SHORT PAPERS

Cell disposition and adhesiveness in the developing chick neural retina

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SUMMARY

The adhesive properties of neural retinal cells located in the ventral and dorsal hemispheres of chick embryonic eyes were investigated. Cell adhesion was monitored using the collision efficiency method; this technique provides a system in which it is possible to identify any preferential adhesions that may occur between cells. In this study no adhesive specificity was detected between cells of the dorsal and ventral retina. This evidence would not support theories which invoke preferential cell adhesion as an explanation of the ordered projection of the neural retina onto the optic tectum during development.

INTRODUCTION

The ordered projection of the neural retina onto the optic tectum (for a review see Gaze, 1970) is generally thought to require some mechanism of selectivity in synapse formation. Sperry (1943, 1965) has proposed that individual neurons possess distinct molecular assemblies at the cell surface which participate in intercellular contact and allow for recognition of proper termination sites. The specificity of retinotectal connexions is at present a matter of some dispute as some experiments point to rigid specificities, while others suggest that the system is rather plastic (Gaze & Keating, 1972; Hunt & Jacobson, 1974). Based on the hypothesis that intercellular adhesion may also mediate cellular recognition (Roth, 1973), Barbera, Marchase & Roth (1973) have shown that the preferential adhesion of neural retina cells to dorsal and ventral optic tectum halves mimics the retinotectal projection found in vivo. This evidence supports an interpretation of neuronal specificity dependent on cell surface adhesive properties (Marchase, Barbera & Roth, 1975).

It is clear that if such a situation exists then cells originating from differing regions of the developing neural retina should display differing adhesive properties. Evidence for such has been presented by Gottlieb, Rock & Glaser (1976) who studied intercellular adhesion in the chick retina. They reported

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the detection of a dorsoventral gradient of adhesive specificity such that the strongest affinity was observed between cells derived from the extremes of the gradient. This finding lends strong support to a model presented by Marchase et al. (1975) relating retinotectal specificity to preferential adhesion.

In this communication I report that, using a cell adhesion assay very different from that employed by Gottlieb et al. (1976), I was unable to confirm the existence of a dorsoventral gradient of adhesive specificity in chick embryo neural retina.

MATERIALS AND METHODS

Preparation of cells

Neural retina tissue from 8-day chick embryos was obtained by careful dissection of the appropriate segment, using the choroid fissure as a guide to the orientation of the eye. Each eye was bisected into dorsal and ventral halves and the retinal tissue adjacent to this cut was discarded. The remaining material, which was retained for use in the experiments, normally comprised 60% of the neural retina most distal from the line of bisection (Fig. 1). Tissue dissociation was performed using two procedures. The first was that described by Gottlieb et al. (1976) in which the disaggregating agent is 600 μg/cm³ trypsin (Difco 1:250) in the presence of 10% heat-inactivated chicken serum and 25 μg/cm³ DNase I (Sigma). The second procedure (Jones, 1974) uses 1 x 10⁻³ M EDTA alone as the disaggregating agent. In both techniques gentle trituration through a Pasteur pipette was sufficient to give cell suspensions with viabilities in excess of 85% as judged by trypan blue exclusion tests. Cell suspensions were forced through nylon cell sieves (Nitex) of 15 μm pore size to ensure that the majority of cell clumps were removed. The cell concentration was determined by haemocytometry and appropriate dilutions made to bring the total particle count to 1 x 10⁶ particles/cm³ in Hanks’ saline.

Suspensions of dorsal neural retina cells (DNR) and ventral neural retina cells (VNR) were either aggregated separately or mixed in a 1:1 ratio by volume prior to aggregation. In all cases cell re-aggregations were performed at 37 °C in Hanks’ saline for 35 min.

Cell adhesion assay

The adhesiveness of the various cell populations was measured using the collision efficiency method (Curtis, 1969; Jones, 1974). In this technique, cells are re-aggregated in a Couette viscometer and samples taken at intervals for calculation of the decline in total particle count with time. A constant shear rate of 10⁻⁶ sec⁻¹ was maintained in the aggregation medium during the course of each experiment. Knowledge of the kinetics of aggregation allows one to calculate a parameter termed the collision efficiency (α) which is a measure of the probability of a collision between two particles resulting in their adhesion.
Cell adhesion in chick neural retina

Fig. 1. Dissection of chick eye. Embryonic eyes were first cut in half perpendicular to the dorsoventral axis (——) and neural retinal tissue from the area around this cut was discarded. Only tissue from the more distal areas of the retina (shaded) was collected for subsequent use as experimental material.

Specificity of adhesion was examined by the technique given by Curtis (1970a, b). In this test, the collision efficiency for the two cell populations is measured for each type separately and then for a 1:1 mixture of the two cell types. If there is no specificity of adhesion the collision efficiency for the mixed cell population will be the mean of the collision efficiencies for both types measured separately. If there is complete specificity such that homologous interactions are preferred to heterologous, then the measured collision efficiency for the mixed cell population will be reduced to 50% of the value for those cell types aggregated separately. If heterologous adhesions are preferred there will be a similar increase in collision efficiency for the mixed cells over that measured for the separate cell types. An advantage of this technique is that no labelling of cells is necessary, as identification of individual cells participating in the aggregation is not required.

RESULTS

Each cell population (DNR, VNR and the 1:1 mixture of DNR and VNR) was examined in eight separate experiments. Of these, four from each group tested cells that had been previously disaggregated with EDTA, the remaining four from each group being trypsinized cell populations. The results are given in Table 1.

