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Sex steroids and parasitism: *Taenia crassiceps* cisticercus metabolizes exogenous androstenedione to testosterone in vitro

Y. Gomez [b], R.A. Valdez [a], C. Larralde [c], M.C. Romano [a,*]

[a] *Dpto.de Fisiologia, Biofisica y Neurociencias, CINVESTAV, Apdo Postal 14-740, 07000, Mexico*
[b] *UPIBI-IPN, Depto. Biofísica y Fisiología, C.P. 07350 Mexico*
[c] *Dpto. de Inmunologia, Inst. de Investigaciones Biomedicas. UNAM, Mexico*

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Abstract

Sex hormones are known to modulate immune responses and may be implicated in sex associated susceptibilities to infections. *Taenia crassiceps* cysticerci grow to larger numbers in female mice than in males. Gonadectomy alters the course of this infection and hormone replacement with 17β-estradiol increases the parasite numbers. However, in chronic *Taenia crassiceps* cysticercosis the sex-hormone profile of males becomes more like that of the females' and progressively loose their sexual behavior. To have further insight in these outstanding endocrinological effects induced by the parasite upon the host, we investigated the parasite's capacity to produce sex steroids. In vitro experiments showed that *Taenia crassiceps* cysticerci transform ^{3}H-Androstenedione to 3_{H}-Testosterone, but not ^{3}H-Pregnenolone. The production of ^{3}H-Testosterone increased when the parasite numbers doubled. A recrystallisation procedure demonstrated that the metabolite identified by TLC was in fact testosterone. Thus, the cysticercus has the ability to use ^{3}H-Androstenedione to make Testosterone possibly by a 17β-Hydroxysteroid deshidrogenase-like activity in the parasite. In vivo, the parasite could use steroid precursors from the host to produce sex hormones, either accidentally or as needed for its own development, and thus alters the host's normal environment with sexual and immunological repercussions.

Keywords: *Taenia crassiceps*; Cysticercosis; Parasite hormones; Sex steroids; 17β-hydroxysteroid dehydrogenase (17β-HSD)

1. Introduction

Reciprocal hormonal interactions between host and parasite are receiving increased attention as they are shown to influence parasite or host success [1–3]. Hormones derived from many glands have now been shown to be involved in the regulation of the immune system, perhaps accounting for differences in sex associated susceptibilities to parasitic diseases [4]. Many examples of reciprocal endocrinological influence between parasites and host have been found. Male sand crabs parasitized with a rhizocefalan [5] have changes in sexual and in secondary sexual characteristics. When the parasitoid *Apanteles kariyai* lays eggs in the insect host *Pseudaletia separata* the larvae do not pupate and low levels of ecdysterone are found [6]. It has been also shown that infection with the trematode *Trichobilharzia ocellata* interferes with reproduction of the snail *Lymnaea stagnalis* through the secretion of a peptide [7]. The reproduction of two filarial (nematodes) species, *Brugia pahangi* and *Dirofilaria immitis*, is modulated by ecdysteroids [8]. The administration of secretory products of *Taenia taeniformis* metacestodes into rats inhibit testosterone production in the rat testis [9] and *Taenia taeniformis* infection alters reproduction in the infected female rat host [10]. Female mice are also more susceptible to infection by *Schistosoma mansoni*: the recovery of adult worms per cercariae being much higher in females than in males indicate that these parasites are more successful in developing into adult worms in the female mice [11]. More detailed attention has received experimental murine cysticercosis caused by *Taenia crassiceps*. This cestode disease of mice has been used to systematically explore the role of

* Corresponding author. Tel.: +52-5-7477000; fax: +52-5-7477105.
E-mail address: mromano@fisio.cinvestav.mx (M.C. Romano).

