STUDIES ON REGULATION OF METABOLISM OF MUCOSUBSTANCES IN THE ALBUMEN GLAND OF S. MACULATA UNDER THE INFLUENCE OF CEREBRAL GANGLIA NEUROHORMONES (CGN)

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KEYWORDS
Mucosubstances
Albumen gland
Cerebral ganglia
Neurohormones
Irrespective of usual mucosubstances, the galactogen an unusual mucosubstance synthesized in the albumen gland of *S. maculata* is under the control of neurohormones secreted in the optic tentacles. The concentration is also influenced by the hormones synthesized in the ovotestis. The present contribution deals with the effect of neurohormones of cerebral ganglia on the synthesis of mucosubstance like galactogen by employing histochemical and biochemical techniques. The accumulation of galactogen in the albumen gland is utilized for the development of perivitelline fluid around the eggs and glycogen in this gland is utilized for the synthesis of galactogen. Such interconversions of mucosubstances are useful during the gametogenesis. Hence results have been discussed in relation to the reproductive physiology of this slug.

**INTRODUCTION**

In many invertebrates Bernard (1850) reported the presence of carbohydrates. He noted an interesting observation that glycogen is most widely distributed mucosubstance in most of the invertebrates but in gastropod mollusks, in addition to glycogen another mucosubstance galactogen also occurs particularly in their albumen glands. Similar observation was reported in the reproductive system of mollusks (Goddard and Martin, 1966). In *Semperula maculata* (Pulmonate, Gastropod) the albumen gland, an accessory sex organ produces two mucosubstances glycogen and galactogen. The presence of galactogen in the albumen gland of this slug was first confirmed by Varute and Nanaware (1972) by employing of metachromatic histochemical technique. Then the efforts were made to investigate the synthetic route of this mucosubstance (Fantan, 1971) and its controlling hormones in the land slug (Nanaware and Yadav, 1993). Several studies have established the endocrine regulation of the synthesis of galactogen in the albumen gland either by the dorsal bodies (DBs) alone or by the DBs and the brain combined (Abeloos, 1943; Laviolette, 1954; Runham et al., 1973; Goudsmit, 1975; Wijdenes et al., 1983; Miksys and Saleuddin, 1985; Yadav and Nanaware, 2010). Since the DBs regulate synthetic activities of the albumen gland, here the attempt has been made to study the induction of galactogen synthesis in the albumen gland following the injection of extract of the cerebral ganglia.

**MATERIALS AND METHODS**

For the present study the slugs were collected from the local gardens in July and August. They were transported immediately to the laboratory and were maintained in plastic troughs filled with soil which was from the same fields and they were fed with their natural food like cabbage, canan leaves etc. The troughs were covered with mosquito curtain cloths and the soil was kept moist so as to provide their natural humidity. Water was always made available to the animals and they were fed daily in the evenings only. As they were kept at room temperature and on normal feeding, they prevented from winter hibernation.

**Preparation of homogenate**

The cerebral ganglia of the *S. maculata* were cut and homogenate was prepared in the molluscan saline solution [NaCl 5.7gm/L, KCl 0.15gm/L, CaCl2 1.11 gm/L, pH 7.5]. The extracts were injected to the animals for 5 days [1mL to each slug every day]. Afterwards the albumen glands were dissected out from the experimental animals after 10th days, 20th days, 30th days, 40th days and 50th days. The albumen gland of control animals and experimental animals were fixed for the histochemical and biochemical observations in the 2% calcium formaline acetate [CAF] (10% formaldehyde containing 2% calcium acetate). The pieces of the glands were fixed for 24h. After fixation the tissues in both the cases were well washed under running water following by routine processing for paraffin embedding and sections at 5-7μ (micron).

**Histochemical Procedures**
METABOLISM OF MUCOSUBSTANCES IN THE ALBUMEN GLAND

For the histochemical detection of glycogen and galactogen the section of albumen gland stained by PAS technique, malt diastase or saliva digestion-PAS, AB [PH 1.0], AB [PH 2.5] and sequential staining techniques such as AB [PH 1.0, 2.5] – PAS (Spicer et al., 1957; Varute and Nanaware, 1972).

