Sexual differentiation in decapod crustaceans: role of the androgenic gland

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Received 4 December 1995; Accepted 28 April 1996

Summary

In male crustaceans — unlike vertebrates — the endocrine and gametogenic functions are clearly separated into distinct organs, the androgenic gland and the testis, respectively. The androgenic gland is thought to be the exclusive source of hormone responsible for sex-differentiation and sexual characteristics in crustaceans. Information on this unique crustacean organ is revised with respect to its structure, secretion and role in the regulation of the expression of sexual characteristics, and intersexuality. Several decapod models are presented for research on sexual differentiation in higher crustaceans.

Key words: Androgenic gland, Decapoda, Crustacea, sex-differentiation, intersexuality

Introduction

The mechanism of sex-determination — the commitment of an embryo to either the female or the male pathway — has not been studied extensively in crustaceans (Katakura, 1989); only limited cytogenetical data are available in decapods (Legrand et. al., 1987). In contrast, there have been numerous attempts to study the regulation of sexual differentiation, which is the overt manifestation of the sex-determination state through the expression of the sex cytodifferentiation genes responsible for the sexual dimorphism of female and male crustaceans. It has been suggested that the androgenic gland is the exclusive source of hormone responsible for sex-differentiation in crustaceans (Charniaux-Cotton, 1954). In male crustaceans — unlike vertebrates — the endocrine and gametogenic functions are clearly separated into distinct organs, the androgenic gland and the testis, respectively (Ginsburger-Vogel and Charniaux-Cotton, 1982; Charniaux-Cotton and Payen, 1988). Thus, the androgenic gland serves as a unique biological model for the study of the endocrine regulation of sex-differentiation. This article sets out to update previous reviews of the androgenic gland (Charniaux-Cotton and Payen, 1985, 1988; Payen, 1990) describing this unique crustacean organ and its role in the regulation of the expression of primary and secondary sexual characteristics, with emphasis on decapod crustaceans. In general, most of the results on sexual differentiation in decapods confirm those obtained beforehand in peracarids and the achievements in decapods are not superior due to longer molting cycle, bigger body and lack of a viable bioassay. This article presents several decapod models that may be useful in future needed research — both for applied and basic reasons — on sexual differentiation of decapods.

Anatomy and Histology of the Androgenic Gland in Decapods

The androgenic gland was first described in the crab *Callinectes sapidus* by Cronin (1947). In decapods the gland is usually located at the subterminal portion of the sperm duct. The cells may be arranged as thin, parallel and anastomosing cords (Carpenter and DeRoos, 1970) or in a compact lobed structure (Kleinholz and Keller, 1979). A combination of the two structures was found in *Macrobrachium rosenbergii*, in which the androgenic gland is composed of strands of cells surrounded by a thin layer of connective tissue, forming a pyramidal cluster loosely associated with the posterior portion of the ejaculatory duct (Veith and Malecha, 1983).

The ultrastructure of the androgenic gland of the crab *Pachyratus crassipes* resembles that of a vertebrate protein-producing cell rather than that of a steroid-producing cell (King, 1964), being characterized by a well developed, granular, endoplasmic reticulum and abundant mitochondria. These cells contain numerous large multivesicular bodies that resemble lysosomes. However, acid phosphatase activity has not been demonstrated in them. The proteinaceous nature of the secretions was confirmed by Taketomi (1986), who reported the existence of two kinds of androgenic gland cells in *Procambarus clarkii*, one of which resembles protein-secreting cells. A gradual enlargement and structural changes in the androgenic gland during sexual differentiation and gonad maturation were described in *P. clarkii* (Taketomi et al., 1996). Veith and Malecha (1983) observed three principal cell types in the androgenic gland of *M. rosenbergii*: type 1 cells are small with a dense cytoplasm, often containing two nuclei; type 2 cells are slightly larger and vacuolated; and type 3 cells are large, with vacuoles filling most of the intracellular space consisting of vacuoles. Certain areas of the gland display cellular degeneration, possibly indicating a holocrine mode of secretion. The androgenic gland of *M. rosenbergii* stained positive for lipids, of which the highest concentration was found both in the gland and the epithelial cells lining the lumen of the ejaculatory duct. The lipids appeared to be evenly distributed throughout the gland and were not confined to any one of the three cell types (Veith and Malecha, 1983).

