cell aggregation: Cells were aggregated as previously described (16, 19). Suspensions of freshly dissociated retina cells were dispersed into 25 ml Erlenmeyer flasks with 3 ml medium (27×10^6 cells/flask), and were swirled on a rotary shaker (70 rpm, 37°C) for 24 hrs or longer.

GS induction: GS was induced by cortisol (hydrocortisone; free base) added to cultures at a final concentration of 900nM (3, 4). Enzyme activity was assayed as previously described (21); specific GS activity was expressed as µmoles of γ-glutamyl-hydroxamate formed per hr per mg of tissue protein at 37°C.

Cytosol preparation: Tissue or cells were washed with TMC-MTG homogenization buffer (0.02 M Tricine, 0.002 M $MgCl_2$, 0.001 M $CaCl_2$ and 12 mM monothioglycerol), and disrupted in a Kontes homogenizer in the same buffer. The homogenate was centrifuged at 140,000xg for 1 hr at 0°C and the supernatant used as cytosol.

TABLE I

GS Specific Activity

	0 hr	24 hr		48 hr		72 hr	
		+ cortisol	- cortisol	+ cortisol	- cortisol	+ cortisol	- cortisol
Retina tissue: organ cultures	1.98 ± .15	15.09 ± 1.35	1.53 ± .21	25.87 ± 2.23	3.04 ± .97	34.66 ± 3.01	3.62 ± 1.01
Monolayer cultures of retina cells	2.02 ± .53	1.67 ± .42	1.40 ± .39	2.43 ± 1.02	2.01 ± .99	1.98 ± .66	1.63 ± .87
Aggregates of retina cells	NA	3.05 ± 1.01	1.37 ± .50	16.73 ± 2.08	3.15 ± 1.12	20.22 ± 2.83	2.69 ± 1.00

Cultures treated, or untreated with cortisol were harvested after 24 or 48 hrs and assayed for GS specific activity. 72-hr cultures were treated with cortisol during the last 48 hrs. 0-hr samples were assayed immediately after tissue dissection or cell dissociation. Results represent averages from 8 experiments. NA - not applicable.

Receptor activity assay: Receptor-hormone complexes were formed by incubating retina cytosols with 1 x 10^{-8}M tritiated cortisol (1,2,6,7-[^{3}H]cortisol, 95 Ci/mM) in the absence(A) or presence(B) of 2.5 x 10^{-6}M unlabeled cortisol at 0°C for one hr (12). Cortisol-binding was determined by charcoal assay (22, 23). The amount of specific receptor activity was measured by the difference of charcoal-resistant radioactivity between tubes A and B, and was expressed as DPM of bound ^{3}H-cortisol per mg protein.

RESULTS

GS inducibility in retina tissue, cell monolayers and cell aggregates: Table I shows that responsiveness of retina cells to GS induction depends on their state of histological association, as previously reported (15, 16). In cultures of whole retina tissue, cortisol induced multifold increases of GS specific acitvity. In dispersed cells maintained in monolayer cultures no significant increase of GS was elicited by cortisol. In cell aggregates GS was inducible by cortisol. Aggregating retina cells take approximately 24 hrs to reconstruct retinotypic tissue architecture (17); significantly, during this period the level of GS increased only slightly, but subsequently rose to more than 60% of that in intact tissue.

Cortisol receptors in retina tissue and in dispersed cells: Measurements of specific cortisol-binding receptor activity in cytosols prepared from whole retina tissue and from dispersed retina cells showed that, soon after the cells were dissociated, their level of receptor activity declined by about

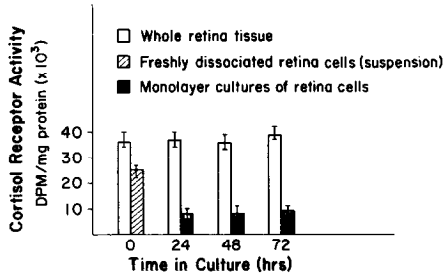

Fig. 1: Cortisol receptor activity in cytosols from whole retina tissue and from dispersed retina cells. Receptor activity was measured at 0 hr and after 24-72 hrs in culture.

25-30%, compared with intact tissue (Fig. 1). In dispersed cells maintained in monolayer culture for 24 hrs or longer receptor activity declined by 75-80% and persisted at this low level. In contrast, in cultures of intact retina tissue receptor activity remained at the original high level.

Since the cells were dissociated in trypsinization, the reduction of receptors in the dispersed cells might have been caused by residual trypsin, even though the cells were thoroughly rinsed with trypsin inhibitors. To test this possibility, retinas were dispersed into cells without using trypsin, by treatment with the calcium-chelating agent EGTA (ethyleneglycol-bis-tetraacetic acid; 0.025% for 25 min., at 37°C). The level of cortisol receptors declined sharply also in cells dispersed with EGTA (Table II), and GS was not inducible in monolayer cultures prepared from these cells. Therefore, the use of trypsin for cell dissociation is not the primary cause for receptor loss in the separated cells, or for their not being inducible.