Biochemical Procedure

For the quantitative estimation of glycogen and galactogen colorimetric method of Caroll et al. (1956) was used.

Observations

Histochemical Observations

The histochemical data on some important staining reactions employed for the detection of mucosubstances in the present investigation on the albumen gland are recorded in Table No. 1; according to the visually estimated staining intensity and shade with four plus (+ + + +) representing the strongest activity. The histochemical study on the albumen gland revealed the following significant facts. The staining reactions observed in this gland are entirely due to glycogen and galactogen granules elaborated in the different parts of the albumen gland. The galactogen granules in the glandular cells showed strongest-PAS reactivity and slight alcino-philia at pH 2.5 but reacted negatively at pH 1.0. In the AB (pH 1.0, 2.5) – PAS sequential staining procedures the globules stained intensely pink.

Biochemical Observations

The biochemical observations of alterations in the glycogen and galactogen and total polysaccharides have been recorded in Table No. 2 and they are shown graphically in graph in which polysaccharide contents have plotted as a function of days after the injections of extract of cerebral ganglia. The injections of extract of cerebral ganglia increased total polysaccharide and galactogen during experimental period, whereas in controlled slugs glycogen goes on decreasing during experimental period but comparatively the increase was less in slugs with tentacles than without tentacles. In the experimental animals the glycogen was decreased and galactogen and total polysaccharides were steadily increased from 10th day to 50th day.

RESULTS AND DISCUSSION

The synthesis and elaboration of neurohormones in the cerebral ganglia appears to be in all the gastropods. It has been pointed out that the role of neuroendocrine caudodorsal cells in the cerebral ganglia to induce polysaccharide synthesis in the albumen gland. The studies on H. duryi showed that hormones of the cerebral ganglia stimulate polysaccharide synthesis in albumen gland. In some other studies the role of neurohormones has been established in the synthesis of galactogen in the albumen gland (Abeloos, 1943; Laviolette, 1954). It was also concluded that the hormones elaborated by brain and tentacular collar cells control the metabolism in certain gastropod species but whole of the stages going from the stimulus to the response have hardly been studied. Thus the field of hormonal control over the activities of albumen gland of hermaphrodite gastropod S. maculata forms as open field for research. Though some ideas concerning the polysaccharide metabolism in the albumen gland have been...
suggested, the literature on the neuro-endocrine control over such activities in the hermaphrodite gastropods is poor. The existing literature indicates the presence of unusual such activities in the hermaphrodite gastropods is poor. The suggested, the literature on the neuro-endocrine control over OTAB = optic tentacle ablated; OTIN = optic tentacle intact; CGEI= Cerebral Ganglia Extract Injection. N.B.* the percentage of glycogen and galactogen were estimated by Caroll (1956). The determination of glycogen in the liver muscle by the use of anthrone reagent. J. Biol. Chem. 220: 583-593. Galaktogen. Miksys, S. and Saleuddin, A. S. M. 1985. Regulation of galactogen synthesis in the slug Arioninae et limacidae. Bull. Biol. Fr. Belg. 193:198. 1. Control 2. OTIN+CGEI 3. OTAB +CGEI 4.63 4.82 4.20 3.70 1.55 3.54 3.00 3.75 4.02 5.13 5.50 5.88 6.02 6.97 6.67 8.05 8.48 9.49 10.27 11.09 2.93 2.52 2.03 1.92 1.55 4.76 5.64 6.14 7.02 6.47 7.28 7.67 8.06 8.57 3.00 3.75 4.02 5.13 5.50 5.88 6.02 6.97 8.05 8.48 9.49 10.27 10.37 11.09 2.50 2.13 2.00 1.84 1.23 3.00 3.75 4.02 5.13 6.91 5.50 5.88 6.02 6.97 8.14 10 20 30 40 50 10 20 30 40 50 10 20 30 40 50 10 20 30 40 50 10 20 0.00 1.23 1.84 2.00 2.13 3.00 3.75 4.02 5.13 5.50 5.88 6.02 6.97 8.14 8.57 9.49 10.27 10.37 11.09

