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ORIGINAL PAPER

Steroid hormones alter AMP hydrolysis in intact trophozoites of *Trichomonas vaginalis*

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Abstract *Trichomonas vaginalis* infection may be influenced by the vaginal concentrations of estrogens. We have investigated the effects of 17β-estradiol and dehydroepiandrosterone sulfate (DHEAS) on the ecto-5′-nucleotidase activity in fresh clinical (VP60) and in long-term-grown (30236 ATCC) isolates of *T. vaginalis*. In vitro exposure to DHEAS and 17β-estradiol did not induce any changes in adenosine monophosphate (AMP) hydrolysis in these isolates. The treatment of parasites in the presence of DHEAS (0.01–1.0 μM) for 2 h inhibited AMP hydrolysis in VP60 isolate, whereas there were no significant changes in nucleotide hydrolysis in the presence of 17β-estradiol. DHEAS and 17β-estradiol (0.01–1.0 μM) for 2 h inhibited AMP hydrolysis in 30236 isolate. The 12 treatment with 0.1 μM DHEAS inhibited AMP hydrolysis, whereas 17β-estradiol did not alter the nucleotide hydrolysis in VP60 isolate. Our findings have shown that the complex effect of steroid hormones and their receptors on *T. vaginalis* may promote changes in ecto-5′-nucleotidase activity during exposure to these hormones.

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Introduction

Trichomoniasis is a commonly known non-viral sexually transmitted disease, caused by *Trichomonas vaginalis*, an amitochondriate parasitic flagellated protozoan. This parasite infects the urogenital tract of both women and men. Trichomoniasis is associated with serious adverse health consequences to women that include infertility (Grodstein et al. 1993), atypical pelvic inflammatory disease (Moodley et al. 2002), preterm birth, and low birth weight infants (Cotch et al. 1997), as well as predisposition to cervical neoplasia (Viikki et al. 2000). Trichomoniasis among men can cause non-chlamydial, non-gonococcal urethritis (Bakare et al. 1999). Recently, serum antibody against *T. vaginalis* was found to be related with prostate cancer (Sutcliffe et al. 2006). Trichomoniasis increases predisposition to human immunodeficiency virus seroconversion for both men and women (Guenthner et al. 2005; Mason et al.

2005). The proteins and glycoproteins in the cell surface of trichomonads play a major role in cytoadhesion, host-parasite interaction, nutrient acquisition, and in the protection from the cytolytic effects (Petrin et al. 1998).

Extracellular adenosine triphosphate (ATP) may act as a signaling compound in cytolytic mechanisms (Filippini et al. 1990; Steinberg and Di Virgilio 1991). The signaling actions of this nucleotide are inactivated by a group of enzymes named nucleoside triphosphate diphosphohydrolases and ecto-5′-nucleotidase (EC 3.1.3.5; Robson et al. 2006). Adenosine monophosphate (AMP) hydrolysis by ecto-5′-nucleotidase represents an important source of extracellular adenosine (Sträter 2006). Adenosine is a regulatory autocoid that is generated as a result of cellular injury or stress, and it interacts with specific G protein-coupled receptors on inflammatory and immune cells to regulate their function (Haskó and Cronstein 2004). 5′-Nucleotidase activity has been described for bacteria and plant cells, and the enzyme is also widely distributed in vertebrate tissues. This enzyme acts on a variety of non-cyclic nucleoside monophosphates, such as AMP, cytidine monophosphate, uridine monophosphate, inosine monophosphate, and guanosine monophosphate, inactivating them to the respective nucleosides and inorganic phosphate (Bianchi and Spychala 2003). Although 5′-nucleotidase has broad substrate specificity, AMP is considered to be the major physiological substrate with K_M values in the micromolar range (Zimmermann 1992). Our laboratory has characterized an ecto-5′-nucleotidase activity in trophozoites of *T. vaginalis* (Tasca et al. 2003) and *Trichomonas gallinae* (Borges et al. 2007), and ultrastructural cytochemical microscopy has shown ecto-5′-nucleotidase activity on the surface of the parasite (Tasca et al. 2004). The presence of an enzyme that hydrolyzes AMP to adenosine provides the nucleoside required for parasite growth due to the lack of de novo purine nucleotide synthesis among all trichomonad species (Wang 1990; Munagala and Wang 2003).

