Temporal Changes in Embryonal Cell Surface Recognition

(plasma membrane/neural retina/optic tectum)

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ABSTRACT Plasma membranes have been prepared from optic tectum and neural retina of chick embryos. These membranes specifically inhibit the aggregation of homotypic cells. Plasma membranes prepared from each of these regions obtained from chick embryos at 7, 8, and 9 days of development show specificity for homotypic cells of the same age and cross-react weakly with homotypic cells of different ages. Thus cell-surface recognition changes with age of development, and most of the cells in each region have a common cell-surface recognition specificity. Tectal cells react weakly with retinal cell membranes, but no inhibition of retinal cell aggregation by tectal cells has been noted.

In a previous communication (1) we have shown that neural retina cells from 8-day-old chick embryos specifically bind plasma membranes obtained from the same cells, but do not bind to cerebellar cell membranes. The binding of homotypic membranes to cells prevents cellular aggregation, both in the case of retinal membranes and retinal cells and cerebellar membranes and cerebellar cells. One may consider two possible situations during development; the first assumes that a given region shows invariant surface specificity and the second that specificity changes with time of development. We report here evidence for the second of these possibilities, using both retinal and tectal cells, strongly suggesting that in these tissues cell-surface changes are a part of normal development. On the basis of studies of membrane inhibition of cell aggregation as a function of development in various regions of the embryonal nervous system we can now operationally define a minimum of eight cell-surface specificities in the chick between the seventh and the ninth day of development.

MATERIALS AND METHODS

Plasma membranes were prepared from isolated single cells as described previously (1), or from whole tissue without trypsinization. In the latter case 40 neural retinas or 20 to 30 tecta were suspended in 10 ml of CMF-CS and the tissue dispersed by aspirating through a pasteur pipette. [CMF, CMF-CS, and CMF-A are Ca^{++} - and Mg^{++} -free Hank's solution unsupplemented, supplemented with 10% heat-inactivated chicken serum, or with 5 mg/ml of bovine serum albumin, respectively. Both solutions were buffered with 0.02 M *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (Hepes), pH 7.4.] The tissue was collected by centrifugation at $300 \times g$ for 5 min at 25° and washed twice by centrifugation with 10 ml of CMF-A. The tissue was finally dispersed in 8 ml of CMF-A

containing 50 $\mu\text{g}/\text{ml}$ of DNase I (Sigma) and broken with 50 strokes of a Dounce type B homogenizer. Plasma membranes were prepared from the homogenate as described previously (1) and dispersed by sonication with a model W140 Branson Sonifier with micro probe for 10 sec at setting 2.5 in 2 ml of CMF at 0° . Excessive sonication will destroy membrane activity.

Cell aggregation assays were carried out by a micromodification of the assay used previously. A cell suspension (0.5 ml containing 5×10^4 cells per ml) in CMF-A was placed in a 5-ml micro Fernbach flask with cell membranes as indicated and incubated at 37° in a New Brunswick rotatory shaker model G76 at 100 rpm. The fraction of single cells remaining was determined at times indicated with a hemocytometer.

RESULTS

Although previous work was carried out exclusively with neural retina and cerebellum, it seemed desirable to extend these observations to other tissues and in particular to one which was available in larger quantities than 8-day cerebellum. In Fig. 1 (*bottom*) we show that plasma membranes obtained from 8-day optic tectum inhibit the aggregation of tectal cells, but even concentrations much higher than those required to inhibit tectal cell aggregation have no effect on the aggregation of neural retina cells. The reciprocal experiment is also shown in Fig. 1 (*top*); neural retinal plasma membranes inhibit retinal cell aggregation and at higher concentration show a small but highly reproducible inhibition of tectal cell aggregation. These data suggest that retinal plasma membranes contain molecules which are recognized by tectal cells, and that these molecules either occur rarely on the retinal cell surface, or if they occur in equal numbers to the retinal recognition sites, they have a poor affinity for tectal cells. These assumptions are necessary because concentrations of retinal membrane that totally prevent homotypic cell aggregation have only a small effect on tectal cell aggregation.

