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Taenia crassiceps WFU cysticerci synthesize corticosteroids *in vitro*: Metyrapone regulates the production

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ABSTRACT

Taenia solium and *Taenia crassiceps* WFU cysticerci and tapeworms have the ability to synthesize sex steroid hormones and have a functional 3β-hydroxisteroid dehydrogenase. Corticosteroids (CS) like corticosterone and dexamethasone have been shown to stimulate *in vitro* estrogen production by *Taenia crassiceps* WFU cysticerci. The aim of this work was to study the ability of *T. crassiceps* WFU cysticerci to synthesize corticosteroids, and the effect of the inhibitor metyrapone on the CS synthesis. For this purpose *T. crassiceps* WFU cysticerci were obtained from the abdominal cavity of mice, thoroughly washed and pre-incubated in multiwells for 24 h in DMEM plus antibiotics/antimycotics. The tritiated CS precursor progesterone (^{3}H-P4) was added to the culture media and parasites cultured for different periods. Blanks containing the culture media plus the ^{3}H-P4 were simultaneously incubated. Blanks and parasite culture media were ether extracted and analyzed by thin layer chromatography (TLC) in two different solvent systems. Corticosterone production was measured in the culture media by RIA. In some experiments metyrapone (0.1–0.5 mM) was added for 24, 48 or 72 h. Results showed that cysticerci mainly synthesized tritiated 11-deoxy corticosterone (DOC) and small amounts of corticosterone that was also detected by RIA. Small amounts of ^{3}H-11-deoxy cortisol were also found. Corticosteroid synthesis was time dependent. The addition of metyrapone significantly inhibited tritiated DOC, deoxycortisol and corticosterone synthesis. These results show for the first time that parasites have the capacity to synthesize CS that is modulated by metyrapone. Data suggest that DOC is the main corticosteroid in the parasites.

1. Introduction

Cortisol, corticosterone and aldosterone are essential hormones found in most tetrapods. Their importance in stress response, immunity, metabolism and differentiation is well known [22]. Glucocorticoids (GCs) are steroid hormones synthesized by the glomerular zone of the adrenal cortex [24] or the interrenal tissues of amphibians, reptiles and fishes. However local CS synthesis has been found in several tissues like the brain and cardiovascular tissues (for a review see Davies and MacKenzie [7]). Furthermore corticosteroid synthesis and corresponding steroidogenic enzymes and their genes were found in thymic epithelial cells [27,19] in the developing lung cells of mammals [20] and in human skin keratinocytes [10].

Corticosteroids exert their effect by binding to specific receptors that share all the characteristics of a large family of molecules that finally recognizes a site called glucocorticoid receptor element in DNA. The binding of the receptor to this site initiates the transcription of several proteins; GC receptors are widely distributed in most cells of vertebrates and are classified as GC receptors (Type II) and mineralocorticoid receptors (Type I), that have great affinity for glucocorticoids and mineralocorticoids, respectively; however Type I receptors act like mineralocorticoid receptors in some tissues but have a high affinity for GC receptors in others; non-genomic actions of GCs have also been reported.

Cortisol is the most abundant glucocorticoid in most vertebrates and corticosterone is the main corticosteroid in rodents, birds and amphibia and has also important mineralocorticoid activities. A wide variety in the type of corticosteroids and their function can be found, for example teleosts lacks aldosterone and in these fishes cortisol may have GC and MC actions. Close et al. [6] provided evidence that 11-deoxycortisol is the main corticosteroid hormone in the lamprey, an agnathan member that belongs to the oldest vertebrate lineage.

Phylogenetic studies on corticosteroid receptors performed by several authors [26,13,3,23] suggested that a MC receptors complex evolved first. Therefore other corticosteroids should be considered as active GCs or MCs and an ancestral hormone, 11-deoxycorticosterone may be important in this complex panorama. In this regard it has been shown that DOC serum concentration

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increases in the rainbow trout during spermiation [4,16] and that DOC is an agonist of the MC in this fish [25].

Effects of corticosterone and dexamethasone on androgen synthesis by mouse Leydig cell has been described by Payne and Sha [18]. Interestingly, corticosteroid action on female and male mammal reproduction aspects seems to be different in chronic and acute stress [1]. Data reported in the literature strongly suggest that these hormones participate in germ cell development as well as on selected ovarian steroidogenic enzymes [15]. Wada [30] published a complete vision of GCs as mediators for vertebrate ontogenic transitions, for example they promote maturation of organs before birth in mammals and hatching in birds, participates in metamorphosis and facilitate acquisition of osmoregulatory ability in fish through Na^+–K^+ ATPase activity and ion transport among other events critical for survival.

We have shown that *Taenia crassiceps* WFU and *Taenia solium* cysticerci and tapeworms, *in vitro* synthesize sex steroid hormones [12,28,21] and have demonstrated the presence and activity of 3β-hydroxysteroid dehydrogenase in their tissues [9].

On the other hand, we have recently demonstrated that the addition of corticosterone or dexamethasone to the culture media of *T. crassiceps* WFU cysticerci increased the 17β-estradiol synthesis in a time and dose dependent-manner [11].