Many hormones, especially the sex steroids, can influence the immune system and the susceptibility for diseases caused by protozoan parasites (Grossman 1984; Olsen and Kovacs 2005; Roberts et al. 2001). Studies have demonstrated an increased incidence of trichomoniasis during pregnancy suggesting that a high-estrogen state may enhance infectivity or symptoms (Brown 1972). Silva-Filho and Bonilha (1992) have shown that α-estradiol and 17β-estradiol increase the parasite adhesion. However, the activity of cell-detaching factor, a virulence factor of *T. vaginalis*, was significant diminished in the presence of 17β-estradiol. This suggests that the symptoms of *T. vaginalis* infection may be influenced by the vaginal concentrations of estrogens (Garber et al. 1991). Studies have shown that estrogen is associated with changes in 5′-nucleotidase activities (Murphy 2001; Ramalingam et al. 1993). Rücker et al. (2005) have demonstrated an increased activity and expression of 5′-nucleotidase from female rat cerebral cortex, following chronic steroid hormone deprivation induced by removal of ovaries.

Considering that trichomonads have no ability to perform purine and pyrimidine synthesis de novo, in this study, we have investigated the effects of 17β-estradiol and DHEAS on ecto-5′-nucleotidase activity, the enzyme activity involved in adenosine production, in fresh clinical (VP60) and long-term-grown (30236 ATCC) isolates of *T. vaginalis*.

Materials and methods

Chemicals

Trizma base, malachite green, ammonium molybdate, polyvinyl alcohol, nucleotides, Coomassie Blue G, bovine serum albumin, calcium and magnesium chloride, 17β-estradiol (cyclodextrin-encapsulated 17β-estradiol), dehydroepiandrosterone sulfate (DHEAS; 5-androsten- 3β-ol-17-one sulfate), and trypan blue dye were purchased from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade.

Parasite culture conditions

Two *T. vaginalis* isolates were used in this study: VP60, a fresh clinical isolate (EU816897; Michel et al. 2006), and 30236 from the American Type Culture Collection (ATCC). Both isolates were cultivated axenically in trypticase-yeast extract-maltose (TYM) medium (Diamond 1957) without agar (pH 6.0) supplemented with 10% (*v*/*v*) heat-inactivated cold serum, penicillin (1,000 IU/ml), and streptomycin sulfate (1.0 mg/ml) in aerobiosis at 37°C (±0.5). Isolates were subcultured every 48 h in TYM medium. Trichomonads, in the logarithmic phase of growth and within 48 h of subculture (exhibiting more than 95% mobility and normal morphology), were harvested and washed with sterile saline solution (NaCl 0.85%; 750×g for 5 min) three times. Parasites were resuspended in saline solution, counted with a hemocytometer, and adjusted to a concentration of 1.5×10^6 trophozoites/ml corresponding to 0.3–0.7 mg/ml protein. All experiments were performed using intact organisms and cellular viability was assessed before and after incubations by motility and trypan blue dye exclusion. The viability was not affected by incubation conditions.

Parasite treatments

The trophozoites were kept in the absence (control) or in the presence of 17 β-estradiol or DHEAS (0.01, 0.1, and

1.0 μM) for 2 or 12 h at 37°C (±0.5). During these pretreatments, the trophozoites were maintained in TYM medium (Diamond 1957) without agar (pH 6.0) supplemented with 10% (*v*/*v*) heat-inactivated serum, penicillin (1,000 IU/ml), and streptomycin sulfate (1.0 mg/ml). After the pretreatments, the trophozoites were washed with sterile saline solution (0.85% NaCl; 750×g for 5 min) three times. The subsequent steps for incubation to measure the nucleotide hydrolysis were the same as described above.

To evaluate the direct effect of hormones on the enzyme activity, 17β-estradiol, or DHEAS, at the final concentrations of 0.01, 0.1, 1.0, 2.5, or 5.0 μM, was added directly in the reaction mixture and was maintained throughout the enzyme assays, as described below. The enzyme assays was performed in the absence of hormones for the control group.

Enzyme assays

Intact trophozoites of *T. vaginalis* were added to the reaction mixture containing 50 mM Tris buffer (pH 7.5) and 3.0 mM $MgCl_2$ for measuring ecto-5′-nucleotidase activity (Tasca et al. 2003). The samples were preincubated for 5 min at 37°C in 200 μl of the reaction mixture. The reaction was initiated by the addition of AMP to a final concentration of 3.0 mM and stopped after 60 min by adding 200 μl 10% trichloroacetic acid. The samples were chilled on ice for 10 min before assaying for the release of inorganic phosphate (Pi; Chan et al. 1986). Incubation times and cellular density were chosen in order to ensure the linearity of the reactions. Controls with the addition of the intact trophozoites after mixing trichloroacetic acid were used to correct non-enzymatic hydrolysis of substrate. Specific activity is expressed as nmol Pi released/min/1.5×10^6 trophozoites. All samples were run in triplicate.

