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Steroidogenic capacity of *Trypanosoma cruzi* trypomastigotes

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ABSTRACT

American Trypanosomiasis is caused by the hemoflagellate *Trypanosoma cruzi* (*T. cruzi*) and affects millions of persons causing variable degrees of digestive and heart disturbances. As far as we concerned, *T. cruzi* capacity to synthesize steroid hormones has not been investigated. Therefore, the aim of this work was to investigate the capacity of *T. cruzi* trypomastigotes to transform tritiated steroid precursors into androgens and estrogens. The *T. cruzi* Tulahuén strain was obtained from mice blood. The trypomastigotes were cultured for 6 and 24 h in Dulbbeco's modified Eagle's medium plus FCS and antibiotics. Tritiated dehydroepiandrosterone or androstendione were added to the culture media and parasites were incubated for 6 or 24 h. The cultures were centrifuged and ether extracted. The steroids were analyzed by thin layer chromatography (TLC) in two solvent systems. After incubation with ^{3}H-androstenedione, *T. cruzi* trypomastigotes synthesized ^{3}H-testosterone (T), ^{3}H-17β-estradiol (E_2) and ^{3}H-estrone (E_1). Metabolism of ^{3}H-DHEA by the parasites yielded ^{3}H-androstendione and ^{3}H-androstendiol at 6 h of incubation. The recrystallization procedure further demonstrated the ^{3}H-androstendiol and ^{3}H-17β-estradiol syntheses. Results indicate for the first time that *T. cruzi* trypomastigotes produce androgens and estrogens when incubated in the presence of steroid precursors and suggest the presence of active parasite steroidogenic enzymes.

1. Introduction

American Trypanosomiasis is caused by the hemoflagellate *Trypanosoma cruzi* (*T. cruzi*) and affects approximately 20 million persons in Latin America [1] (World Health Organization, 2007). The human infection usually goes through three phases: an acute episode, which is often self-resolving and leads to the indeterminate phase; the chronic form, which develops in one-third of asymptomatic infected people and is characterized by a variable degree of digestive and heart disturbance; heart damage, which is thought to result in part from the generation of autoimmune reactions against myocardial tissues [2].

A bulk of evidence indicates that sex steroid hormones influence the development and course of parasitic infections. The impact of the differences in the immunological response of males and females and the association with sex steroids were reviewed [3–5]. Besides this information a wealth of evidence has accumulated indicating that gender is relevant for the susceptibility, and the course of parasitic infections [6,7]. The effect of hormones on the development of *Schistosoma* has been reviewed [8,9].

Reproductive organs and their function may be influenced by the *T. cruzi* infection. In this regard, male reproductive organs are affected in experimental Chagas disease [10], whereas testosterone secretion is impaired within 4 weeks of *T. congolense* infection in sheep [11]. On the other hand, ovariectomy increased the *T. cruzi* parasitism in mice [12]. In experimental *T. congolense* infection, plasma progesterone concentrations were lower in the infected West African Dwarf goats [13]. *T. evansi* also induced infertility in dromedary bulls, causing changes in steroids concentration, a drop in testosterone and increased 17β-estradiol serum concentrations, and altered semen characteristics [14].

The exogenous sex steroid hormones administration influenced *T. cruzi* infection. For example, 17β-estradiol has been shown to modulate the acute phase of *T. cruzi* infection in mice [15] and dehydroepiandrosterone administration decreases parasitemia in rats [16]. Parasitemia was lower in orchiectomized *Calomys callosus*, a wild rodent found naturally infected with *T. cruzi*, and testosterone replacement raised the parasitemia to control levels [17]. Steroid-related hormones as the sesquiterpenoid juvenile hormone that have morphogenetic and gonadotropic physiological functions in insects have effects on the growth of *T. cruzi* [18].

A parasite such as *Schistosoma mansoni* synthesizes, incorporates and metabolizes lipids [19,20]. This parasite also synthesizes isoprenoic compounds [21], and produces different molecular

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species of phospholipids [22]. Helminths also produce ecdysteroids [23–30]. The production and physiological significance of male and female sex steroids hormones synthesized by parasites have received poor attention (for a review, see [31]). The synthesis of steroid hormones by *Schistosoma mansoni* was investigated by Briggs [32], who found that this parasite has the capacity to perform several steps in the steroidogenic pathway. We have investigated the ability of *T. crassiceps* ORF strain cysticerci to synthesize steroid hormones *in vitro* [33] and found that the parasites have the capacity to transform ^{3}H-androstendione into ^{3}H-testosterone. We have also demonstrated that *T. solium* cysticerci synthesized androgens and small quantities of estrogens *in vitro* [34]. Recent results from our laboratory also showed that *T. solium* and *T. crassiceps* cysticerci as well as the adult worms synthesized steroid hormones from tritiated progesterone and dehydroepiandrosterone [35,43]. These results strongly suggest the presence and activity of steroidogenic enzymes in cysticerci.