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biological factors involved in host-parasite interactions [12]. Genetic background and immune status of the host affect the growth of this parasite [13–19]. Endocrinologically, the murine host's gender is of great consequence for the rate of reproduction of *T. crassiceps*: infected females carry larger parasite loads than males, in early infection but later in very chronic infections males also become massively parasitized [14]. The reasons for the early parasites's preference for the female environment is far from clear, although some findings are suggestive of estrogen-mediated immulogical modulation in favor of TH_2 mediated immune response (mostly responsible of antibody synthesis) and depression of TH_1 mediated immunity (mostly responsible of cellular immune responses [20]. The sluggish massive colonization of male mice by *T. crassiceps* prompted the hypothesis that the parasite induced sex hormone changes leading to feminization of the male host that resulted in their carrying as large parasite loads as females. Indeed, it was later shown that *T. crassiceps cysticerci* triggers a feminization process in the infected male host, characterized by significantly reduced testosterone and increased estradiol serum levels [21]. Concomitantly, infected males progressively loose their sexual behavior and by 16 weeks of infection none of the infected males show any sexual response toward female mice [22]. Complete restoration of the sexual behavior of the infected males is obtained after the administration of testosterone [22]. At this point it was thought important to determine if the cysticercus has any capacity to produce sex hormones. The cysticercus is the larvae form of the adult tapeworm and it is well known that in many species the gonads are able to synthesize sex hormones at early stages of embryological development. Here we report on the ability of *T. crassiceps* cysticerci to use radiolabeled steroid precursors to produce testosterone in vitro: a seemingly unique finding, this of a primitive parasite producing a sex-steroid active in more evolved mammals.

2. Material and methods

2.1. Parasites

The fast growing ORF strain of *T. crassiceps* isolated by Freeman [23] supplied by Dr. B. Enders (Beringwerke, Marburg, Germany) was used to infect mice. The parasites have been maintained in female Balb/c mice by i.p. sequential inoculation of metacestodes in their peritoneal cavity for over 15 years now. The cysticerci used in the experiments were harvested from the peritoneal cavity of ether anesthetized female mice after 3–5 months of infection: all cysticerci were mobile and translucent cystic structures approximately 0.5 cm in diameter.

Two hundred microliters of cysticerci suspension, equivalent to 20.25 ± 1.73 parasites were placed in Dulbecco's Modified culture medium (DMEM) containing 0.1% BSA and washed twice with the same media. Parasites were incubated in DMEM plus 0.1% BSA for 1 h at 37°C in a shaking water bath and the media was then discarded. Cysticerci were then placed in each scintillation vial containing DMEM plus 0.1% BSA and 0.1 μCi of ^{3}H-Androstenedione (1,2,6,7-^{3}H-(*N*)-androst-4-ene-3, 17-dione, 93.0 Ci/mmol, Dupont New Products, Boston MS) or ^{3}H-Pregnenolone ([7-^{3}H-(*N*)-Pregnenolone, 23.5 Ci/mmol], Dupont Boston, New Products, MS) and incubated for 1 h at 37°C in a shaking bath. The tracer was also added to culture media free of parasites and incubated as described above as a blank. Cold diethyl ether (2 volumes) was then added to the culture and immediately thawed. The organic phase was then transferred to new vials and evaporated under nitrogen atmosphere at 37°C. The samples were reconstituted with 0.1 ml of absolute ethanol.

2.2. Thin layer chromatography

Testosterone, Androstenedione, 17β-Estradiol and Estrone were used for internal standards in the experiments where ^{3}H-Androstenedione was the steroid precursor, and the same plus 17-OH-P4, 17-OH-P5 and DHEA when ^{3}H-Pregnenolone was the precursor. TLC was carried out using Silica gel 60 F_{254} pre-coated sheets plates (Merck, Darmstadt, Germany). Aliquots of 40 μl of the ethanolic samples were supplemented with standard steroids and further fractionated in two independent TLC systems. Some plates were developed with a mixture of methylene chloride and ethyl acetate (8:2 v/v) and other with a mixture of benzene-acetone (3:1 v/v). The standard steroids (Steraloids, Wilton, NH) were detected in the plates by ultraviolet light and exposed to 10% H_2SO_4 followed by heating at 120°C. Regions corresponding to authentic standards were cut and placed in vials containing scintillation liquid and radioactivity counted in a liquid scintillation spectrometer. The recovery of radioactivity was estimated by comparing the difference between initial and final cpm.

Results are expressed as the percentage of substrate transformation for each identified metabolite, after 60 min of incubation in the presence of the precursor. Since the recovery of the radioactivity of each precursor was higher than 85%, no corrections were made. Results were submitted to analysis of variance and Student's *t*-tests.

2.3. Production of testosterone measured by RIA

Parasites were prepared as previously described (see parasites). Four hundred microlitres of parasites were

Table 1
Percentage transformation of ^{3}H-Androstenedione by *Taenia crassiceps* cysticerci[a]

	$\bar{X} \pm$ SD ($n = 7$)
Testosterone	34.58 ± 2.93
Remanent	55.25 ± 2.14

[a] Metabolism of radioactive androstenedione by cysticerci yielded ^{3}H-testosterone as the only product. The TLC plates were developed with methylene chloride–ethylacetate (8:2 v/v). Results are expresed as the percentage of substrate transformation after 1 h incubation at 37°C. Data are the mean ± SD of three different experiments made at least by duplicate.