Protein determination

Protein was measured by the Coomassie Blue method (Bradford 1976) using bovine serum albumin as standard.

Statistical analysis

Statistical analysis was conducted by one-way analysis of variance followed by Duncan test as a post hoc, considering a level of significance of 5%.

Results

In the present study, we have investigated the effects of the 17β-estradiol and DHEAS on the ecto-5′-nucleotidase activity in intact trophozoites of *T. vaginalis*. Cellular integrity and viability were assessed before and after the reactions by the motility of trophozoites and trypan blue dye exclusion. The integrity of the trophozoites was not affected by any of the conditions used in the assays. In order to evaluate a direct effect on ecto-5′-nucleotidase activity, we have tested in vitro 17β-estradiol or DHEAS concentrations varying from 0.01 to 5.0 μM in VP60 and 30236 isolates. AMP hydrolysis has demonstrated no difference between the investigated isolates (data not shown).

VP60 isolate treated with DHEAS for 2 h presented a significant inhibition of 43%, 52%, and 24% ($P<0.05$) for AMP hydrolysis in all tested concentrations (0.01, 0.1, and 1.0 μM), whereas 17β-estradiol did not significantly change AMP hydrolysis in VP60 isolate (Fig. 1).

Figure 2 shows the effect of the treatment with DHEAS and 17β-estradiol for 2 h in the 30236 isolate on ecto-5′-nucleotidase activity. AMP hydrolysis, at 0.01, 0.1, and 1.0 μM, was significantly inhibited in the presence of

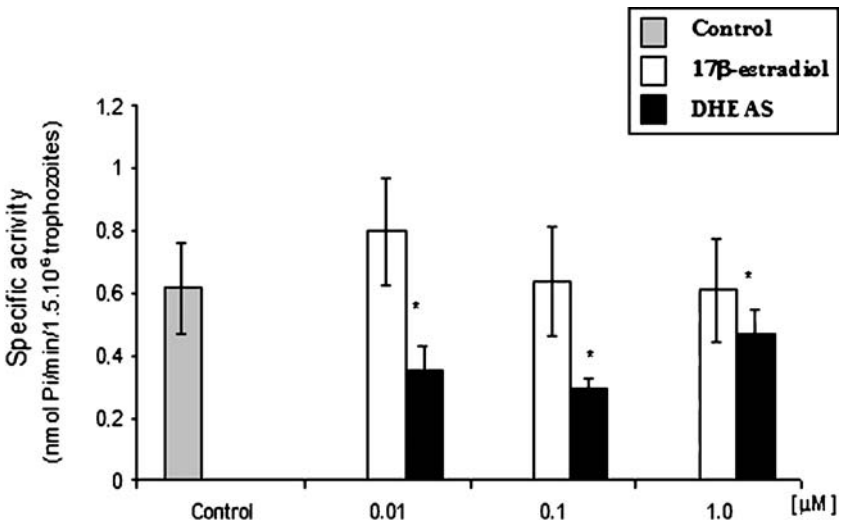

Fig. 1 Effect of 17β-estradiol and dehydroepiandrosterone sulfate treatment during 2 h on adenosine monophosphate hydrolysis in VP60 isolate. *Bars* represent the mean±SD of four experiments ($n=4$) using different parasite suspensions, each in triplicate. *Asterisk* indicates significant difference from controls by one-way analysis of variance, followed by Duncan test as a post hoc ($P<0.05$)

