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Steroid hormone production by parasites: the case of *Taenia crassiceps* and *Taenia solium* cysticerci☆

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Abstract

Many examples of reciprocal endocrine interactions between parasites and hosts have been found in insects, arthropods and mammals. Cysticercosis produced by *Taenia solium* metacestodes is a widely distributed parasite infection that affects the human and the pig. *Taenia crassiceps* experimental murine cysticercosis has been used to explore the role of biological factors involved in host–parasite interactions. We had shown that *T. crassiceps* cysticercosis affects the serum concentration of steroid hormones and the reproduction behavior of the male mice host. In an effort to understand the biology of the parasite, we had investigated the parasite capacity to produce sex steroids. For this purpose, *T. crassiceps* cysticerci were incubated in the presence of different steroid precursors. TLC and recrystallization procedures showed that testosterone is produced from ^{3}H-androstenedione in cysticerci. The conversion of ^{3}H-testosterone to androstenedione, although present is much less significant. In addition, we had studied the production of testosterone by *T. solium* cysticerci. For this purpose, cysticerci were dissected from pork meat and incubated as above described. The results showed that *T. solium* cysticerci also produce testosterone. We have speculated about the importance of androgens in the growth of *T. crassiceps* cysticerci and found that the addition of the antiandrogen flutamide to the culture media of the parasites significantly decreased ^{3}H-thymidine incorporation. We therefore hypothesized, that the ability of cysticerci to produce testosterone from steroid precursors might be important for the parasite growth and development.

Keywords: Parasites; *Taenia crassiceps*; *Taenia solium*; Cysticercosis; Sex steroids

1. Introduction

Reciprocal interactions between host and parasites is a key point to understand parasite success and therefore to find the way to control those infections that cause diseases in the host. Parasites produce substances that produce changes in the host tissues and even can reach the host circulation. In the other hand, the host's immune system and hormonal milleau affect the development of the parasite. This interplay between the parasite and the host defines the severity and intensity of parasite infections and even affects the host behavior [1–4]. The products secreted by parasites have been demonstrated to induce changes in the host. Several cases are found in the literature. As examples of this fact, we can mention those related to worms and Taenias. The injection of products secreted by cultured *Taenia taeniformis* metacestodes into rats inhibit testosterone production by the testis [5]. The infection with these parasites produced an adverse effect on the fertility of both male and female rats [6]. Parasites seem to regulate the immune response of the host through products secreted to their environment [7]. These authors found that excretory/secretory products from *Taenia crassiceps* larvae suppressed T cell proliferative responses in vitro as well as the production of interferon gamma (IFN-γ) and IL-4. *T. crassiceps* metacestodes increased apoptosis of co-cultured T *lymphocytes* [8]. Furthermore, released products from *T. crassiceps* metacestodes obtained from the peritoneal cavity of infected mice inhibited the in vitro degranulation of mice and rat mast cells and the in vivo degranulation in rats [9]. The presence of male schistosomes is necessary for the sexual maturation of the female worms and an induction of vitellogenesis have been obtained using extracts of the male parasites (for a review, see [10]). Proopiomelanocortin (POMC)-derived factors, renin–angiotensin system (RAS) elements and morphine-like susbstances have been demonstrated in *Schistosoma mansoni* and hematophagous leeches; the secretion of these substances may influence the host

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immune and neuroendocrine system (for a review, see [11]).

Steroid production occurrence is an ancient fact. Many microorganism and plants have steroidogenic enzymes that can transform a wide variety of steroids. The physiological relevance of this transformation is not known, but is some cases it functions as a detoxificant system that transforms a harmful steroid to an innocent one. Adrenal corticosteroids as well as male and female sex steroid hormones have been found in all vertebrates studied, but the occurrence of steroid hormones in the invertebrate is less known. Therefore, their physiological significance remains unclear in several cases.