Table 2: Effect of Injection of Cerebral Ganglia Extract On Polysaccharides In The Albumen Gland of Semperula Maculata*

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Experimental Methods</th>
<th>Percentage Of Polysaccharides**</th>
<th>Glycogen</th>
<th>Galactogen</th>
<th>Total Polysaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycogen</td>
<td>Galactogen</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td></td>
<td>2.50</td>
<td>2.13</td>
<td>2.00 1.84 1.23 3.00</td>
</tr>
<tr>
<td>2.</td>
<td>OTIN+CGEI</td>
<td></td>
<td>2.93</td>
<td>2.52</td>
<td>2.03 1.92 1.55 3.54</td>
</tr>
<tr>
<td>3.</td>
<td>OTAB+CGEI</td>
<td></td>
<td>4.63</td>
<td>4.82</td>
<td>4.20 3.70 3.04 3.85</td>
</tr>
</tbody>
</table>

Methods


** The percentage of glycogen and galactogen are expressed as mg/100 mg of wet tissue; OTAB = optic tentacle ablated; OTIN = optic tentacle intact; CGEI = Cerebral Ganglia Extract Injection.

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REFERENCES


Miksys, S. and Saleuddin, A. S. M. 1985. The effect of the brain and dorsal bodies of Helisoma duryi [mollusa, pulmonata] on albumen gland but also their variations under the influence of neuroendocrine secretions of cerebral ganglia. The injections of extract of cerebral ganglia homogenate caused a decrease of glycogen from 2.50 to 1.23 mg/100 mg of wet tissue in the albumen gland and an increase of galactogen from 3.00 to 6.91 mg/100 mg of wet tissue. The increase was steady from 10th to 50th day after injection of extract of cerebral ganglia.

Thus from the results of the present investigation it seems that the galactogen synthesis in the albumen gland under the control of neurosecretory hormones in the cerebral ganglia.

According to literature on albumen gland, it seems that only a few gastropod species the detailed histochemical and biochemical studies have been carried out to determine the exact role of the hormones elaborated by dorsal body, optic tentacle or any other neurohormones are involved in the control of the activity of the albumen gland of a terrestrial slug, S. maculata. In this experiment the histochemical findings are in agreement with the biochemical studies. The presence of galactogen in the albumen gland of gastropods has been shown by some investigators. May (1934) report that the galactogen synthesis rises during the late prebreeding period and it is employed in egg coat formation. Goudsmit (1975) demonstrated that galactogen synthesis in albumen gland of H. pomatia is stimulated by a factor of cerebral ganglia. But origin of the factor is not clear. Similar observations were also reported by Meenakshi and Scheer (1968) and Nanaware (1974).

According to literature on albumen gland, it seems that only a few gastropod species the detailed histochemical and biochemical studies have been carried out to determine the exact role of cerebral ganglia neurohormones (CGN) in the control of the activity of albumen gland. Research in this area is hampered by the fact that it is not exactly known whether the cerebral ganglia neurohormones or any other factor control the activity of albumen gland. Therefore, in order to confirm the effects of various experimental conditions created due to neuroendocrine secretions on the qualitative changes in the polysaccharides, glycogen and galactogen of the albumen gland, the present biochemical studies were performed. The quantitative biochemical estimation of the total polysaccharides, glycogen and galactogen coincided with the histochemical qualitative results observed in the albumen gland. From the critical survey it was noticed that the study involving the nutrients like total polysaccharides, glycogen and galactogen of the albumen gland in one and the same species of gastropod has not so far been carried out. Therefore, the present study is the first effort to bring about the percentage of chemical components of this gland. The present study not only points out the percentage of chemical components of


