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An active and selective molecular mechanism mediating the uptake of sex steroids by prostate cancer cells

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ABSTRACT

Steroid hormones play important roles in normal physiological functions and diseases. Sex steroids hormones are important in the biology and treatment of sex hormone-related cancer such as prostate cancer and breast cancer. Cells may take up steroids using multiple mechanisms. The conventionally accepted hypothesis that steroids cross cell membrane through passive diffusion has not been tested rigorously. Experimental data suggested that cells may take up sex steroid using an active uptake mechanism. ^{3}H-testosterone uptake by prostate cancer cells showed typical transporter-mediated uptake kinetic. Cells retained testosterone taken up from the medium. The uptake of testosterone was selective for certain steroid hormones but not others. Data also indicated that the active and selective uptake mechanism resided in cholesterol-rich membrane domains, and may involve ATP and membrane transporters. In summary, the present study provided strong evidence to support the existence of an active and selective molecular mechanism for sex steroid uptake.

1. Introduction

Steroid hormones regulate numerous functions that include differentiation, development, metabolism, reproduction, and immune response, and also play important roles in diseases (Simons, 2008). Steroid hormones include androgens, estrogens, progestins, glucocorticoids, mineralocorticoids. Androgens, estrogens and progestins are also called sex steroids, of which the most common ones are progesterone, testosterone (T) and dihydrotestosterone (DHT), and estradiol (E2), respectively. Sex steroids are important to the biology and treatment of sex hormone related cancer (Groner and Brown, 2017). Targeting the androgen signaling and the estrogen signaling has been a main modality of treatment for prostate cancer and breast cancer, respectively (Dai et al., 2017; Stuchbery et al., 2017; Snaterse et al., 2017; Castrellon, 2017; Africander and Storbeck, 2017; Jordan and Brodie, 2007). Sex steroids also are involved in ovary and endometrial cancer, and non-sex hormone-related cancer such as colon cancer (Chuffa et al., 2017; Kamal et al., 2016; Plaza-Parrochia et al., 2017; Jeon et al., 2016; Gharwan et al., 2015; Diep et al., 2015; Lin et al., 2011; Barzi et al., 2013). Adrenal glands produce androgens that are named adrenal androgens. Dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) are the predominant adrenal androgens in the circulation (Rainey and Carr, 2004; Rainey et al., 2002). DHEA and DHEAS are substrates for T and DHT production through the steroidogenesis pathway (Labrie et al., 1998). Adrenal androgens have multiple functions of their own that are independent of AR (Traish et al., 2011; Maninger et al., 2009).

Most of sex steroids in the plasma are bound with proteins, mainly with sex hormone-binding globulin (SHBG) (Hammond, 2011; Joseph, 1994; Mendel, 1992; Rosner et al., 2010). The steroids bind with respective nuclear receptors to regulate expression of genes upon entering the cells. Progesterone, T/DHT, and E2 bind with progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER), respectively (Grimm et al., 2016; Bain et al., 2007; Hilton et al., 2017; Heemers and Tindall, 2007; Heinlein and Chang, 2002; Wilson, 2009). The ligand-bound receptors translocate to the nucleus to regulate expression of sex hormone-targeted genes. A wealth of knowledge has been accumulated with regard to the production, metabolism, and circulation of sex steroids, as well as nuclear receptor-mediated biological functions and cell signaling pathways. No nuclear receptors have been identified for the adrenal androgens.

Cells may take up steroid using multiple mechanisms, which include passive diffusion and more selective or more active, transporter-mediated mechanisms. The conventional belief is that sex steroids cross the bi-phospholipid layer of the cell membrane through passive diffusion due to their hydrophobic property (Oren et al., 2004). However, cellular transport of other hydrophobic molecules including fatty acids and cholesterol is mediated by mechanisms other than passive diffusion (Klaassen and Aleksunes, 2010; Ikonen, 2006; Altmann et al., 2004).

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Abbreviations

AR	androgen receptor
ATCC	American Type Culture Collection
cpm	counts per minute
CS-FBS	charcoal-stripped fetal bovine serum
DHEA	dehydroepiandrosterone
DHEAS	DHEA sulfate
DHT	dihydrotestosterone
E2	estradiol
ER	estrogen receptor
epiT	epitestosterone
FBS	fetal bovine serum
OATP	organic anion-transporting polypeptide
OST	organic solute and steroid transporter
PBS	phosphate buffered saline
PR	progesterone receptor
PGRMC1	progesterone receptor membrane component 1
RLU	relative luminescent unit
RLB	reporter lysis buffer
SHBG	sex hormone-binding globulin
SLCO	solute carrier organic anion
T	testosterone

There have been reports that uptake of steroids may be mediated by molecular mechanisms, although the contribution of these proposed mechanisms to the uptake of sex steroids remains unknown. T, DHEAS, or estrogens are known to bind with cell membrane-bound, G-protein coupled receptors including Gnα11, ZIP9, and GPER-1 to augment non-classical or non-genotropic functions of the steroids (Shihan et al., 2014; Bulldan et al., 2016; Shihan et al., 2013; Carmeci et al., 1997; Funakoshi et al., 2006; Shihan et al., 2015). Membrane receptor-mediated endocytosis also may be involved in the internalization of sex steroids, although the role of endocytosis in nuclear receptor-mediated functions of the steroids is not clear (Lin and Scanlan, 2005; Hammes et al., 2005; Porto et al., 1995). The organic solute and steroid transporter (OST) proteins OSTα-OSTβ was proposed to be a newly identified putative steroid transporter (Ballatori, 2005). The organic anion-transporting polypeptide (OATP) superfamily member solute carrier organic anion (SLCO) family member SLCO1B3 was reported as a T transporter (Hamada et al., 2008). More data are needed to describe accurately the uptake of sex steroids by cells in order to understand fully the transport and tissue distribution that may affect the function of the steroids.