In the case of invertebrates, steroid hormones are documented to exert physiological functions in the development and reproduction of several species. Arthropods produce ecdysones, which are steroid hormones involved in the insect moulting, these steroids might function as androgen or estrogen-like hormones in the insects [12]. Ecdysterone can be measured in the sera of infected hosts parasitized by a trematode, *Schistosoma* that produces and releases the hormone to the host's circulation [13]. Ecdysterone production have been reported in several helmynth species, including the parasitic nematodes *Dirofilaria inmitis*, *Brugia pahangi*, *Ascaris suum* and *Anisakis simplex*, the cestodes *Moniezia expansa*, *Echinococcus granulosus* and *Hymenolepis diminuta*, and the trematodes *S. mansoni* and *Fasciola hepatica* [14–21]. Water beetles excrete testosterone [22] while preparations of androgenic glands of blue crabs convert progesterone to 11-deoxy-corticosterone [22]. Estrogens were found in *Schistosoma bullata* [23] and in the ovaries of a silkworm, *Bombyx mori* [24]. Estradiol have been detected in the eggs of the lobster [25], while the testis of this invertebrate can convert androstenedione to testosterone [26]. Finally homogenates from schistosomes convert a variety of steroids to its metabolites [27].

2. *Taenia solium* and *T. crassiceps* cysticercosis

Cysticercosis is a disease caused by the larval stage of cestodes of the family Taeniidae that affects many hosts, including humans. Cysticercosis is a serious public health problem in Mexico, Central and South America, India, Subsaharan Africa and is endemic in many Asiatic countries. Neurocysticercosis is the most common cause of adult seizures in many underdeveloped countries and the major reason that epilepsy is twice as common in developing countries as compared to developed countries (for a review, see [28]).

T. solium is a Platyhelminth that belongs to the class Cestoda. Cestodes or tapeworms have a long, flat body, made up of many segments called proglottids. Each proglottid is a reproductive unit that contains testis and ovary capable of producing gametes. Male organs mature first and produce sperm, which is stored until maturation of the ovaries occurs (Fig. 1). After fertilization, the gravid proglottids detach from the worm in intestine of the host and are shed in the feces. The intermediate host, normally the pig, is infected by ingestion of parasite eggs or proglottids from human feces. The eggs are induced to hatch by gastric and intestinal fluids. The hatched larvae (oncospheres) penetrate the intestinal mucosa and invade the host and migrate via the blood stream. The production of excretory/secretory peptidases by the oncospheres may facilitate invasion [29]. The oncospheres lodge preferentially in the pig muscle where mature into cysticerci causing a tissue reaction that depends of the larval stage [30]. The life cycle is completed when humans consume undercooked pork meat containing the cysts. Man can also act as an intermediate host for *T. solium* (for a detailed review, see [28]).

Characteristics of helminths:

- **Complex evolutive cycles.**
- **Larval status different from the adult.**
- **Body surface covered by a tegument: Absorption and metabolic exchange.**
- **Hermaphrodites. Proglottides contain ovaries and testis.**
- **The eggs have a larva that can infect the host (oncosphere).**
- **In the host tissue the larva develops into a cysticercus.**

Fig. 1. *Taenia solium* is a Platelminth that belongs to the class Cestoda. These tapeworms and other related to them are the cause of widely extended diseases that affects the human and the domestic animals.

The laboratory manipulation of another *taenia*, called *Taenia crassiceps* have allowed a substantial progress in the knowledge of cysticercosis. *T. crassiceps* is a tapeworm whose adult form is usually found in the intestine of European and North American red foxes. The rodent is the natural intermediate host for this parasite that multiply by budding in the peritoneal cavity (Fig. 2). The cysts also multiply in the peritoneal cavity of balb/c mice that are excellent hosts for *T. crassiceps* cysticerci, and therefore a useful model for the study of cysticercosis. Several strains of *T. crassiceps* have been isolated and maintained under laboratory conditions [31,32]. The ORF has become the most widely used because of its ability to multiply rapidly in the mouse peritoneal cavity. Endocrinologically, the murine host's gender is of great consequence for the rate of reproduction of *T. crassiceps*. In early infection, females carry larger parasites' loads than males, but later in chronic infections male also become massively parasitized [33] The reasons for the early parasite's preference for the female environment are still unclear. Immunendocrine interactions involving sex steroid hormones are candidates that might explain the sex susceptibility observed in *T. crassiceps* infections [34]. The questions were: Is the hormonal female's environment facilitating the sluggish parasite load found in the female mice? Which is the parasite's role in the shift from a low susceptibility of the male in early infections, to a massive invasion in a very chronic infection? We have shown that males chronically infected with *T. crassiceps*