It is relevant to point out that in a very interesting paper Barbera *et al.* (2) have shown that isolated retinal cells will adhere to the intact optic tectum, and that selective adhesion can be demonstrated between cells of the dorsal and ventral regions of the retina, which adhere preferentially to the ventral and dorsal regions of the optic tectum. These observations were made with 8- and 12-day-old neural retinal cells adhering to 12-day tectal fragments, and are therefore not directly comparable to our results.

The specific molecules involved in recognition are not known. The data in Fig. 2 show that the treatment of neural retinal plasma membranes with crystalline trypsin abolishes their ability to inhibit cell aggregation, suggesting that one

Abbreviations: CMF, CMF-CS, and CMF-A, Ca^{++} - and Mg^{++} -free Hank's solution unsupplemented, supplemented with 10% heat-inactivated chicken serum, or with 5 mg/ml of bovine serum albumin, respectively.

FIG. 1. Time course of cell aggregation in the presence of membranes. The top portion of the figure shows the time course of cell aggregation of 8-day retinal and tectal cells in the presence of retinal membranes. $\circ$ — $\circ$, no added membranes; $\bullet$ — $\bullet$, 31 μg of retinal membrane protein; Δ — Δ , 62 μg of retinal membrane protein. The bottom of the figure shows similar data with tectal membranes with the quantity of membrane protein indicated.

of the membrane components involved in cell recognition is a protein. Similar sensitivity to trypsin has been noted with membranes prepared from the optic tectum.

In contrast to the optic tectum, which reproducibly yields cells which aggregate well, cells obtained from the telencephalon frequently aggregate poorly although they show specificity for homotypic membranes.

In Fig. 3 we show the results of examining the inhibition of aggregation of neural retinal cells by membranes obtained from isolated 8-day retinal cells allowed to recover from trypsinization (1). The membranes show striking preference for inhibition of the aggregation of 8-day retinal cells. Even at higher concentrations than required to inhibit the aggregation of 8-day cells, the aggregation of 7-day retinal cells or the aggregation of 9-day retinal cells is only partially inhibited,

FIG. 2. Effect of trypsin on membrane activity. A suspension (0.1 ml) of 8-day retinal membranes (0.1 mg of protein) in CMF was incubated for 10 min at 37° with 0.1 mg of crystalline trypsin. Soybean trypsin inhibitor (0.25 mg) was added and the membranes assayed for their ability to inhibit aggregation of 8-day retinal cells. $\circ$ — $\circ$, no addition; $\blacktriangle$ — $\blacktriangle$, intact membranes (0.1 mg protein); $\bullet$ — $\bullet$, trypsin-treated membranes; Δ — Δ , membranes treated with a preincubated mixture of trypsin and trypsin inhibitor.

FIG. 3. Effect of retinal cell age on membrane inhibition. Neural retinal cells were obtained from 7-, 8-, and 9-day embryos. The cells were allowed to aggregate for 30 min under standard conditions either alone, or with the indicated concentration of 8-day retinal membranes. The data show the percent inhibition of aggregation by the addition of membranes. Cells (80–90%) aggregate in 30 min in the absence of membranes.

suggesting that only a fraction of these cells recognize the membranes prepared from 8-day cells.

These observations suggest that the cell surface recognition specificity changes rapidly during development. A less likely possibility is that different cell populations are extracted as single cells from 7-, 8-, and 9-day retina. We have examined for this latter possibility in two ways. First we have prepared a plasma membrane fraction from the residual tissue after preparation of single cells by trypsinization and shown that it has the same temporal specificity as plasma membranes obtained from single cells (data not shown), and second we have prepared plasma membranes from whole tissue (Fig. 4) and they clearly show the same temporal specificity as membranes prepared from isolated single cells.

