Source of the tentacular hormone in terrestrial pulmonates

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Summary. Collar cells around the optic tentacles of pulmonate gastropods were shown to have a stimulating effect on spermatogenesis in vivo and in vitro. No hormonal action was found in the tentacles other than in collar cells. Therefore, collar cells are considered to be the source of the so-called tentacular hormone in stylommatophoran pulmonates. We propose to name the collar cell group 'optic gland'.

There is experimental evidence that the optic tentacles of pulmonata stylommatophora produce a hormone that is involved in the regulation of gametogenesis. In some species of slug, such as *Arion ater, Arion subfuscus* and *Milax* sp., the optic tentacles exert a stimulating effect on spermatogenesis and an inhibitory effect on oogenesis[1-5]. This effect also occurs in other slugs, *Limax flavus, Limax marginatus*[6-8] and *Lavicaulis alte*[9], and in the snails *Euhadra peliomphala*[10-12], *Achatina fulica*[13], and *Cryptozona belageri*[14]. In the slugs *Deroceras raticulatus* and *Limax flavus,* tentacular homogenate was shown to inhibit egg-laying[6-8]. On the other hand, in the slug *Ariolimax californicus,* the tentacles have an inhibitory influence on spermatogenesis[15]. In *Ariolimax columbianus,* indirect evidence was found for an inhibitory effect of the tentacles on the growth and synthetic activity of the albumen gland[16]. Although studies on the hormonal action of the optic tentacles on gametogenesis are sometimes contradictory, the existence of a so-called tentacular hormone controlling sexuality has been well demonstrated. Therefore, many studies have been devoted to the identification of the hormone-producing center in the optic tentacles[17-23]. Up to the present, however, no particular cells have been implicated as sites of hormone production. The present report deals with the demonstration of such an endocrine center in the optic tentacles of some terrestrial stylommatophoran pulmonates.

The animals used in this study were the slugs *Limax flavus, Limax marginatus, Incialia confusa, Incialia fruhstorferi, Nipponarion carinatus* and *Milax gagates,* and the snails *Euhadra peliomphala, Euhadra callizona, Euhadra questa, Acusta despecta sieboldiana, Bradybaena similaris* and *Achatina fulica.* The optic tentacle of these animals contains the eye and the optic nerve connecting it to the cerebral ganglion. Closely apposed to the eye is a large tentacular ganglion. No neurosecretory cells were found in the tentacular ganglion of these pulmonates at any time during the year. Therefore, we attempted to check all the other issue in the optic tentacles such as collar cells, 2 types of lateral cells and the gland cells of the dermatomuscular layer. Among these, the collar cells are intimately connected with the tentacular ganglion and are arranged in groups. They are large cells, about 60 µm in diameter, ovoid or pear-shaped, with small spherical nucleoli, and they send a fine cytoplasmic projection into the ganglion. Furthermore, they are full of droplets and granules, and undergo some histological changes during the year in relation to reproduction. The nuclear volume and the amount of secretory substances in the cytoplasm increased during reproduction. Their glandular nature is clearly apparent in electron microscopy as well as in light microscopy. They are characterized by a well-developed ergastoplasm, extensive Golgi regions and large secretory inclusions, different from neurosecretory granules.

Homogenates of the optic tentacles have been shown to induce spermatogenesis and to inhibit oogenesis in these slugs. As shown in table 1, the hormonal effects were most evident on spermatogenesis. Removal of the tentacles including collar cells did not induce spermatogenesis. In *Limax flavus,* especially, no spermatozoa were found in the acini after their removal. After the injection of collar cell homogenate, spermatogenic activity returned and gradually increased. On the other hand, tentacular homogenate including nerve tissue other than collar cells had no hormonal effect. Therefore, neurohormone was shown not to be concerned with spermatogenesis. Induction of spermatogenesis by the injection of collar cell homogenate was dose-dependent (fig. A). Muscle and tentacular ganglion homogenates had no hormonal effect even when their titers were the same as those of collar cells. The hormonal effect was then studied with the organ culture technique[24,25]. The culture medium used was previously developed for snails. A piece of hermaphrodite gland was cultured in this medium containing collar cells. Detailed culture methods have been described previously[26]. Results are shown in table 2. Collar cell homogenate was shown to have a direct stimulating effect on spermatogenesis. No hormonal activity was found in the tentacles other than in collar cells. Induction of spermatogenesis by collar cells was dose dependent also in vitro (fig. B). From these results, it was shown that collar cell homogenate acted directly on spermatogenesis as a gonadotropic hormone. Similar results were also found in other species used. Based on these results, collar cells in the optic tentacles are considered to

Table 1. Effects of collar cell (optic gland) homogenate on spermatogenesis in vivo in the slug, *Limax flavus* and the snail, *Euhadra peliomphala*