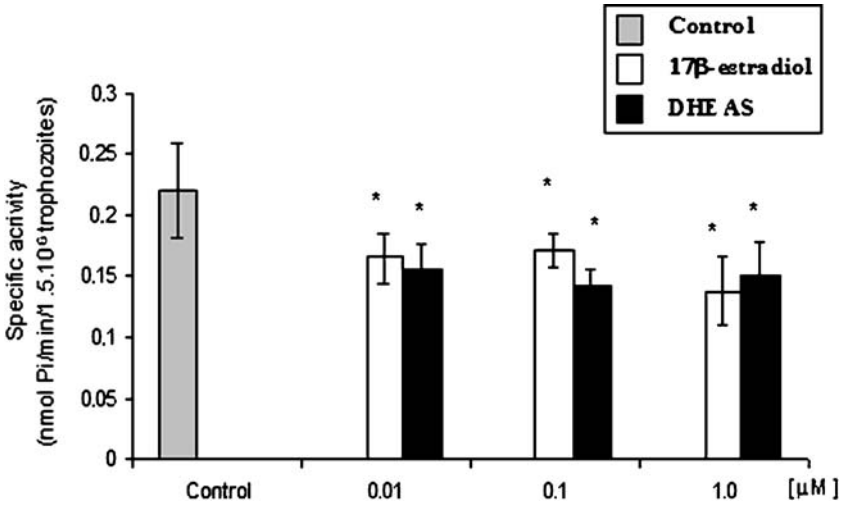

Fig. 2 Effect of 17β-estradiol and dehydroepiandrosterone sulfate treatment during 2 h on adenosine monophosphate hydrolysis in 30236 ATCC isolate. *Bars* represent the mean±SD of four experiments (n=4) using different parasite suspensions, each in triplicate. *Asterisk* indicates significant difference from controls by one-way analysis of variance, followed by Duncan test as a post hoc (P<0.05)

DHEAS (29%, 36%, and 31%, respectively; P<0.05). The 30236 isolate treated with 17β-estradiol presented a significant inhibition of 25%, 22%, and 37% (P<0.05) for AMP hydrolysis in all tested concentrations (0.01, 0.1, and 1.0 μM).

The treatment with DHEAS for 12 h induced a significant inhibition in AMP hydrolysis (32%, P<0.05) at 0.1 μM in VP60 isolate (Fig. 3). In a similar treatment, 17β-estradiol did not significantly change AMP hydrolysis in VP60 isolate (Fig. 3). The treatment with DHEAS and 17β-estradiol for 12 h did not significantly change AMP hydrolysis in 30236 isolate (data not shown).

Discussion

The results demonstrated the influence of DHEAS and 17β-estradiol on ecto-5′-nucleotidase activity in intact trophozoites of *T. vaginalis*. The importance of testing two different isolates is explained by the fact that fresh clinical isolates have demonstrated higher extracellular nucleotide hydrolysis and different nucleotide hydrolysis ratios when compared to long-term growth isolates (Tasca et al. 2005). We have investigated the effect of hormones in VP60 and ATCC 30236 isolates in order to observe if steroids could differentially modulate the adenosine production. The control of adenosine levels is important because this nucleoside is the primary precursor of the entire purine nucleotide pool in *T. vaginalis*, and adenine is converted to GMP via adenosine (Munagala and Wang 2003). Our findings have shown that VP60 is more sensitive to the effects induced by steroid hormones since the short-term treatment (2 h) and a lower DHEAS concentration (0.1 μM) promoted the most intense inhibition on AMP hydrolysis. The 12-h treatment also decreased AMP hydrolysis in the presence of 0.1 μM DHEAS, even though the effects were less expressive due to adaptive mechanisms developed by the parasites to resist these environmental conditions. These results reinforce the idea that fresh clinical isolates are more susceptible to environmental changes induced by factors, such as hormones. In contrast, it is important to highlight that ATCC 30236 isolate also presented a decrease in AMP hydrolysis during 2 h treatment, whereas there was no significant response in

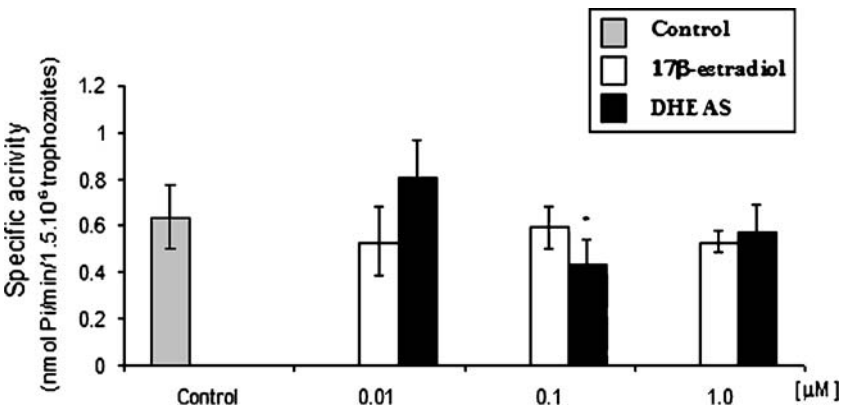

Fig. 3 Effect of 17β-estradiol and dehydroepiandrosterone sulfate treatment during 12 h on adenosine monophosphate hydrolysis in VP60 isolate. *Bars* represent the mean±SD of four experiments (n=4) using different parasite suspensions, each in triplicate. *Asterisks* indicates significant difference from controls by one-way analysis of variance, followed by Duncan test as a post hoc (P<0.05)

