In vitro synthetic activity of the juvenile ovotestis of *Helix aspersa*: Influence of the brain and the dorsal bodies

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Summary. A bioassay was developed in *Helix aspersa* to study the effect of known endocrine centers on the two-month-old gonad, using organ culture. The incorporation of ^{14}C leucine and ^{3}H fucose in the juvenile gonad almost doubled over control levels, in the presence of the brain and the dorsal bodies.
Key words. Bioassay; organ culture; *Helix aspersa.*

To study the neuroendocrine control of reproduction in *Helix*, it is necessary to develop a rapid bioassay which will produce quantifiable and reproducible results. We have therefore utilized an in vitro organ culture technique to study the synthetic activities of the gonad using radioactive precursors [1]. The results from our laboratory have shown an increased activity of protein and carbohydrate synthesis in the gonad of two-month-old snails compared with other stages [1]. We have therefore chosen to use juvenile gonads to study whether the factor(s) from the known endocrine centers stimulate these synthetic activities.

Preliminary studies from our laboratory revealed that gametogenesis was stimulated in organ culture in the presence of the brain [2]. It is well established that in *Helix aspersa* the endocrine dorsal bodies, which are under the inhibitory control of the brain [3], stimulate oocyte maturation [4]. In the present paper, we provide evidence that the brain/dorsal bodies and the dorsal bodies do stimulate protein and carbohydrate synthesis in the juvenile gonad of *Helix aspersa.*

Materials and methods

The experiments were done on the ovotestis of two-month-old *Helix aspersa* raised in the laboratory at 20 °C under 18L:6D photoperiod. Either brain (cerebral ganglia and the associated dorsal bodies) or dorsal bodies embedded in the connective tissue sheath of the cerebral ganglia were isolated from six-month-old reproductively active snails raised under the above laboratory conditions.

Four experimental groups were chosen, each consisting of 10 replicates and the whole experiment was performed twice. The four groups consisted of

1) gonad with the brain,
2) gonad with the dorsal bodies,

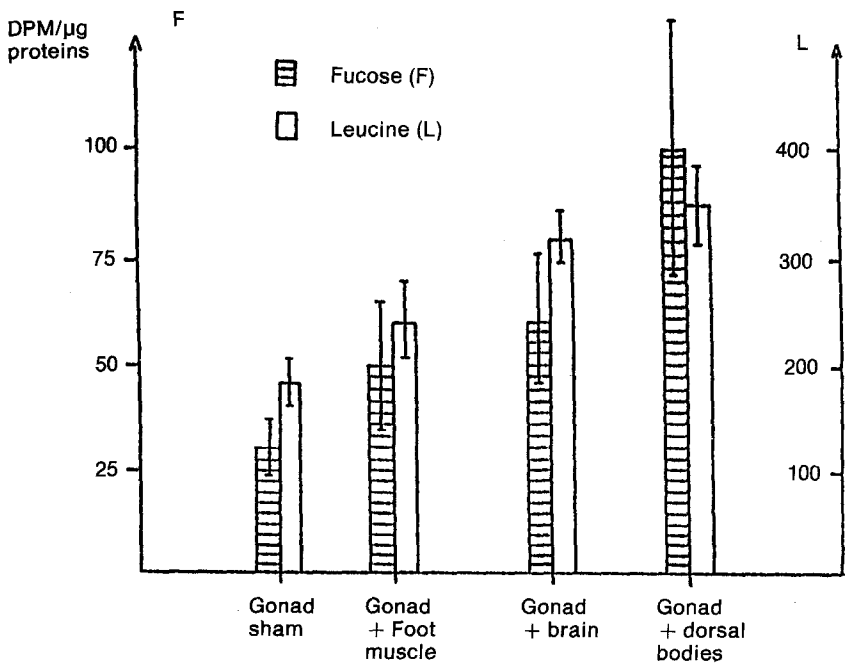

Figure 1. Effects of some organs on the incorporation rates of leucine and fucose in gonadal explants.

3) gonad alone (control),
4) gonad with the foot muscle.

For organ culture, the gonads alone or with associated organs were placed in culture dishes containing medium 199 at pH 7.6 and the osmotic pressure of 260 mOsm/l. Penicillin (100 IU/ml) and streptomycin (50 µg/ml) were added to the medium. ^{3}H L-fucose (27 Ci/mM) and ^{14}C L-leucine (300 mCi/mM) were added to the medium at a concentration of 5 mCi/ml. The total fluid volume in each culture was 500 µl and the duration of the culture period was 60 h in total darkness. At the end of the experiment, a piece of the gonad was washed in a non-radioactive chase solution for one hour, solubilized and the radioactive incorporation was measured in a scintillator counter. The incorporation was expressed as dpm/mg of protein. The protein was estimated according to Lowry et al.[5]. Data was statistically analyzed using the Mann and Whitney test[6]. Pieces of the gonad were also fixed for the cutting of semi-thin and ultra-thin plastic sections and ultrastructural observations[7].