Comparison of the data shows that trypsinized cells are less adhesive than cells treated with EDTA; this finding is not unexpected in view of the well documented effect of trypsin on the rate of cell aggregation (Steinberg, Armstrong & Granger, 1973). The lag period for recovery from trypsinization under
Table 1. Aggregation of neural retina cells

Cell adhesiveness is expressed as collision efficiency (\(a\)) %. All experiments were performed at 37 °C in Hanks’ medium at a shear rate of 10·64 sec\(^{-1}\).

<table>
<thead>
<tr>
<th>Disaggregation agent</th>
<th>Collision efficiency (%) and s.d.</th>
<th>(iii) DNR + VNR (1:1 mixture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(i) DNR</td>
<td>(ii) VNR</td>
</tr>
<tr>
<td>EDTA</td>
<td>16·81</td>
<td>16·43</td>
</tr>
<tr>
<td></td>
<td>s.d. 1·23</td>
<td>s.d. 0·41</td>
</tr>
<tr>
<td></td>
<td>(i)–(ii) (P &gt; 0·01), (i)–(iii) (P &gt; 0·01), (ii)–(iii) (P &gt; 0·01)</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>6·84</td>
<td>6·99</td>
</tr>
<tr>
<td></td>
<td>s.d. 0·54</td>
<td>s.d. 0·71</td>
</tr>
<tr>
<td></td>
<td>(i)–(ii) (P &gt; 0·01), (i)–(iii) (P &gt; 0·01), (ii)–(iii) (P &gt; 0·01)</td>
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Probabilities (\(P\)) are derived from Student’s \(t\) test.

the conditions given above is normally 10 min, but cells do not recover their maximal adhesiveness for some time after this. Pre-incubation of trypsinized cells for 20 min prior to aggregation raised the collision efficiency to around the 10 % value (unpublished findings), but these results may be unreliable as it was often necessary to redisperse these cell suspensions on a whirlimixer in order to start the aggregation with a single cell population.

Whatever the conditions for tissue dissociation it was observed that no significant differences could be measured between the collision efficiency for the mixed DNR and VNR cell population and that of either pure cell population (Table 1). If the results of Gottlieb et al. (1976) were to be confirmed, the collision efficiency of a 1:1 mixture of DNR and VNR would need to be greater than the collision efficiency of either alone. It may be concluded that, using this assay system at least, no dorsoventral gradient of adhesive specificity in the developing neural retina has been found.

**DISCUSSION**

The results presented in this communication conflict with those given by Gottlieb et al. (1976) which suggest the existence of adhesive specificities between cells of the dorsal and ventral retina. The most obvious reason for this discrepancy must lie in the nature of the adhesion assays used in the two studies. The collision efficiency method is a refinement of adhesion assays based upon measuring the kinetics of cell aggregation from a single cell suspension. The success of aggregation kinetics in measurements of cell adhesiveness may be seen by its use (with minor variations) by many workers in the field (see Curtis, 1973 for review). In their study, Gottlieb et al. (1976) use a variation of the cell adhesion assay of Walther, Ohman & Roseman (1973). In this system, radioactive isolated cells are incubated for various time periods with a confluent
monolayer and the rate of adhesion is determined from the amount of radioactivity present in the monolayer and adherent cells after removal of the cell suspension. In this type of assay, cell-substrate and cell–cell contacts within the monolayer may give rise to difficulties with interpretation of the results from the adhesion assay. This problem is acknowledged by Gottlieb et al. (1976) in a discussion of their findings. As the collision efficiency method requires all cells participating in the assay to be in a state of suspension, none of these complications arise and it may be argued that artifactual results are less likely to occur. A second possible explanation for the discrepancy between the two studies may lie in the period of time allowed to elapse between trypsin treatment of the cells and their use in the adhesion assays. Cell monolayers are prepared for the assay of Gottlieb et al. (1976) within 1 h of removal of the tissue from the embryo (Gottlieb & Glaser, 1975). At least half of this time is taken up in trypsinizing of the tissue and washing the resulting cell suspension before plating out. It is therefore probable that in both techniques, the time allowed for recovery of cells from trypsinization is roughly similar. This argument does not affect the results relating to EDTA-treated cells also investigated in this study.

If the conclusions presented in this communication are correct, then the model proposed by Marchase et al. (1975) must be considered an unlikely candidate for explaining neuronal specificity. There is however one way in which this model and my results may be reconciled. In both the monolayer and collision efficiency assays the majority of cells whose adhesion is studied are not the ganglion cells which project into the optic tecta, since these are a minor component of the total cell population of the retina. Further, those areas of the cell plasma membrane which are morphologically most closely involved with retinotectal connexions (the axons and growing tips) are not present at all in either adhesion assay. This situation is a result of the ripping away of ganglion cell bodies from their axons in the process of dissection of the neural retinae. Given that the plasma membrane is a fluid mosaic (Singer & Nicolson, 1972) and that there is a strong possibility of the segregation of specific molecules into discrete patches on the membrane, it would seem quite possible that any adhesive recognition molecules involved with retino-tectal specificity (Marchase et al. 1975) may be located only at the growing tip and axon. Thus any adhesion studies using only the retinal cell bodies would be expected to give negative results concerning gradients of adhesive specificity.

To follow this possibility further it would seem necessary to investigate the adhesive properties of ganglion cell axons alone. The feasibility of such an experiment is being considered at present.

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REFERENCES


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