the long-term treatment (12 h) for any concentrations of hormones tested. The lower effects observed for this isolate might be related to its long-term adaptation to in vitro cultivation and, therefore, to a lower responsiveness to environmental conditions.

T. vaginalis has specific androgen and estrogen receptors, which suggests that steroid hormones could directly affect the parasite (Ford et al. 1987). Sugarman and Mummaw (1988) showed that estrogen decreases the growth of *T. vaginalis.* Another report showed that the application of vaginal estradiol pellets appeared to ameliorate the clinical symptoms of vaginitis in 33 women tested (Lirosi and Guarascio 1972). Furthermore, direct effects of hormones on growth and viability have been shown in several parasite organisms. The adrenal hormone DHEA has been shown to mediate only inhibitory actions on parasites *Schistosoma mansoni* (Morales-Montor et al. 2001) and *Plasmodium* sp. (Ayi et al. 2002). Carrero et al. (2006) presented evidence on the direct in vitro effect of DHEA on growth and viability of *Entamoeba histolytica* trophozoites. In vivo evidence on the anti-malarial effect of DHEA came from the inverse correlation found in young women from Kenya, between the levels of blood-circulating DHEA and the parasitemia (Leenstra et al. 2003). The mechanisms by which DHEA improves the immune response against the parasites are not well defined; however, some reports have demonstrated a role for androgens in the regulation and modulation of the activity of certain immune cell types, such as T cells, natural killer, and B cells (Olsen and Kovacs 2005).

Contradictory reports are found in the literature considering the relationship between estrogen and 5′-nucleotidase. Some reports have demonstrated an increase in plasma 5′-nucleotidase activities in women with *hyperemesis gravidarum*, which is characterized by enhanced release of pregnancy-related hormones (Depue et al. 1987; Abell and Riely 1992; Goodwin et al. 1992). An increase in plasma 5′-nucleotidase activities may be partly attributed to elevations of pregnancy-related hormones suggesting changes in the purine metabolism in women with hyperemesis gravidarum (Yoneyama et al. 2002). Spychala et al. (2004) demonstrated that estradiol, acting through the estrogen receptor, strongly down-regulates the expression of ecto-5′-nucleotidase. However, an increase in AMP hydrolysis and in the expression of ecto-5′-nucleotidase has been shown in cerebral cortex of ovariectomized rats (Rücker et al. 2005). Therefore, the presence of enzymes involved in ATP hydrolysis to adenosine may play an important role in growth of the parasite in a hostile environment under constant alterations due to the lack of de novo purine nucleotide synthesis.

In summary, our findings have shown that the complex effect of steroid hormones and their receptors on *T. vaginalis* may promote changes in ecto-5′-nucleotidase activity during exposure to these hormones. Therefore, our results present an additional contribution for the complex scenario of hormone effects on nucleotide hydrolysis and their influence in pathophysiological conditions.

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References

Abell TL, Riely CA (1992) Hyperemesis gravidarum. Gastroenterol Clin North Am 21:835–849

Ayi K, Giribaldi G, Skorokhod A, Schwarzer E, Prendergast PT, Arese P (2002) 16alpha-bromoepiandrosterone, an antimalarial analogue of the hormone dehydroepiandrosterone, enhances phagocytosis of ring stage parasitized erythrocytes: a novel mechanism for antimalarial activity. Antimicrob Agents Chemother 46:3180–3184

Bakare RA, Ashiru JO, Adeyemi-Doro FA, Ekweozor CC, Oni AA, Okesola AO, Adebayo JA (1999) Non-gonococcal urethritis (NGU) due to *Trichomonas vaginalis* in Ibadan. West Afr J Med 18:64–68

Bianchi V, Spychala J (2003) Mammalian 5′-nucleotidases. J Biol Chem 278:46195–46198