Another possibility was that the low level of receptor activity in cytosols from dispersed and from monolayer-cultured cells was due to release during cell homogenization of endogenous proteases which inactivated these receptors. To test this possibility, cells from 24 hr monolayer cultures were homogenized in buffer with added DFP (diisopropylfluorophosphate; a potent protease inhibitor previously used in similar studies, 24). However,

TABLE II

Measurements of Cortisol Receptors in Cytosols
(specific cortisol-binding activity)

SOURCE OF CYTOSOL	0 hr		after 24 hrs in culture	
	Activity (DPM/mg protein)	% of Activity	Activity (DPM/mg protein)	% of Activity
Whole retina tissue	35850 ± 2867	100	36312 ± 2484	101
Trypsin-dissociated retina cells (0 hr) and monolayer cultures (24 hrs)	24844 ± 2004	70	8262 ± 682	23
EGTA-dissociated retina cells (0 hr) and monolayer cultures (24 hrs)	25984 ± 904	73	10361 ± 594	29
Monolayer cultures homogenized in buffer with 2.5 mM DFP	NA	NA	8320 ± 732	23
Monolayer cultures homogenized in buffer with 5.0 mM DFP	NA	NA	8273 ± 784	23

Retina tissue was dissociated into cells following treatment with trypsin or EGTA; cortisol receptor activity was determined in cells and cultures and compared with activity in freshly isolated whole retina tissue (100%).
NA - not applicable.

inclusion of DFP in the cytosol preparation did not result in significantly higher levels of receptor activity (Table II).

Receptor activity in cell aggregates: The above results suggested that the decline in the level of cortisol receptors might be due to disruption of normal cell-cell contacts and to separation of the cells. This implied that restitution of such contacts might reverse the decline. This possibility was tested by reaggregating the dispersed cells, i.e., allowing them to re-establish histotypic juxtapositions and relationships. The process of cell aggregation involves formation of multicellular clusters in which the different cell types gradually become sorted out, associated and organized in a pattern typical of normal retina tissue architecture (17, 18). In such aggregates, inducibility for GS increases coordinately with their advancing histological organization (2, 16).

Fig. 2 shows that the level of cortisol receptor activity increased progressively in aggregating cells and in cell aggregates; in 72 hr aggregates

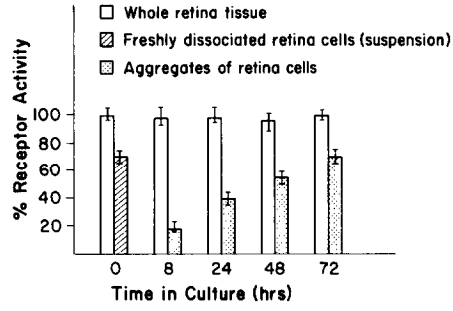

Fig. 2: Cortisol receptor activity in cytosols from whole retina tissue and from aggregates of retina cells after various culture times. Activity (DPM/mg protein) is expressed as percent of the level present in freshly dissected whole retina tissue.

receptor activity rose to 65-75% of that found in intact retina tissue cultured for the same time. Therefore, in contrast to dissociated cells maintained in a dispersed state, in histologically reassociated cells there was "recovery" of receptor activity and of inducibility for GS.

DISCUSSION

Our results show that, when embryonic neural retina cells are dissociated from their tissue framework and maintained in a dispersed state, there is a very rapid and marked reduction in the level of cytoplasmic receptors for cortisol, concommitantly with loss of inducibility for GS. When the dispersed cells are made to reaggregate and reconstruct three-dimensional tissue-type associations, receptor decline is reversed and followed by a substantial increase in receptor level and in inducibility for GS. We interpret these results as suggesting that cell-cell contacts are involved in the maintenance of cytoplasmic cortisol receptors in embryonic retina cells. It cannot be stated with certainty that the loss of GS inducibility in dispersed retina cells is due directly or exclusively to the decline in the level of cortisol receptors; since this decline is not complete, and since still other intracellular changes may be triggered by cell separation, the relationship between these two effects requires further investigation.

The exact cause for the reduction in the level of cortisol receptors in dissociated and separated retina cells is presently unknown. The hypothetical possibility of "selective leakage" of these receptors was considered; however, various attempts to detect and to prevent it (if it occurs), failed to provide support for such an interpretation. Our present working hypothesis is that cortisol receptors (receptor activity) in embryonic retina cells is susceptible to regulation by specific cell-cell contacts. The possibility that cytoplasmic hormone receptors are modulated by changes in contact-relationships between cells is consistent with the concept that specific cell contacts and interactions play an important role in the control of macromolecules involved in cell differentiation (for other examples see 26-28).

There is much evidence that various cell functions are affected by modifications of the cell surface due to changes in cell contacts or in cell shape (29). It is a reasonable assumption that cell separation alters cell surface characteristics, and that such alterations signalled to within the cells might trigger changes in internal control mechanisms. Loss or inactivation of cortisol receptors due to cell separation may represent an example of this. It is also conceivable that cell separation prevents some metabolic cell interactions which are mediated by cell contacts or cell junctions, and which have a regulatory role in differentiation mechanisms. In the retina, such contact-dependent cell interactions may be required for maintaining cortisol receptors at a level necessary for GS induction.

While these and additional interpretations remain to be explored, our results have demonstrated that, in the embryonic retina, histotypic cell-cell associations are involved in maintaining the stability (activity) of cortisol-binding cytoplasmic constituents. It would be of interest to determine if a similar situation exists in other tissues in which specific gene expressions are regulated by steroidal inducers.

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