As far as we concerned, *T. cruzi* capacity to synthesize steroid hormones has not been investigated. However, this parasite has the capacity to synthesize lipids. In this regard, it has been reported that *T. cruzi* trypomastigotes synthesize and shed lipids into the culture media [36], and have the capacity to synthesize and remodel inositol phosphoceramide during differentiation [37]. These authors suggest a role for inositol phosphoceramide in *T .cruzi* differentiation.

The aim of this work was to investigate the capacity of *T. cruzi* trypomastigotes to transform tritiated steroid precursors into androgens and estrogens.

2. Materials and methods

2.1. Animals and parasites

Animals raised and maintained in our breeding facilities were used for the experiments, which were conducted following the Institutional Experimental Guidelines for Animal Studies. The *T. cruzi* Tulahuén strain was maintained by subcutaneous passages of trypomastigotes in male Cbi mice. After 8 days of infection, mice were bled and parasites were obtained by differential centrifugation. After the steps of purification, parasites were counted in Neubauer's camera and the final concentration was adjusted with culture medium.

2.2. Trypanosome cruzi culture and conditions

The trypomastigotes (10^{7} *T. cruzi*/ml) were cultured in polystyrene tubes (TPP) for 6 and 24 h in Dulbbeco's modified essential medium (DMEM, Gibco) plus 2% FCS (Neutroquímica), 100 μg/ml penicillin (Gibco), 100 UI/ml streptomicin (Gibco), 20 μg/ml gentamicin (Gibco), 0.2 mM 2-mercaptoethanol and HEPES 10 mM (Gibco).

Tritiated dehydroepiandrosterone (^{3}H-DHEA-(1,2,6,7-^{3}H(N) dehydroepiandrosterone, 74 Ci/mmol, NEN) or tritiated androstendione (^{3}H-A_4-(1,2,6,7-^{3}H(N) androstendione, 93 Ci/mmol, NEN) was added to the culture media (50,000 cpm/ml), and parasites were incubated for 6 or 24 h at 37 °C under 5% CO_2. Blanks containing only culture media and the corresponding tritiated steroids (50,000 cpm/ml) were simultaneously incubated. Both precursors were dissolved in absolute ethanol and the final ethanol concentration in the culture media was less than 0.1%.

After the incubation period, the cultures were centrifuged at 1200 × *g* and the supernatant was reserved. For the extraction procedure cold diethyl ether (2 volumes) were added to the media and immediately thawed. The organic phase was transferred to new vials and evaporated under nitrogen atmosphere at 37 °C. The samples were reconstituted with 0.1 ml absolute ethanol.

2.3. Thin-layer chromatography

Blanks and parasites culture media were analyzed by thin layer chromatography (TLC). 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 steroid and further on fractionated in a mixture of methylene chloride–ethyl acetate (8:2, v/v) or benzene–methanol (9:1, 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 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 incubation in the presence of the precursor.

2.4. Recrystallization of steroids

The trypomastigotes were prepared as described above and cultured for 24 h in the presence of tritiated DHEA to recrystallize androstendiol or tritiated androstenedione to recrystallize 17β-estradiol. The samples were ether extracted and dissolved in ethanol. A pool of the ethanolic samples was prepared and dried under nitrogen atmosphere. For androstenediol recrystallization the dried extracts were dissolved in methanol containing 22 mg of cold androstendiol. For 17β-estradiol recrystallization the dried extracts were dissolved in ethanol containing 22 mg 17β-estradiol so that the solution was nearly saturated with the steroid. HPLC–quality water (1:1 for androstendiol and 1:2 for 17β-estradiol) was added by drops until the solution became turbid and crystals began to appear. The solution was kept at 4 °C overnight to allow complete crystallization of the steroid. The weight and radioactivity of the crystals were measured and the specific activity was calculated and expressed as cpm/mg [42]. The recrystallization was conducted four times.

2.5. Statistical analysis

Two rounds of experiments were performed ($n = 4$). Statistical comparisons were made by the Mann–Whitney *U*-test.

3. Results

Fig. 1A shows the transformation of ^{3}H-A_4 into metabolites by *T. cruzi* incubated for 6 h in the presence of the steroid precursor when the plates were developed in methylene chloride–ethyl acetate (8:2, v/v). The parasites yielded ^{3}H-testosterone (T), ^{3}H-17β-estradiol (E_2) and ^{3}H-estrone (E_1). After 24 h of incubation, the synthesis of testosterone and estrogens increased, particularly the synthesis of E_2 (Fig. 1B). Fig. 1C shows the results obtained when the TLC plates were developed in benzene:methanol (9:1, v/v). In this case the trypomastigotes were incubated for 24 h in the presence of the tritiated precursor and the results basically corroborated the ones found with the methylene chloride–ethyl acetate system.

The metabolism of ^{3}H-DHEA by the parasites is shown in Fig. 2. In Fig. 2A and B the plates were developed in methylene chloride–ethyl acetate (8:2, v/v). The incubation of *T. cruzi* in the presence of DHEA for 6 h yielded mainly the androgens, ^{3}H-androstenedione and ^{3}H-androstenediol (Fig. 2A). ^{3}H-testosterone and ^{3}H-17β-estradiol were almost undetectable (Fig. 2A). After