Borges FP, Gottardi B, Stuepp C, Larré AB, Tasca T, De Carli GA, Bonan CD (2007) Characterization of an ecto-5′-nucleotidase (EC 3.1.3.5) activity in intact trophozoites of *Trichomonas gallinae*. Vet Parasitol 143(2):106–111

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quanties of protein utilizing the principle of protein-dye binding. Anal Biochem 72:218–254

Brown MT (1972) Trichomoniasis. Practitioner 209:639–644

Carrero JC, Cervantes C, Moreno-Mendoza N, Saavedra E, Morales-Montor J, Laclette JP (2006) Dehydroepiandrosterone decreases while cortisol increases in vitro growth and viability of *Entamoeba histolytica*. Microbes Infect 8:323–331

Chan KM, Delfert D, Junger KD (1986) A direct colorimetric assay for Ca2+ -stimulated ATPase activity. Anal Biochem 157:375–380

Cotch MF, Pastorek JG, Nugent RP, Hillier SL, Gibbs RS, Martin DH, Eschenbach DA, Edelman R, Carey JC, Regan JA, Krohn MA, Klebanoff MA, Rao AV, Rhoads GG (1997) *Trichomonas vaginalis* associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. Sex Transm Dis 24:353–360

Depue RH, Bernstein L, Ross RK, Judd HL, Henderson BE (1987) Hyperemesis gravidarum in relation to estradiol levels, pregnancy outcome, and other maternal factors: a seroepidemiologic study. Am J Obstet Gynecol 156:1137–1141

Diamond LS (1957) The establishment of various trichomonads of animals and man in axenic cultures. J Parasitol 43:488–490

Filippini A, Taffs RE, Sitkovsky MV (1990) Extracellular ATP in T-lymphocyte activation: possible role in effector functions. Proc Natl Acad Sci USA 87:8267–8271

Ford LC, Hammill HA, DeLange RJ, Bruckner DA, Suzuki-Chavez F, Mickus KL, Lebherz TB (1987) Determination of estrogen and androgen receptors in *Trichomonas vaginalis* and the effects of antihormones. Am J Obstet Gynecol 156:1119–1121

Garber GA, Lemchuk-Favel LT, Rousseau G (1991) Effect of beta-estradiol on production of the cell-detaching factor of *Trichomonas vaginalis*. J Clin Microbiol 29:1847–1849

Goodwin TM, Montoro M, Mestman JH, Pekary AE, Hershman JM (1992) The role of chorionic gonadotropin in transient hyperthyroidism of hyperemesis gravidarum. J Clin endocrinol Metab 75:1333–1337

Grodstein F, Goldman MB, Cramer DW (1993) Relation of tubal infertility to history of sexually transmitted diseases. Am J Epidemiol 137:577–584

Grossman CJ (1984) Regulation of the immune system by sex steroids. Endocr Rev 5:435–455

Guenthner PC, Secor WE, Dezzutti CS (2005) *Trichomonas vaginalis*-induced epithelial monolayer disruption and human immunodeficiency virus type 1 (HIV-1) replication: implications for the sexual transmission of HIV-1. Infect Immun 73:4155–4160

Haskó G, Cronstein BN (2004) Adenosine: an endogenous regulator of innate immunity. Trends Parasitol 25:33–39

Leenstra T, Ter Kuile FO, Kariuki SK, Nixon CP, Oloo AJ, Kager PA, Kurtis JD (2003) Dehydroepiandrosterone sulfate levels associated with decreased malaria parasite density and increased hemoglobin concentration in pubertal girls from western Kenya. J Infect Dis 188:297–304

Lirosi G, Guarascio A (1972) Effeti delle modificazioni ormonali dell ambiente vaginala nella terapia dell vaginiti specie de *Trichomonas*. Minerva Ginecol 24:23–27

Mason PR, Fiori PL, Cappuccinelli P, Rappelli P, Gregson S (2005) Seroepidemiology of *Trichomonas vaginalis* in rural women in Zimbabwe and patterns of association with HIV infection. Epidemiol Infect 133:315–323

Michel R, Borges F, Wiltuschnig R, Vieira PB, Tasca T, De Carli GA (2006) Prevalência da tricomonose em mulheres residentes na Vila dos Papeleiros em Porto Alegre, RS. Rev Bras Anal Clin 38:127–130

