Neural Induction in Amphibians
Transmission of a Neuralizing Factor

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Summary. The neural plates of very early neurula stages of Triturus alpestris were removed, the material which is released from the extracellular space between mesoderm and neural plate to the medium in which the embryos were dissected was isolated and extracted with phenol. The protein isolated from the phenol layer showed neural inducing activity. Proteoglycans isolated from the aqueous layer did not show such inducing activity. These results together with previously published experiments (Wilhelm Roux’s Arch 184:285–299) suggest that a neuralizing factor which is released from the mesoderm acts on the inner surface of the overlying dorsal ectoderm.

Key words: Neuralizing factor — Transmission — Mesoderm — Extracellular material — Triturus embryo

Introduction

During gastrulation the dorsal mesoderm induces in amphibians in the overlying dorsal ectoderm the neural plate, the primordium of the neural system. Substances which induce neural tissues have been extracted from amphibian embryos as well from heterologous tissues (Tiedemann et al. 1961; Tiedemann et al. 1963). A partially purified neuralizing protein from chicken embryos does not lose its inducing activity after covalent binding to BrCN-Sepharose particles, which prevents the factor from being taken up by the ectoderm cells (Tiedemann and Born 1978; Born et al. 1980). This suggests that the factor interacts with binding sites on the surface of the competent ectoderm cells. After centrifugation of a homogenate from Xenopus gastrulae some neutralizing factor is found in the 100,000 × g supernatant. This factor could be derived from the cytosol, but could also include factor which may be transmitted through the extracellular space from mesoderm to competent dorsal ectoderm.

To test this hypothesis we have isolated material from the extracellular space between mesoderm and neural ectoderm and tested for its inducing activity.

Materials and Methods

Dissection of embryos, extraction and biological test of extracellular material.

Offprint requests to: H. Tiedemann at the above address
(1969). The amount of RNA was determined photometrically from the ultraviolet spectra.

**Histological Examination**

The embryos and sandwich explants were examined under the dissecting microscope, embedded in paraffin and stained with aniline blue-orange G for histological examination.

**Results and Discussion**

Neuroectoderm was dissected from very early neurula stages when the interspace between the inducing chordamesoderm and the reacting neuroectoderm still exists, so that few cells are damaged by the dissection procedure. The material from the extracellular space between chordamesoderm and the dorsal neuroectoderm which is released into the medium was extracted with phenol (s. methods). The protein fraction (which may also contain glycoproteins) isolated from the phenol layer has neural inducing activity (Table 1). A large portion of the induced neural tissue could not be assigned to certain regions of the neural system, but in two cases diencephalon and eye (archencephalic inductions) could be identified. To evaluate the inducing activity it must be taken into consideration that the very small amount of protein isolated from the extracellular material was diluted in a proportion of about 1:3 with an inert non-inducing protein (γ-globulin; Table 1) to handle the material. The protein nature of the neuralizing factor is also supported by earlier observations which have shown that neuralizing activity extracted from amphibian embryos is inactivated by proteolytic enzymes (Tiedemann et al. 1961).

It was shown that ectoderm can be induced by soluble factors (Becker et al. 1959) and in transfer experiments (Saxén and Toivonen 1962; Toivonen et al. 1975). Whether in the embryo direct cell contacts are involved or substances which migrate over short distances is however an open question. The experiments reported in this communication suggest that in normal development a factor is released from the mesoderm, probably by exocytosis. It is however possible that some factor remains loosely attached to the surface of mesodermal cells before it binds to the plasma membrane of ectoderm cells.

The material isolated from the extracellular space contains besides protein a relatively large amount of polysaccharides. The polysaccharides are enriched after phenol extraction in the aqueous phase and have no inducing activity (Table 1). They contain about 50–60% neutral sugars, 15% uronic acids and 5% aminohexoses suggesting the presence of proteoglycans besides neutral polysaccharides. We did not find RNA in the material from the extracellular space, thus indicating little contamination from broken cells.

It has been shown by light and electron microscopy that in late gastrulae tightly packed small metachromatic (Johnson 1977) granules (Toivonen et al. 1975), which probably contain hyaluronic acid (Tarin 1973) appear in the extracellular space between mesoderm and dorsal ectoderm and in other parts of the embryo. Chordamesoderm cultured for four days releases acidic macromolecules which incorporate [3H]leucine, [14C]glucosamine and [14C]acetate (Kaska and Triplet 1980). The function of these molecules is not yet known. The proteoglycans may facilitate adhesion and migration of cells but could perhaps also modulate the activity of inducing factors. It was shown in previous experiments that a proteoglycan which was isolated from chick embryos forms complexes with vegetalizing as well neuralizing inducing factors and thereby inhibits their inducing activity, when applied in larger excess (Tiedemann et al. 1969; Niebel et al. 1973). Whether complexes of the neural inducing protein with proteoglycans are involved in the transmission of the factor from chordamesoderm to the reacting dorsal ectoderm needs further investigation.

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**References**


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**Table 1. Inducing activity of protein and polysaccharides isolated from the extracellular space between mesoderm and dorsal ectoderm of early neurulae**

<table>
<thead>
<tr>
<th>Inducer</th>
<th>No. of cases</th>
<th>Positive %</th>
<th>Induced tissues (%)</th>
<th>Diencephalon</th>
<th>Eye</th>
<th>Unidentified brain and other neural tissue</th>
<th>Neuriod</th>
<th>Mesenchyme + melanophores</th>
<th>Frontal gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular Proteins (diluted with γ-globulin)</td>
<td>32</td>
<td>75</td>
<td>6*</td>
<td>3</td>
<td>44b</td>
<td>22</td>
<td>69</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Extracellular Polysaccharides (combined with γ-globulin)</td>
<td>25</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>γ-globulin (control)</td>
<td>61</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2c</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The extracellular protein was tested by the implantation method on gastrulae

* Medium size
b 13% medium size; 31% small size
c Two balancers were induced by the extracellular polysaccharides and one balancer by the γ-globulin control