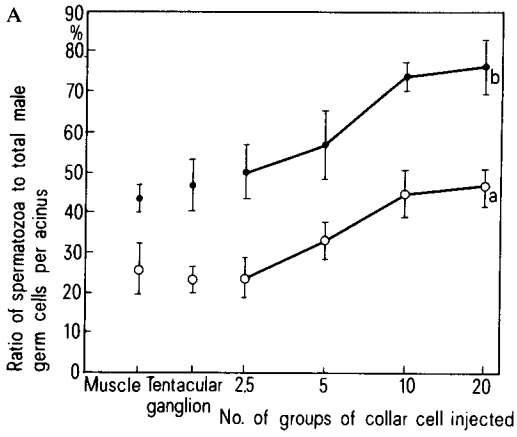
Species	Germ cells	Control	Tentacular removal, including collar cell (OGR)	OGR+ collar cell homogenate (OGH)[a]	OGR+ tentacular homogenate other than collar cell (TH)
Limax flavus	Spermatocyte	46.3±4.9	92.4±2.4	41.9±2.0	87.1±1.7
	Spermatid	31.0±2.2	7.1±2.3[b]	31.3±2.5	12.0±3.1
	Spermatozoon	22.7±4.7	-	25.8±2.7[c]	0.9±0.1
Euhadra peliomphala	Spermatocyte	52.0±1.3	86.4±2.5	69.0±1.5	84.7±2.5
	Spermatozoon	48.0±1.3	13.6±2.5[b]	31.0±1.5[c]	15.3±2.5

[a] Collar cell (optic gland) homogenates in a physiological salt solution have been injected into the body cavity of animals (0.05 ml per 2 days for 40 days: a total of 10 collar cell groups, optic glands) whose optic tentacles, including collar cell (optic gland), were cut off 3 days before injection. After 40 days, the hermaphrodite gland was examined histologically. The total number of each germ cell per 1 sectioned acinus was counted and expressed as a percentage (%, mean±SE, n=30). [b] Significantly different from control group by analysis of variance $p < 0.01$. [c] Significantly different from OGR and OGR + TH groups by analysis of variance $p < 0.01$.

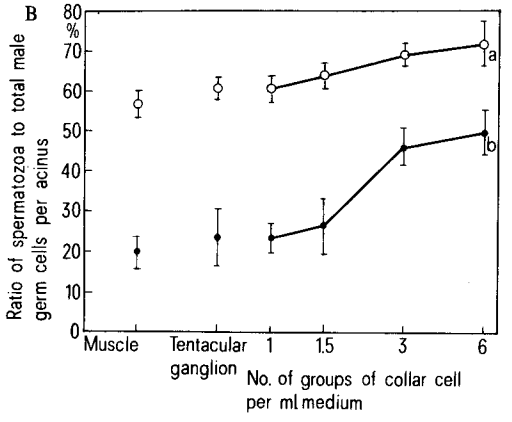
Table 2. Effects of collar cell (optic gland) homogenate on spermatogenesis in vitro in the slug, *Limax flavus* and the snail, *Euhadra peliomphala*

Species	Germ cell	Control	Collar cell homogenate[a]	Tentacular homogenate other than collar cell
Limax flavus	Spermatocyte	22.5±1.8	19.3±1.1	21.5±2.4
	Spermatid	20.0±1.7	13.3±1.0	22.0±1.5
	Spermatozoon	57.5±2.1	67.4±1.6[b,c]	56.5±2.1
Euhadra peliomphala	Spermatocyte	79.5±4.0	56.3±3.0	80.5±2.3
	Spermatozoon	20.5±4.0	43.7±3.0[b,d]	19.5±2.3

Cultures were kept at 25 °C for 10 days. The total number of each germ cell per 1 sectioned acinus was examined histologically and expressed as a percentage. [a] 3 groups of collar cell/1 ml medium (%, mean±SE, n=30). [b] Significantly different from other groups by analysis of variance. [c] $p<0.05$; [d] $p<0.01$.



A Effects of collar cell (optic gland) homogenate on spermatogenesis in vivo in the slug, *Limax flavus* (a) and the snail, *Euhadra peliomphala* (b). Collar cell (optic gland) homogenates in a physiological salt solution were injected into the body cavity of animals (0.05 ml per 2 days for 40 days). After that the hermaphrodite gland was examined histologically. The ratio of spermatozoa to male germ cells per acinus is expressed as a percentage. (%, mean ±SE, n=30).



B Effects of collar cell (optic gland) homogenate on spermatogenesis in vitro in the slug, *Limax flavus* (a) and in the snail, *Euhadra peliomphala* (b). Cultures were kept at 25 °C for 10 days. After that the hermaphrodite gland was examined histologically. The ratio of spermatozoa to male germ cells per acinus is expressed as a percentage. (%, mean ±SE, n=30).

be the source of the tentacular hormone. Recent work has suggested that spermatogenesis in *Limax flavus* and *Euhadra peliomphala* is controlled directly by testosterone secreted from the hermaphrodite gland[6-8,26]. Therefore, collar cell homogenate may act as a gonad stimulating hormone in some pulmonates.