Moodley P, Wilkinson D, Connolly C, Moodley J, Sturm AW (2002) *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. Clin Infect Dis 34:519–522

Morales-Montor J, Mohamed F, Ghaleb AM, Baig S, Hallal-Calleros C, Damian RT (2001) In vitro effects of hypothalamic-pituitary-adrenal axis (HPA) hormones on *Schistosoma mansoni*. J Parasitol 87:1132–1139

Munagala NR, Wang CC (2003) Adenosine is the primary precursor of all purine nucleotides in *Trichomonas vaginalis*. Mol Biochem Parasitol 127:143–149

Murphy BM (2001) Hormonal control of enzyme activity during the plasma membrane transformation of uterine epithelial cells. Cell Biol Int 20:5420–5430

Olsen NJ, Kovacs WJ (2005) Gonadal steroids and immunity. Endocr Rev 17:369–384

Petrin D, Delgaty K, Bhatt R, Garber G (1998) Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin Microbiol Rev 11:300–317

Ramalingam V, Krishnamoorthy G, Govindarajulu P (1993) Plasma membrane enzymes in human breast carcinoma: relationship with serum hormones. Neoplasma 40:363–367

Roberts CW, Walker W, Alexander J (2001) Sex-associated hormones and immunity to protozoan parasites. Clin Microbiol Rev 14:476–488

Robson SC, Sévigny J, Zimmermann H (2006) The E-NTPDase family of ectonucleotidases: structure function relationships and pathophysiological significance. Purinergic Signal 2:409–430

Rücker B, Pochmann D, Fürstenau CR, Carneiro-Ramos MS, Battastini AM, Barreto-Chaves ML, Sarkis JJ (2005) Effects of steroid hormones on synaptosomal ectonucleotidase activities from hippocampus and cortex of adult female rats. Gen Comp Endocrinol 140(2):94–100

Silva-Filho FC, Bonilha VL (1992) Effect of estrogens on the adhesion of *Trichomonas vaginalis* to epithelial cells in vitro. Braz J Med Biol Res 25:9–18

Spychala J, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS (2004) Role of estrogen receptor in the regulation of ecto-5′-nucleotidase and adenosine in breast cancer. Clin Cancer Res 10:708–717

Steinberg TH, Di Virgilio F (1991) Cell-mediated cytotoxicity: ATP as an effector and the role of target cells. Curr Opin Immunol 3:71–75

Sträter N (2006) Ecto-5′-nucleotidase: structure function relationships. Purinergic Signal 2:343–350

Sugarman B, Mummaw N (1988) The effect of hormones on *Trichomonas vaginalis*. J Gen Microbiol 134:1623–1628

Sutcliffe S, Giovannucci E, Alderete JF, Chang TH, Gaydos CA, Zenilman JM, De Marzo AM, Willett WC, Platz EA (2006) Plasma antibodies against *Trichomonas vaginalis* and subsequent risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 15:939–945

Tasca T, Bonan CD, De Carli GA, Battastini AMO, Sarkis JJF (2003) Characterization of an ecto-5′-nucleotidase (EC 3.1.3.5) activity in intact cells of *Trichomonas vaginalis*. Exp Parasitol 105:167–173

Tasca T, Bonan CD, De Carli GA, Sarkis JJ (2004) *Trichomonas vaginalis*: cytochemical localization of a NTPDase1 and an ecto-5′-nucleotidase and effects of adenine nucleotides on cellular viability. Parasitol Res 93:300–303

Tasca T, Bonan CD, De Carli GA, Sarkis JJ, Alderete JF (2005) Heterogeneity in extracellular nucleotide hydrolysis among clinical isolates of *Trichomonas vaginalis*. Parasitology 131:71–78

Viikki M, Pukkala E, Nieminen P, Hakama M (2000) Gynaecological infections as risk determinants of subsequent cervical neoplasia. Acta Oncol 39:71–75

Wang CC (1990) Nucleic acid metabolism in *Trichomonas vaginalis*. In: Honigberg BM (ed) Trichomonads parasitic in humans. Springer-Verlag, New York, pp 84–90

Yoneyama Y, Kobayashi H, Kato M, Chihara H, Yamada T, Otsubo Y, Araki T (2002) Plasma 5′-nucleotidase activities increase in women with hyperemesis gravidarum. Clin Biochem 35:561–564

Zimmermann H (1992) 5′-Nucleotidase: molecular structure and functional aspects. Biochem J 285:345–365