Results and discussion

The incorporation of the radioactive precursors in the gonad as measured by scintillation is shown in figure 1. The synthetic activity of the gonad increased significantly over controls in the presence either of the brain or the dorsal bodies. Furthermore the dorsal bodies alone caused an even greater increase than the brain/dorsal bodies complex. When a physiological concentration of the methionine-enkephalin peptide was added to the culture medium, the incorporation of both leucine (L) and fucose (F) in gonads almost doubled over control levels (fig. 2).

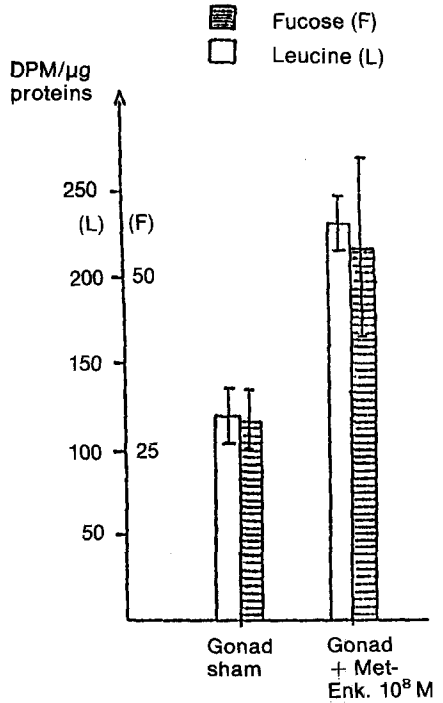

Figure 2. Effects of met-enkephalin on the incorporation rates of leucine and fucose in gonadal explants.

Cytological and ultrastructural observations revealed that medium 199 alone is unfavorable for organ culture work using the gonad because germinal cells usually showed necrosis. The addition of foot muscle did not alter the situation. However, in the presence of dorsal bodies and especially of brain, the acinar structure was similar to that before culture (figs 3, 4). The vesicular tissue was maintained, the male and female cells appeared normal and hardly any necrosis was observed within the culture time in this study (fig. 4). The cellular membranes and the basal laminae proliferated (figs 5, 8), and the acinar epithelium was very developed (fig. 5), compared to the control (figs 6, 7).

The gonadotrophic effect of the neuro-endocrine organs (brain/dorsal bodies) using a longer organ culture period has been shown previously[2]. However, the present study using a shorter culture period provides an in vitro bioassay method to evaluate quantitatively the synthetic activities of the juvenile gonad in the presence of neuro-endocrine organs in *Helix aspersa*. The brain/dorsal bodies or the dorsal bodies alone stimulated the incorporation of both ^{14}C leucine and ^{3}H fucose in the gonad whereas the presence of foot muscle in the culture medium did not alter the incorporation compared to controls. In crayfish, it was found that muscle extract did not influence vitellogenesis but that brain extracts did[7].

In *Helix*, the dorsal bodies alone were more effective in increasing the synthetic activities of the gonad than the brain/dorsal bodies complex. Since the dorsal body cells are under the inhibitory control of the cerebral green cells located in the brain[3], the isolation of the dorsal bodies from the brain removes this inhibition, and presumably causes a greater release of hormone from the dorsal body cells. The release of hormone from dorsal body cells by exocytosis will depend on the equilibrium between positive and negative influences of the brain. Miskys and Saleuddin[9] reported that in *Helisoma duryi*, galactogen synthesis in vitro in the albumen gland could be stimulated by an extract from the dorsal bodies in 17 h whereas it took longer (72 h) when the whole brain containing the dorsal bodies was used.

It was interesting to note that the in vitro incorporation of ^{3}H fucose was close to that measured in vivo[1]. The increased incorporation of ^{3}H fucose, either in presence of the dorsal bodies or after the addition of methionine-enkephalin to the culture medium, is interesting. By immunocytochemistry this opioid neuropeptide has been localized in the dorsal bodies of *Helix*[10]. It should be mentioned that in vertebrates, opioid peptides influence sugar metabolism either directly or indirectly, by the intervention of catecholamines[11].

