In Vitro Culture of Serotonergic Neurons from Fetal Rat Brain

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Principle of Technique

Monoaminergic neurons are among the first to differentiate in the central nervous system (CNS) (4), and they innervate in a diffuse fashion many cortical and subcortical structures throughout the CNS. The exact function of this early innervation is poorly understood, although it appears through recent studies (3) that it may have some trophic effect on the multiplication and differentiation of target neurons.

Tissue culture studies are well-suited to analyze, in a simplified environment, the influence of serotonergic neurons on a target structure such as the hippocampus. A comparison of some parameters of growth and differentiation between separated cultures of 5-hydroxytryptamine (5-HT) neurons and their target, and cocultures of the two, may shed some light on their interaction (6).

Specific Instrumentation

Maximow Assembly

Maximow depression slide; Belgo Labo, Belgium; Rossignol, France

40 mm square cover slips; Chance Propper, U.K.

22 mm round cover slips; Chance Propper, U.K.

Dissecting Instruments

Dumont Forceps no. 7; Dumont (Switzerland)

Feather surgical blades no. 11; Feather Industries (Japan)

Inverted Microscope with phase contrast and interference contrast; Reichert—American Optical "Biovert"

Chemicals

Tissue Culture Medium

A. Explant cultures

Adult human serum; 30%; prepared in the lab

Minimum Eagle’s medium + glutamine, 40%; GIBCO

Hanks’ balanced salt solution (BSS), 30%; GIBCO + 600 mg/100 ml glucose.

B. Dissociated cultures

Adult human serum, 20%

Minimum Eagle’s medium + glutamine, 45%

10-day-old chick embryo extract, 5%

Hanks’ BSS, 20%

Nonessential amino acids, 10%

C. Collagen: prepared from rat tail tendons (2)

Histology

Electron Microscopy

Glutaraldehyde (50% biological grade); Polysciences

Paraformaldehyde; Merck

Uranyl acetate (“en bloc” staining); Prolabo

Light Microscopy (Golgi-Cox)

Potassium bichromate; Merck or Prolabo

Potassium chromate; Merck or Prolabo

Mercuric chloride; Merck or Prolabo

Potassium sulfite; Merck or Prolabo.

Procedure

Cocultures of Explants

Raphe explant. The brain of E 15–E 16-day embryos is dissected in Hanks’ BSS, and the region of the brain stem between the pontine and the mesencephalic flexures is cut out. After a median section, two explants are prepared from each embryonic brain, including roughly the B4-B9 groups. Each explant is plated on a collagen-coated cover slip (2).

Hippocampal explant. From the same embryonic brain, hippocampus primordia are dissected out, and one explant of hippocampus is plated close to each raphe explant. The cultures are then nourished with a drop of nutrient and sealed in the maximow assembly.
Control cultures consist of isolated explants of raphe and hippocampus.

Cocultures with Dissociated Raphe

Raphe explants prepared as above are pooled and mechanically dissociated with repeated pipetting and filtering through a steel sieve (40 μm mesh). The dissociated cells are plated on a collagen-coated cover slip on which a hippocampal explant has been already plated. Each cover slip receives the quantity of dissociated cells corresponding to one explant.

Control cultures consist of explants of hippocampus and dissociated raphe neurons grown separately.

Examination of Living Cultures

Cultures are examined daily under phase and interference microscopy, and representative samples are monitored for periods of several days with time-lapse microcinematography (5). This type of examination is particularly suited for dissociated raphe neurons, whose behavior in the presence and in the absence of specific target can be compared. Similarly, the out-
growth of raphe and hippocampus explants can be monitored under both conditions.

**Histological Procedures**

Golgi-Cox impregnation of explant cultures is performed according to Wolf and Dubois-Dalcq (8). Fixation for electron microscopic examination is carried out on representative cultures at progressive intervals after explantation, routinely from 4 to 40 days.

**Specific Histological Procedures**

In order to analyze the interaction between serotonergic neurons and their target at the cellular level, specific identification of the former are required. For this purpose, the following two techniques are used.

Specific uptake of 3H serotonin. Detailed technical data have been given elsewhere (1). Cultures are incubated for half an hour with a mixture of tritiated serotonin and cold noradrenaline. After several rinses in cold phosphate buffered saline (PBS), the cultures are fixed and processed for radioautography. Following radioautographic procedures, the cultures are dehydrated and mounted in toto in Permount, and examined with phase or interference contrast microscopy.

Immunocytochemical labeling of serotonergic neurons. Specific anti-5-HT (gift of Dr. Lauder) is used as a primary serum in the peroxidase-antiperoxidase technique of Sternberger (7). After fixation in a solution of 4% paraformaldehyde, cultures are incubated "in toto" for 48 hr at 4°C, and processed as indicated by Sternberger (7). Following immunocytochemical procedure, they are dehydrated in alcohol and xylene, mounted in Permount, and examined without counterstaining in interference-contrast microscopy.

**Critical Evaluation**

This in vitro approach is best suited to detect qualitative differences in the maturation of raphe and hippocampal neurons when grown separately and in coculture.

General cytoarchitecture is detected by Golgi impregnation and ultrastructural details of axonal and dendritic specializations can be analyzed with electron microscopy. Labeling of 5-HT neurons either with radioactive tracer or specific antiseraum, may allow a comparison of patterns of innervation with the in vivo condition.

However, such techniques are time consuming, as tissue culture experiments imply a number of incompletely controlled parameters that can be eliminated only by extensive sampling. Additional procedures such as radioautography and immunocytochemistry contribute to this pitfall. Moreover, quantitative estimates can hardly been obtained, for the above-mentioned reason, and this is a severe limitation of this approach.

**Literature Cited**

3. Lauder JM, Krebs H: Serotonin as a differentiation signal in early neurogenesis. Dev Neurosci 1:15, 1978