Table 2
Percentage transformation of ^{3}H-Androstenedione by *Taenia crassiceps* cysticerci[a]

	Percentage of transformation $\bar{X} \pm$ SD ($n = 6$)
Testosterone	35.46 ± 1.84
Remanent	47.7 ± 1.93

[a] To further corroborate the *in vitro* transformation of ^{3}H-androstenedione to ^{3}H-testosterone by the parasites, TLC plates were developed in bencene:acetone (3:1 v/v). Again ^{3}H-testosterone was the only significant metabolite produced by the parasites. Results are expressed as percentage of substrate transformation. Date are the mean ± SD of an experiment made by sextuplicate.

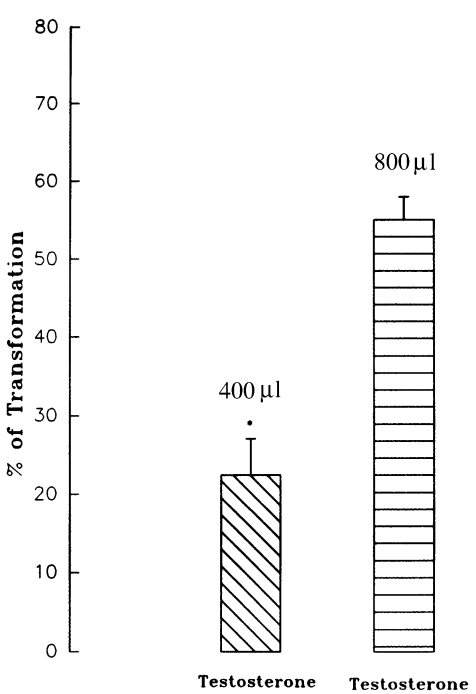

Fig. 1. The amount of testosterone produced is related to the number of cysticerci in the culture medium. Four hundred or 800 µl of cysticerci/tube were incubated for 1 h at 37°C. The production of radioactive testosterone is expressed as the percentage transformation of ^{3}H-Androstenedione. Data are the mean ± SD of an experiment containing four samples per group.

placed in each of 42 scintillation vials containing DMEM plus 0.1% BSA and Androstenedione (0.1 mg/ml) and incubated for 3 h. The samples were immediately ether- extracted as above described, transferred to new vials, dried and then dissolved in ethanol. The samples were then combined in two pools and dried under nitrogen atmosphere. For testosterone measurement, 4 ml of buffer RIA were added to each vial pool and testosterone measured by RIA as described previously [24].

2.4. Recrystallization of testosterone

The parasites were prepared as described above and incubated in the presence of 0.1 µCu of ^{3}H-Androstenedione for 1 h. The samples were immediately ether-extracted and dissolved in ethanol. The samples were then combined in a pool and dried under nitrogen atmosphere. The recrystallization procedure was done as follows. Four consecutive crystallization procedures were performed in the presence of 25–30 mg unlabeled testosterone that was mixed with the pool of extracted incubation media. A mixture of ethanol and water (1:1) was used as the solvent. As controls 25–30 mg of estrone or estradiol authentic standards were mixed with aliquots of the radioactive parasites samples and crystallized four times from the same mixture. The mother liquors and the final crystals were assayed for radioactivity [25]. Final identification of the metabolite was obtained by constant specific activities of crystals. The specific radioactivity of the crystals was expressed in cpm/mg.

3. Results

Metabolism of ^{3}H-androstenedione by cysticerci yielded testosterone as the only one significant product when methylene chloride-ethyl acetate was used as the eluent (Rf = 0.24) (Table 1). To corroborate this fact, samples were developed in a benzene:acetone mixture with similar results (Rf = 0.51) (Table 2). No significant transformation of ^{3}H-Androstenedione to 17β-Estradiol nor to Estrone could be detected. Nor were *Taenia crassiceps* cysticerci able to metabolize ^{3}H-pregnenolone since no metabolites could be detected in the incubation media (negative data not shown).