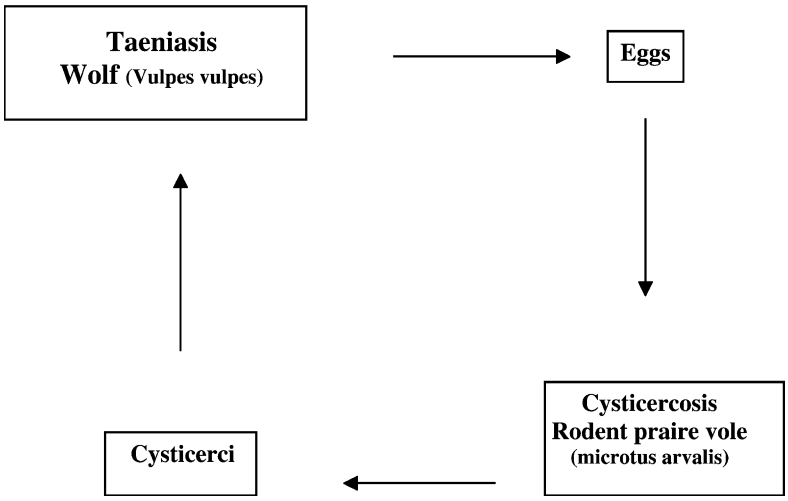

Fig. 2. Life cycle of *Taenia crassiceps*. *Taenia crassiceps* infection occurs naturally in wild animals. The laboratory manipulation of this Cestode that growths in the mice have facilitated the study of the biology of cysticercus and the host–parasite interactions.

cysticercus have a significant reduction in testosterone and increased serum estradiol levels [34]. Interfering the estrogen production by the administration of an antiaromatase to male mices resulted in a decrement of the parasite load [35]. In addition, we have demonstrated that after several weeks of infection, males progressively lose their sexual behavior and by 16 weeks of infection none of the infected males show any sexual response toward female mice [36].

3. Production of testosterone by cysticercus

At this point, we became interested in the parasite's capacity of reproduction. The cysticercus is the larvae form of an adult hermaphrodite tapeworm. It is well known that sex steroid hormones are produced at early stages of embryonic development in many species, however the occurrence and physiological significance of male and female sex steroid hormones in parasites have received poor attention. Furthermore, there was not any information about steroid production in the cysticercus. We therefore have investigated the ability of *T. crasssiceps* cysticerci to synthesize steroid hormones in vitro [37]. The fast growing ORF strain isolated by Freeman [31] supplied by Enders was maintained in female balb/c mice by i.p. sequential inoculation of the metacestodes in their peritoneal cavity. The cysticerci were aseptically harvested from the peritoneal cavity of mice after 3–5 months of infection. Aliquots of preincubated parasites were placed in vials containing sterile defined culture media and were cultured for 1 h at 37 °C in the presence of different radiolabeled steroid precursors. The culture media was extracted and processed by thin layer chromatography (TLC) in two different solvent systems. This methodology has demonstrated that *T. crassiceps* cysticercy transform ^{3}H-andostenedione to ^{3}H-testosterone. Testosterone production by the parasites was also measured by radioimmunoanalysis (RIA) of the extracted culture media. In order to confirm the nature of the metabolite found, a recrystallization procedure was done. The procedure corroborated the ability of cysticerci to convert androstenedione to testosterone. Parasites did not yield testosterone when incubated in the presence of ^{3}H-pregnenolone [37]. Parasites were also incubated in the presence of ^{3}H-testosterone and the media investigated by TLC. The method showed that the incubation of parasites in the presence of ^{3}H-testosterone yields small amounts of androstenedione (Media of transformation $= 0.72\%$, $n = 17$, each vial contained 1 ml of parasites). In most cases, transformation of testosterone to androstenedione was between 1.5 and 2%; not any transformation was detected in 20% of the vials. The finding of a poor transformation of testosterone to androstenedione strongly suggests that androstenedione→testosterone is the preferential pathway in the cysticerci.

We have speculated about the potential importance of androgens in the growth of the parasites and therefore cultured the *T. crassiceps* cysticerci in the presence of an antiandrogen. For this purpose, we had incubated the parasites for 48 h in a defined culture media (DMEM plus antibiotics) in the presence of different doses of flutamide and studied the ^{3}H-thymidine incorporation. We found that the addition of increasing doses of flutamide to the culture media significantly affected the growth of the parasite estimated as ^{3}H-thymidine incorporation (Fig. 3). Preliminary experiments also indicate that after 48 h of treatment with flutamide, changes in the microscopic aspect of the cultured parasites began to be evident (not shown).