The Androgenic Gland, Development of Sexual Characters and Somatic Growth

Charniaux-Cotton (1954) was the first to suggest a regulatory role for the androgenic gland. She showed that bilateral androgenic gland ablation (andrectomy) in *Orchestra gammaria* blocked differentiation of secondary male characteristics and resulted in decreased spermatogenesis (Charniaux-Cotton, 1954). Once the external male sexual characteristics are formed in gonochoristic shrimp, the androgenic gland is not needed for their maintenance (Touri, 1977a). The role of the androgenic gland in the regulation of the development of decapod external sex characters is well established. It was recently illustrated by injecting the crayfish *P. clarkii* with androgenic gland extracts, which effected the appearance of external male characters in the form of reversed spines. Injections of sperm duct extracts as control produced no such effect (Taketomi et al., 1990). A strong correlation was also found in *P. clarkii* males between the development of the androgenic gland and the morphological differentiation of the abdominal appendages (Taketomi et al., 1996). Masculinization of the external characteristics of female crayfish was observed after the implantation of androgenic gland (Nagamine and Knight, 1987a). A high degree of feminization, which included initiation of oogenesis and development of oviducts and female gonopores, occurred in maturing *M. rosenbergii* males that had been andrectomized in the youngest developmental stage. Males andrectomized in later developmental stages were either partially feminized or not feminized at all (Nagamine et al., 1980a). Reimplantation of the androgenic gland into andrectomized *M. rosenbergii* males reversed the effect of the andrectomy. Androgenic gland implantation masculinized female recipients, as manifested by the development of the *appendices masculina*, the male gonopore complex, mature masculine chelipeds and initiation of spermatogenesis in the ovaries (Nagamine et al., 1980a, 1980b). A wide range of abnormalities in gonadal development was observed in andrectomized males, depending on the age at which the andrectomy was performed. Development of reduced testes was observed in males andrectomized at a relatively old age. In younger andrectomized males partly testicular and partly ovarian gonads ("ovotestes", Fig. 1) or abnormally lobulated ovaries developed (Snir, 1992). Sagi and Cohen (1990) observed complete sex reversal as a result of surgical removal of the androgenic gland in juvenile *M. rosenbergii*, leading to the development of functional females capable of mating and producing progeny (Fig. 2). Functional sex reversal of female *M. rosenbergii* by implanting androgenic glands into the youngest and smallest prawns that could be identified as females has been reported by Malecha et al. (1992). In both cases, progeny was obtained when fertile sex-
Fig. 1. Gonads of an andrectomized *Macrobrachium rosenbergii* male. Part of the gonad resembles the testis ("TST") and part the ovary ("OV"). The ovarian component contains yolk but no oocytes. The prawn was andrectomized 64 days after the metamorphosis and weighed less than 1.0 g at the time of andrectomy. Bar represents 2.5 mm.

Fig. 2. An andrectomized *M. rosenbergii* male which has transformed into a functioning female carrying fertile eggs (arrow). This prawn, andrectomized 30 days after metamorphosis, is the first case of a full and functional sex reversal ever reported for a decapod crustacean.

reversed animals were crossed with normal prawns, and the sex ratio of the offspring supported the homogametic male theory, in keeping with Katakura (1989). Implantation of ovarian tissue into andrectomized crayfish males resulted in the development of female secondary sexual characteristics (Nagamine and Knight, 1987b); this finding suggested that the androgenic gland prevents the appearance of female characteristics only in the early stages via inhibition of differentiation of the juvenile gonad into an ovary.
Fig. 3. Growth of andrectomized *M. rosenbergii* males vs. sham-operated males and intact females. Vertical lines indicate the standard error.

It was demonstrated (Sagi et al., 1990) that androgenic gland ablation affects growth rates and morphotypic differentiation into the three distinctive adult male morphotypes that coexist in *M. rosenbergii* populations (Kuris et al., 1987; Sagi and Ra’anana, 1988). Andrectomy of small-males did not prevent transformation into the orange-claw morphotype but did prevent further transformation into the blue-claw morphotype. However, andrectomy of orange-claw males did not prevent transformation into the blue-claw morphotype. The growth rates of the andrectomized small and orange-claw males were significantly lower than those of the control prawns (Sagi et al., 1990). In *M. rosenbergii* the male growth rate was considerably higher than the female rate (Sagi et al., 1986). The somatic growth of andrectomized males was significantly lower than that of sham-operated and normal males and very similar to that of normal females (Fig. 3).

**The Androgenic Gland, Intersexuality and Early Sex Differentiation**

Intersexuality in gonochoristic decapods may shed some light on the role of the androgenic gland in early sex differentiation. Nagamine and Knight (1987a) stated that: “knowing that the androgenic gland can masculinize genotypic females makes the presence of a bilateral gynandromorph a paradox since the androgenic gland at the male half should be capable of masculinizing the contralateral female half”. The expression of intersexuality in *Cherax quadricarinatus* — in which one half has male internal characters and the contralateral half has female characters, while the secondary external characters are masculine on both sides (Sagi et al., 1996) — conforms with the hypothesis that early male differentiation in decapods is mediated by a secretion from the androgenic gland primordial which diffuses along the genital tract (Charniaux-Cotton and Payen, 1988). This may explain the presence of the male reproductive system and the absence of the female system on the same side, i.e., the side on which the androgenic gland exerts its local effect through diffusion. On the other side, in the absence of an androgenic gland, differentiation of an ovary is permitted (Charniaux-Cotton, 1959; Sagi et al., 1996). Contradictory findings were recorded by Nakamura and co-workers who studied the time schedule of organogenesis of the genital organs and androgenic gland in *Penaeus japonicus* (Nakamura, 1992 and Nakamura et al., 1992). These studies suggested that the androgenic gland is inactive during organogenesis of the genital organs, leading to their conclusion that the differentiation of the testes is induced genetically, without the participation of the young androgenic gland. On the other hand, the authors also suggested that some other factor (from the ejaculatory bulb) is responsible for male differentiation of the primordial gonad (Nakamura et al., 1992). Earlier, a brain factor was thought to be responsible for
male differentiation, based on reports from other decapods (shrimp and crab) that the maintenance of the testicular germinal zone, sperm duct and androgenic gland were dependent on a brain factor (Tourie, 1977b).