The production of Testosterone was roughly proportional to the number of cysticerci present in the media. As shown in Fig. 1 the percentage of transformation of ^{3}H-Androstenedione to Testosterone was doubled when twice the number of cysticerci were placed in the vials. The presence of Testosterone in the parasites incubation media was also found by way of specific RIA (21.16 ± 2.6 pg/ml)

Table 3
Recrystallization of testosterone in aliquots of incubation media from *Taenia crassiceps* cysticerci[a]

	Consecutive crystallization (cpm/mg)			
	C1	C2	C3	C4
Testosterone	3855	3880	3285	3491
Mother's liquors	10711	646	326	300

[a] Data show specific activity obtained in authentic standard crystals formed in the presence of the pool of extracted radiactive incubation media from parasites. A mixture of ethanol and water was used as the solvent. Specific radioactivity of the crystals are expressed in cmp/mg of testosterone. Radioactivity of mother's liquors is expressed as total cpm/ml.

A recrystallization procedure was performed in order to demonstrate the nature of the metabolite characterized as Testosterone in TLC. The results of Table 3 show that when Testosterone authentic standards were mixed with radioctive parasite samples a constant specific activity of crystals is found in the consecutive recrystallization steps. On the other hand, Estradiol and Estrone authentic standards did not recrystallize to constant specific activity when mixed with radioactive parasite samples (not shown).

4. Discussion

The transformation of tritiated Androstenedione and Pregnenolone to steroid metabolites was assayed in *T. crassiceps* cysticerci incubated in vitro. A significant transformation of ^{3}H-androstenedione occurred, showing that the cysticercus has the ability to use the supplied substrate to make Testosterone. No significant transformation of ^{3}H-Androstenedione to Estrone was found in the present study, although our experiments do not complety rule out the possibility of traces of aromatase activity in the cysticercus. The fact that specific activity of samples subjected to repeated crystallization was constant corroborates that the metabolite identified by TLC was indeed Testosterone. On the other hand, the incubation of parasites with ^{3}H-Pregnenolone did not yield any measurable product. This fact suggests that the parasites lack 3β-hydroxy-steroid dehydrogenase /Δ^{5-4} isomerase (3β-HSD). The enzyme involved in the synthesis of testosterone from Androstenedione is 17β-hydroxysteroid dehydrogenase (17β-HSD) [26]. Thus, our results indicate that a 17β-HSD like enzyme is present and active in the cysticercus (or larval stage) of the cestode *T.crassiceps* if exogenously supplied precursor is available. No traces of endogenous testosterone were found in the absence of exogenous precursor at least by RIA. The fact that the parasite did not transform ^{3}H-Pregnenolone strongly suggests that it lacks the complete steroidogenic pathway.

There are no previous reports on the production of sex steroids by *T.crassiceps* cysticercus or by any other taenids. In fact, a computer search for literature in the intersection of sex-steroid X all parasites could not find a single report of a parasite synthesizing sex-steroids in the last 25 years. However, the existence of other steroid hormones in parasites have been documented. Ecdysteroids and related metabolites, which are steroid hormones involved in the molting process of insects have been shown in the strobila of the sheep cestode *Moniezia expansa*, [27] and in protoscoleces and hydatid cyst fluid of *Echinococcus granulosus* [28]. Ecdysteroids have also been found in the trematode *Schistosoma mansoni*, where it is assumed they may be involved in the parasite's development and reproduction [29].

It is well known that Androstenedione is a weak androgen produced by the adrenal glands and gonads. It is used as a precursor to synthetize Estrogens in the placenta and is also an intermediate metabolite in the synthesis of Testosterone and Estrogens in the gonads. On the other hand, Testosterone is a hormone known to be involved in the development of the primary and secondary male characteristics during development, at least in mammals and birds, and when aromatized to Estrogen mediates feminization and other events in females and males. Notwithstanding an accidental misplacement in phylogeny of such an important gene as that coding for 17-HSD in the cestode *T. crassiceps*, we speculate that it could be strategic: Testosterone playing a crucial role in *T. crassiceps* biology. One possibility being its affecting the parasite's sexual development itself, in a manner similar to that of mammals. The other possibility, not disjunctive with the first, that of helping-out the male host with additional Testosterone: a most convenient tactic of the cysticercus to ensure host's estrogenization and its immunological consequences of favoring the TH2 immune system (antibodies) which would in turn downregulate the antiparasitic role of TH1 immune system (cellular immunity)[30].

Thus, our results are an example of the hormonal relationship between host and parasite that may allow the parasite to develop, reproduce and survive by using the host's more complete endocrinological system. In return, the cysticerci provides the male host with additional quantities of precursor (Testosterone) to aromatize it to the excess Estrogen found in chronically infected mice (21). Evidently, there not being a significant aromatase activity in the cysticercus, it must be the male host's aromatase the one responsible for the high levels of Estrogen found in chronically infected male mice.

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