The data in Fig. 4, obtained with a large excess of membranes so as to maximize cross-reactivity, show that plasma membranes prepared from 7-, 8-, and 9-day neural retina show high specificity for inhibiting aggregation of cells obtained from tissue of the same age. They also show preference for tectal cells obtained from embryos at the same age as the neural retina from which the membranes were prepared. Thus

FIG. 4. Effect of embryonal age on membrane specificity. All experiments were carried out with membranes prepared from whole neural retina, obtained from 7-, 8-, or 9-day-old embryos. The membranes were used to inhibit aggregation of either retinal or tectal cells of the ages indicated. One hundred micrograms of membrane protein was used in each assay to maximize cross-reactivity.

FIG. 5. Age specificity of tectal cells. In the *left panel* is shown the inhibition of cell aggregation obtained with 8-day tectal membranes (90 μ g of protein) using 7-, 8-, and 9-day-old tectal cells, and 8-day cerebellar cells. In the *right panel* is shown the inhibition obtained at two different protein concentrations when 9-day tectal membranes were tested with 7- and 9-day tectal cells.

during this period of development the changes in surface specificity of the retina and tectum are synchronous.

Fig. 5 shows that tectal plasma membranes also show temporal specificity, and 7-, 8-, and 9-day tectal cells are clearly distinguished by 8- and 9-day tectal plasma membranes. In addition, cerebellar cell aggregation is not inhibited by tectal cell membranes.

DISCUSSION

The observations presented in this paper show that the surface specificity responsible for homotypic cell aggregation changes rapidly during development. It is at the moment very difficult to decide unequivocally whether these changes reflect changes in cell type or changes in the surface of preexisting cells. Detailed studies have been carried out on the development of the neural retina (3-5) and optic tectum (6, 7) in the chick embryo between day 7 and 9, and these data would suggest that the majority of the neuronal cells have ceased to divide by day 7, while glial cells continue to proliferate. Although the fraction of the total cells in the tissue that have ceased to divide is not clear, the sum of the available evidence suggests that some of the observed changes must represent changes in the surface of preexisting cells rather than the generation of new cells. In the neural retina this is the time period in which the inner and outer plexiform layers are being formed, and it is perhaps not surprising that this massive production of cell-surface membranes is accompanied by a change in cell-surface specificity.

It may seem surprising that different cell types in one tissue retain a common surface specificity, but this observation is not without precedent. Elegant studies by Goldschneider and Moscona (8) have shown that neural retinal cells have common antigenic determinants, which differ from those of any other neural tissue. The antigenic determinants detected by this work are presumably different from those reported here, since they are present in neural retina from 5-day-old chick embryos as well as from 28-day-old chickens.

The cell-surface specificity that is detected by the membrane assay is presumably important in histogenesis and is too

general to be responsible for the much more precise specifications required for synaptogenesis. It is nevertheless highly interesting that retinal cell surfaces are recognized by the optic tectum, just as intact retinal cells are recognized by the tectum (2). This recognition shows polarity because retinal cells do not recognize tectal membranes. This polarity mimics the fact that the retina will send axons to the tectum, although no direct relationship between the two events has been established. Interestingly enough, cross reactivity between the two tissues appears to be synchronous between day 7 and 9.

It is important to make a distinction between aggregation factors and the phenomenon we have been investigating. Aggregation factors in sponges appear to be multivalent glycoproteins acting as ligands between cells of the same species (9-12). Aggregation factors obtained from chick embryos by Moscona and coworkers are obtained from cells in culture (13, 14) and enhance the size of 24-hr aggregates. In our system we are looking at initial recognition which occurs on a much shorter time scale, and there is no obvious relation between the two types of measurements. We should note, however, that an aggregation factor of the type seen in sponges, if not released by the cell membrane, would simply become a specific membrane recognition site. Interesting differences in size and pattern of cell aggregates from neural tissues have been noted by Garber and Moscona (15, 16) as a function of embryonal age. It is also known that the agglutinability of embryonal cells with lectins changes with age (17). The differences reported in this paper, to the best of our knowledge, are the first indication of rapid changes in cell surface specificity during development.

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