We thus speculate that cysticerci's testosterone production might be important for the own development. Since it is well known that testosterone is involved in the development of the gonads of mammals and birds, it might be possible that the

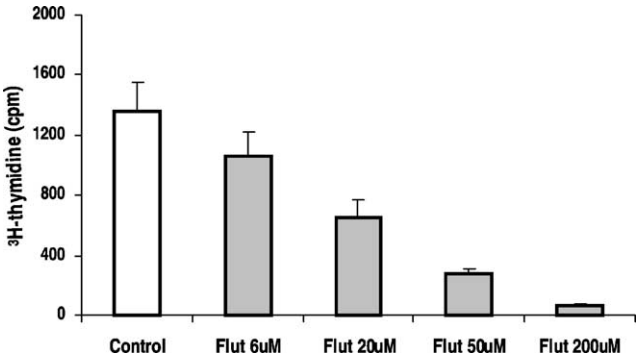

Fig. 3. Effect of flutamide on ^{3}H-thymidine incorporation by *Taenia crassiceps* cysticerci. Thirty microliters of cysticerci obtained from the peritoneal cavity of female mice were incubated for 24 h in a multiwell containing 1 ml of DMEM plus 10% fetal bovine serum and antibiotics (1 ml/100 ml of media of a solution containing penicillin 10,000 U/ml, streptomycin 10,000 μg/ml and amphotericin B 25 μg/ml). After this period, culture media were changed by other composed of DMEM, antibiotics and ^{3}H-thymidine. Flutamide at different concentrations or the solvent were added to the culture media and the cysticerci were cultured for additional 48 h. The cysticerci were washed for three times with PBS and dissolved in 0.5 M NaOH; radioactivity was measured in a scintillation counter. Each value represents media + S.D. of five wells of a representative experiment made three times.

development and/or the differentiation of the *T. crassiceps*' gonads is dependent on the presence of androgens.

It was also interesting to investigate if the androgen production found in *T. crassiceps* cysticerci is present in the cysticerci that affect the human and the pig. For this purpose we have dissected *T. solium* cysticerci from the muscle of infected pigs, and separated the fibrous capsule that surrounded each parasite under a dissection microscope. Parasites were thoroughly washed in sterile culture media and cultured in defined media (DMEM plus antibiotics) for 1 week at 37 °C in an atmosphere of 5% CO_2 and 95% air. After this period, they were washed with sterile culture media and further incubated for 3 h in the presence of ^{3}H-androstenedione. The culture media were extracted and steroids separated by TLC using methylene chloride–ethylacetate (8:2) as the solvent system. Results showed that *T. solium* cysticerci have the ability to transform androstenedione to testosterone with a high efficiency (Table 1).

Table 1
Percentage transformation of ^{3}H-androstenedione by *Taenia solium* cysticerci

Percentage of transformation ($X \pm$ S.D.)	
Testosterone	26.28 ± 3.097

In vitro transformation of ^{3}H-androstenedione to ^{3}H-testosterone by *T. solium* cysticerci. TLC plates were developed with methylene chloride–ethylacetate (8:2, v/v). ^{3}H-testosterone was the only significant metabolite produced by the parasites. Results are expressed as percentage of substrate transformation. Date are the mean ± S.D. of a representative experiment made by sextuplicate.

4. Conclusions

These findings indicate that the *T. solium* and *T. crassiceps* tapeworms are able to produce an androgen early in their embryonic life. Therefore, a 17β-hydroxysteroid dehydrogenase (HSD) activity is present in the cysticercus. In vivo, 17β-HSD types 1 and 3 catalyze the synthesis of active steroids with NADPH as the co-enzyme, while types 2 and 4 catalyze the inactivation of active steroids with NAD+ as the co-enzyme. The importance of these enzymes in regulating androgen and estrogen action has become subject of interest in the last decade [38]. The activity of this enzyme family has been found in bacteria, plants and animals [39,40]. The capacity of *T. solium* and *T. crassiceps* cysticerci to produce testosterone, plus the effects of blocking androgen receptors with flutamide, suggest that at least in the case of *T. crassiceps*, the endogenous production of testosterone is important for the larva development.

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