In contrast to the present findings, no effects of tentacular removal on gametogenesis were observed in the snails *Helix aspersa*[27] and *Helix pomatia*[28] or in the slug *Vaginulus borellianus*[29]. These contradictory findings seem to arise from experimental conditions or species-specific reactions to the removal of the optic tentacles. The gonadotropic function of the optic tentacles may change with developmental state or with season. As the optic tentacles are important as photoreceptors and for orientation to food, indirect effects of tentacular removal on reproductive activity cannot be excluded. As the regeneration capacity of the tentacles is high, removal sould be performed repeatedly. Special attention must be paid to these points. The tentacles of gastropods other than stylommatophoran pulmonates have also been reviewed from this point of view. There is no indication of an endocrine role of the tentacles in basommatophora. In prosobranchia, e.g., *Patella vulgata*, the tentacles produce an inhibitory factor for spermatogenesis druing the male phase[30]. However, the hormone-producing center has not been discovered. In *Calyptera sinensis* and *Crepidula fornicata*, the right optic tentacles in the male phase promote morphogenesis of the male tract including the penis and induced regression of the female tract. This hormone was shown to be released fromt the right pedal ganglion into the hemolymph and accumulated in special hemal lacunae[31,32]. Therefore, these effects were demonstrated to be due to neurosecretion. Thus, the source of tentacular hormone may differ from species to species. In addition to these, the possibility of neurosecretory activity by the eye of *Aplysia californica* (opisthobrachia) was suggested by morphological findings[33].

It is well known that in cephalopoda other than nautiloidae, such as *Octopus vulgare*, maturation of the gonads is controlled by the optic gland. The optic glands are the source of a hormone that simulates the maturation of the gonadal system[34]. The cells of the optic gland were shown to contain large amount of lipofuscin, which is characteristic of molluscan neurons, and it is suggested that they are of nervous origin. Collar cells in pulmonates have also been thought to be a special kind of peculiar neuron, with their process corresponding to the axon of nerve cells. By analogy with the Octopus optic gland we propose to name the collar cell group around the tentacular ganglion in these pulmonates 'optic gland'.

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Regulation of feeding and ovipositional success of *Amblyomma americanum* ticks[1]

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Summary. Duration of the adult female *Amblyomma americanum* feeding period was found to be dependent upon male receptiveness to mating, which was in turn dependent upon when males were placed with females. Prolonging the time to mating by 5 days had no effect on female engorgement weight or length of the preovipositional period, but prolonging mating by 10 days substantially decreased the number of ovipositing females and ablated egg viability. In the absence of males, females were severely stunted in size and had to be forcibly removed from the host.

Regulation of tick activity in the environment, either daily or seasonally, is fairly well understood[2,3]. However, knowledge of the biology of ticks when on the host is only now beginning to be understood. It is known that some tick species have preferred attachment sites to the host[4], and aggregation of the sexes on the host results from chemical (pheromone) communication[5,6]. However, basic information pertaining to events leading to engorgement, a process dependent upon mating, are still poorly understood.

Materials and methods. Female albino Hartley guinea-pigs weighing 500-600 g were used as hosts. Adult *Amblyomma americanum* ticks were confined to hosts using the capsule technique[7]. Female ticks were placed on all hosts at day 0, and male ticks were added on day 0, 5 or 10. One group of guinea-pigs had only females. All ticks were individually marked with yellow (females) or red (males) acrylic paint on the dorsal surface to facilitate observations on migratory and pairing behavior while inside the capsules. Hosts were housed in plastic cages (43×20×18 cm) and given food (guinea-pig chow) and water ad libitum. During the tick feeding period, ticks were observed daily for changes in attachment sites, initiation of, or termination of pairing behavior, and detachment. Detached female ticks were weighed and placed in individual vials held at 85% relative humidity, 25 °C and 12 h L/D, and observed daily for oviposition. Once oviposition was complete, egg massess were weighed and observed daily for larval eclosion.

For each experimental group, the mean ± SE of the female feeding and preoviposition period, and engorgement and egg mass weight was determined. In addition, the number of engorged females that oviposited and the viability of the eggs, as assessed by subsequent hatch, were recorded.

Results. When males were added to infested hosts by day 5 of the female feeding period, or earlier (groups A and B), all females oviposited and weighed in excess of 300 mg (table). Deposited egg massess had a mean weight of 136-179 mg and were viable. However, if males were added later than day 5 (group C), there was a substantial decrease (40%) in

Effect of prolonging mating behavior on the feeding success, ovipositional success and egg viability of female *Amblyomma americanum* ticks

Group	Combination (sex) $n=10$	Feeding time (days)	Mean weight (mg)	Oviposition (%)	Mean time to oviposition (days)	Mean egg mass weight (mg)	Egg hatch
A	Female + male day 0	9.7 ± 2.3[a]	327 ± 14.5	100 (10/10)	13.3 ± 1.3	136 ± 10.8	+
B	Female + male day 5	13.2 ± 1.0	376 ± 75.2	100 (10/10)	16.2 ± 0.5	179 ± 44.5	+
C	Female + male day 10	19.8 ± 0.8	362 ± 76	60 (6/10)	14.7 ± 1.2	136 ± 19.2	-
D	Female only	20.0 ± 0[b]	93 ± 3.3	10 (1/10)	18.0[c]	18.0[c]	-

[a] Mean ± SE; [b] all ticks were removed on day 20; [c] only one tick oviposited.