Finally we hope that the bioassay reported here can be extended to study the physiological roles of known endocrine centers in *Helix* and other molluscs. Work is in

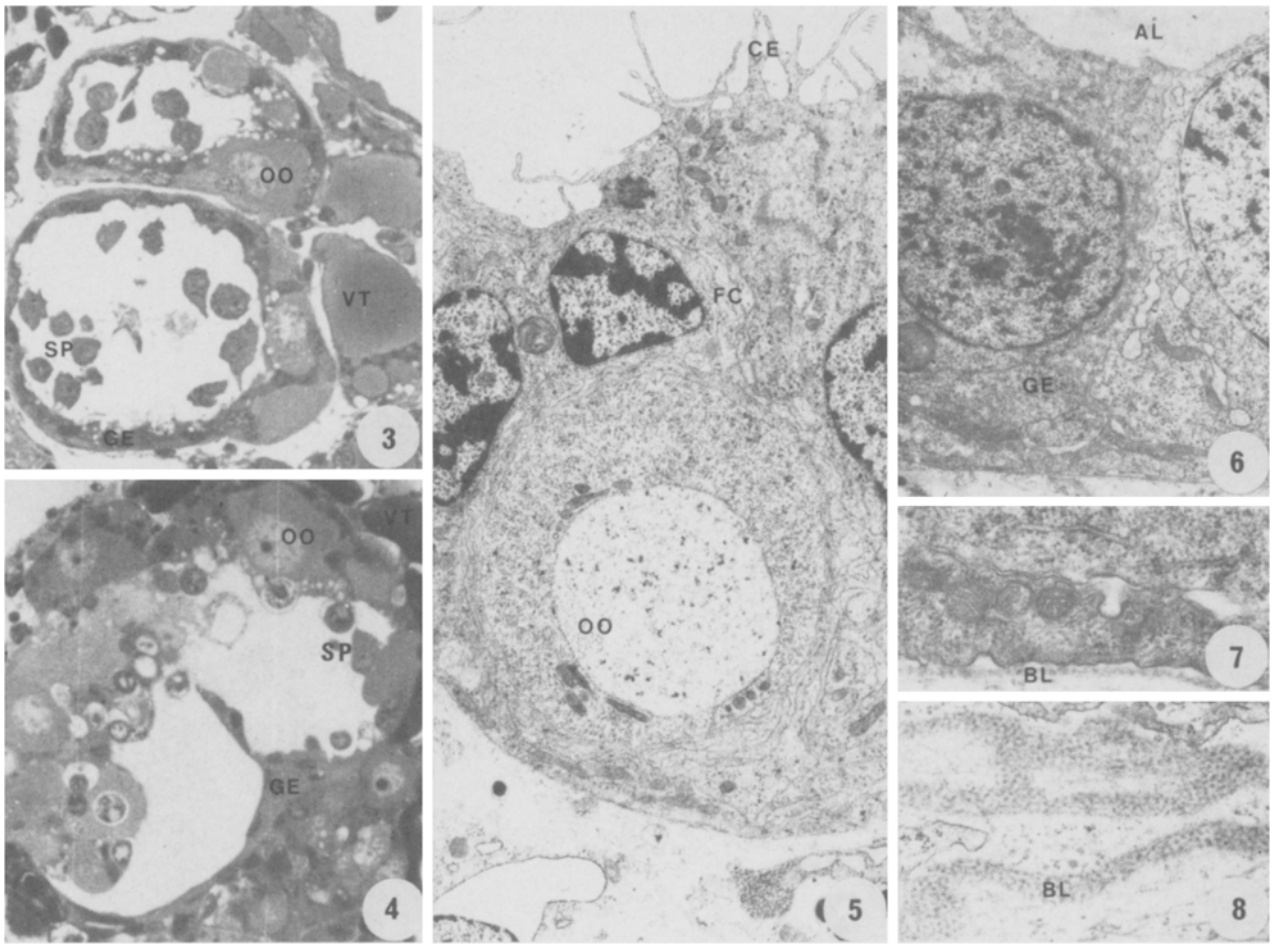

Figure 3. Semi-thin section of gonad colored with toluidine blue: cytological state before the culture (× 100).
Figure 4. Semi-thin section of gonad associated with brain after 60 h of culture and colored with toluidine blue (× 100).
Figure 5. Electron micrograph showing the germinative epithelium of the gonad cultured 60 h with brain (× 7000).
Figure 6. Electron micrograph showing the germinative epithelium of the gonad sham cultured 60 h (× 7000).
Figure 7. The basal lamina (BL) of the gonad sham (× 25 000).
Figure 8. The basal lamina (BL) of the gonad cultured 60 h with brain (× 25 000).
AL: acinar lumen; CE: cytoplasmic expansions (arrows); FC: follicular cells; GE: germinative epithelium; 00: oocyte; SP: spermatocytes; VT: vesicular tissue.

progress to determine whether the bioassay is applicable to adult organs in *Helix aspersa*.

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