**Biochemical Nature of Active Substances from the Androgenic Gland**

The only androgenic substances purified thus far from the androgenic gland of decapod crustaceans were lipidic in nature. Veith and Malecha (1983) found that the androgenic gland of *M. rosenbergii* stained positive for lipids. Berreur-Bonnenfant et al. (1973) extracted a lipoidal substance with a molecular weight of 200–250 daltons from the androgenic gland of the crab *Carcinus maenas*. Injection of that substance every second day inhibited vitellogenesis in sexually active female *Orchestia*. A carotenoid pigment in the second antenna, a secondary male characteristic, appeared as early as the sixth day after similar injections in *Talitrus* females. However, spermatogenesis was not induced. The active molecule, characterized by Ferec et al. (1978) as farnesylacetone, was shown to be synthesized by the androgenic gland. The action of farnesylacetone at low concentrations is rapid and organ specific (being expressed in the gonads), and does not exhibit any species specificity. Farnesylacetone affects protein and RNA synthesis in its target organs (Berreur-Bonnenfant and Laurence, 1984).

In contrast to the findings described above, the ultrastructure of the androgenic gland of the crab *Pachygrapsus crassipes* and the presence of a considerable amount of protein in the secretory vesicles of the cytoplasm suggest that the androgenic hormone may be a protein or a polypeptide (King, 1964). Similarly, the ultrastructure of the androgenic gland of *P. clarkii* suggests a peptidergic-proteinaceous secretion (Miyawaki and Taketomi, 1978; Taketomi, 1986). So far, no proteinaceous androgenic hormones have been identified in decapod crustaceans. However, such hormones have been purified and partially characterized in terrestrial isopods (Katakura et al., 1975; Juchault et al. 1978; Katakura and Hasegawa, 1983; Hasegawa et al., 1987). Recently, an androgenic hormone, in the range of 17,000–18,000 daltons, was purified from the androgenic glands of intersexed *Armadillidium vulgare* (Martin et al., 1990). This androgenic hormone appears to be an N-glycosylated protein with two chains linked by disulfide bridges (Martin et al., 1995). It seems very likely that, similarly to isopods, the androgenic hormone in decapod crustaceans is also proteinaceous in nature. This hypothesis presents a promising line for future research.

**Endocrine Regulation of the Androgenic Gland**

Reproduction in crustaceans is neuroregulated by hormones from the central nervous system. Eyestalk neuropeptides such as gonad-stimulating hormone (GSH) and gonad-inhibiting hormone (GIH) apparently act directly on the female ovaries (for reviews, see Charniaux-Cotton and Payen, 1988; Quackenbush, 1991; Fingerman, 1995), whereas in males their action on the testes appears to be indirect via a direct effect on the androgenic gland (Adiyodi, 1984; Gupta, 1989; Hasegawa et al., 1993). Bilateral destalking of the protandric shrimp *Pandalus platyceros* caused an increase in RNA synthesis in one particular cell type (the C cell type) in the androgenic gland (Brockenbrough-Foulks and Hofman, 1974). Based on experiments with bilateral eyestalk ablation and eyestalk extract injection, Kulkarni et al. (1984) concluded that hormones released from the neuroendocrine system regulate androgenic gland activity. Sarojini et al. (1994) presented the hypothesis that in *P. clarkii* serotonin stimulates the release of GSH, which in turn acts upon the androgenic gland, which releases the androgenic hormone.

To conclude, it is reasonable to expect that the coming years will bring about the determination of the biochemical structure of the androgenic gland hormone in decapod crustaceans, as well as its encoding gene sequence. To clarify the regulatory picture, the question whether the androgenic gland hormone is the sole sex-differentiation agent in *Crustacea* should be addressed. Description of the regulatory mechanism of sex differentiation will pave the way for a better understanding of the molecular mode of genetic sex-determination in crustaceans. Since many cultured decapod crustaceans exhibit sexual dimorphic growth patterns, it is obvious that tremendous applied significance lies in the understanding of the regulation of both the sexual determination and differentiation processes.

**Acknowledgements**

Our study of the androgenic gland was supported by a United States–Israel Binational Science Foundation (BSF) grant no. 93-00231.
References