Corticosteroid presence and function is not well understood in the case of most invertebrates and to our knowledge is unknown in parasites. To this end we have investigated the ability of *T. crassiceps* WFU cysticerci, to synthesize corticosteroids *in vitro*. In addition we have investigated the effect of metyrapone, an inhibitor of CS synthesis on corticosteroid synthesis.

2. Materials and methods

2.1. Parasites

Cysticerci from the WFU strain of *T. crassiceps* were collected from the peritoneal cavity of female Balb/c mice after 5–7 months postinfection and rinsed five times in phosphate-buffered-saline, pH 7.2 (PBS). Cysticerci were pre-incubated for 24 h at 37 °C in an atmosphere of 5% CO_2 and air, in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO BRL, Grand Island, NY, USA), plus 0.1% bovine serum albumin (BSA, Sigma–Aldrich Chemical Co., St. Louis, MO, USA), 25 mM of *N*-[2-hidroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES; Sigma) and 1% antibiotic–antimycotic solution (penicillin at 10,000 U/ml, plus streptomycin at 10,000 μg/ml, amphotericin 25 μg/ml; GIBCO).

Following the pre-incubation period, the parasites were washed with DMEM and transferred to new wells (250 μL cysticerci/well) containing fresh culture medium. Some cultures were treated for 24, 48 or 72 h with different concentrations of metyrapone (0.1–0.5 mM) solved in ethanol, this solvent was added to control groups at a final concentration of 0.6%. Finally, fresh medium containing 0.1 μCi of ^{3}H-progesterone (3H-progesterone, 1, 2, 6, 7-^{3}H progesterone 93.0 Ci/mmol, Amersham Pharmacia Biotech) and the cysticerci further incubated for different periods. Blanks containing the culture media plus the ^{3}H-P4 were simultaneously incubated. At the end of the experiments, culture media were recovered and ether extracted. The samples were reconstituted in 100 microliter of absolute ethanol [12].

2.2. Thin layer chromatography

17OH-progesterone, 11-deoxycorticosterone, corticosterone, 11-deoxycortisol, and cortisol were used as internal standards. Thin layer chromatography (TLC) was carried out using Silica gel 60 F_{254} pre-coated sheet plates (Merck, Darmstadt, Germany) as described previously for *T. crassiceps* cysticerci [12]. Aliquots of 20 μl of the ethanolic samples were supplemented with standard steroids and further fractionated in a TLC system. The plates were developed in two different solvent systems (benzene:acetone 50:50 v/v or toluene:acetone:methanol 78:20:2 v/v; Merck, Darmstadt, Germany). 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 was counted in a liquid scintillation spectrometer. The recovered radioactivity was estimated by comparing the difference between initial and final cpm.

Results were expressed as the percentage of substrate transformation for each metabolite, after 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. Radioimmunoanalysis (RIA)

In another experiments cysticerci were precultured as described above and further incubated for 48 h in DMEM with 1 mM cold pregnenolone as the precursor to determine corticosterone in the cysticerci culture media. The pool of culture media was ether extracted to obtain enough samples to measure corticosterone by RIA. For costicosterone RIA, tritiated corticosterone (^{3}H-corticosterone (1, 2, 6, 7-^{3}H(N)) New England Nuclear USA 250 μCi 70 Ci/mmol) was used as the tracer and the anti-corticosterone antibody was purchased from Sigma (Product # 8784, Saint Louis Missouri, USA). The percent of the antibody cross-reactivity was: corticosterone 100%, 11-deoxycorticosterone 20%, cortisol 4.5%, progesterone 15.7%, 20α-hydroxyprogesterone 8.8%, 20β-hydroxyprogesterone 5.2%, testosterone 7.9%, androstenedione 2.6%, aldosterone 4.4%. The experiments were repeated three times.

2.4. Statistics

Statistical analysis were performed using Prism version 4 2003 (GraphPad Software Inc.). Data are presented as means ± SD. Probability values of $p < 0.05$ were considered to be significant. One-way analysis of variance Anova followed by Dunnet's test was used to compare all metyrapone dose columns vs. control column.

3. Results

Fig. 1 shows the corticosteroid metabolites found in cysticerci culture media after 24, 48 or 72 h in the presence of ^{3}H-progesterone. The plates were developed in benzene:acetone 50:50 v/v (Fig. 2A) or in toluene:acetone:methanol 78:20:2 v/v (Fig. 2B). Tritiated progesterone was mainly metabolized to ^{3}H-11 DOC and ^{3}H-11-deoxicortisol, small quantities of corticosterone and cortisol were also found. Data in Fig. 1 shows that the corticosteroid synthesis by *T. crassiceps* WFU was time dependent.

The corticosteroid synthesis was similar using the two solvent systems which confirms the nature of metabolites found (Fig. 2).

The effects of metyrapone on corticosteroid synthesis by cysticerci is shown in Figs. 3 and 4. The addition of the inhibitor significantly decreased the synthesis of ^{3}H-11 DOC, but the effect was not evident after 72 h, whereas ^{3}H-11-deoxicortisol was still significantly depressed after 48 and 72 of exposition to metyrapone (Figs. 3 and 4). The highest dose of metyrapone decreased ^{3}H-corticosterone synthesis at 24 h, no significant effect of the drug was observed after this incubation time (Fig. 5).