It was found that prostate cancer cells were able to accumulate intracellular T at 20–50-fold of that was added in the culture medium (Wu et al., 2013), which indicated that the cells acquired exogenous T using an active uptake mechanism. In the present study, the uptake of T was examined using a ^{3}H-T as substrate using prostate cancer cell lines. Progesterone, E2, epitestosterone (epiT), DHT, DHEA and DHEAS were evaluated for competition for the uptake of ^{3}H-T. Activation of AR was compared between T and the steroids that blocked T uptake. Potential molecular mechanisms were investigated using specific chemical inhibitors. An active and selective uptake mechanism was described.

2. Materials and methods

2.1. Reagents

T, epi-T, DHT, DHEA and DHEAS were purchased from Steraloids (Newport, RI). Progesterone, E2, (2-hydroxypropyl)-β-cyclodextrin (2HP-β-CD), AG-205, and bafilomycin A1 were purchased from Sigma-Aldrich (St. Louis, MO). 1,2,6,7-^{3}H(N) T (^{3}H-T), 1,2,6,7-^{3}H(N) progesterone (^{3}H-progesterone) and 6,7-^{3}H(N) E2 (^{3}H-E2) were purchased from PerkinElmer (Waltham, MA). Specific activity of the ^{3}H-T, ^{3}H-progesterone, and ^{3}H-E2 were 83.4, 96.6, and 60.0 mCi/μmol, respectively. The concentration of ^{3}H-T, ^{3}H-progesterone, and ^{3}H-E2 were 12, 10.4, and 16.7 μM respectively, based on the specific radioactivity 1 μCi/μl provided by the manufacturer.

2.2. Cell culture

Human prostate cancer VCaP cell line was purchased from American Type Culture Collection (ATCC) (Manassas, VA). Human prostate cancer LAPC-4 cell line was established by Dr. Charles Sawyers group (Klein et al., 1997). VCaP and LAPC-4 cell lines express wild type AR (Sobel and Sadar, 2005a,b). Cell lines were propagated in medium supplemented with 10% fetal bovine serum (FBS) (Atlantic Biologicals, Atlanta, GA). VCaP cells were maintained in DMEM medium (Thermo Fisher Scientific, Waltham, MA). LAPC-4 cells were maintained in RPMI1640 medium (Thermo Fisher Scientific). All media contained 2 mM L-glutamine (Corning Life Sciences, Tewksbury, MA), 100 U/ml penicillin and 100 μg/ml streptomycin (Corning Life Sciences). For LAPC-4 cells, tissue culture vessels were coated with 1.7 μg/ml poly-D-lysine (Sigma-Aldrich) in H_2O at 0.076 ml/cm^2, at room temperature for 15 min, and followed by aspiration of the coating reagent and overnight air dry. Phenol red-free version of each medium was used for pre-culture and treatments. Cells were incubated at 37 °C, in an atmosphere with 95% air and 5% CO_2.

2.3. Uptake of ^{3}H-T

Cells were seeded at 5×10^4 per well in 0.5 ml medium supplemented with 10% FBS on 24-well plates, and cultured for 3 days or 7 days for LAPC-4 or VCaP, respectively. Cell culture medium was replaced with medium supplemented with 10% charcoal-stripped (CS-FBS) to remove androgens (Fiandalo et al., 2017). Cells were cultured for 1 day before treatment. Cells were treated in 0.25 ml of treatment medium. Cells were rinsed 4 times with 0.25 ml phosphate buffered saline (PBS). Cells in each well were lysed in 0.125 ml lysis buffer (2% SDS, 10% glycerol, 10 mM Tris-HCl, pH6.8) and incubated at 37 °C for 30 min. At the end of lysis, 0.5 ml of SOLVABLE (PerkinElmer) was added to each well. The content was mixed thoroughly using gentle swirling, and transferred into a 25 ml scintillation vial that contained 5 ml Ultra Gold Scintillation fluid (PerkinElmer). Radioactivity of the lysate was measured on a scintillation counter. Each experiment was set up in 4 replicates. Two types of controls were included in every experiment. The background control cells was not treated with ^{3}H-T nor T to control the background radiation readings. The non-specific binding controls cells were treated with ^{3}H-T in the presence of T that was 300-fold of the concentration of ^{3}H-T. Radioactivity of the non-specific binding controls, counts per minute (cpm), were subtracted from the cpm reading of each treatment for ^{3}H-T-specific total radioactivity.

2.4. Release of intracellular ^{3}H-T

Cells were seeded on 6-well plate at 4×10^5 cells per well in 2 ml medium supplemented with 10% FBS and cultured for 3 days or 7 days for LAPC-4 or VCaP, respectively. Cells were cultured for 1 day in 2 ml phenol red-free medium with 10% CS-FBS, and treated with 1 nM ^{3}H-T for 20 min. Cells were rinsed 3 times with phenol red-free medium with 10% CS-FBS. One set of treated cells were lysed in 0.2 ml lysis buffer, mixed with 0.5 ml SOLVABLE and 5 ml Ultra Gold Scintillation fluid, and analyzed on the scintillation counter for total radioactivity. The total activity of the cell culture was used as the baseline total cellular ^{3}H-T. The remaining sets of cells were cultured in 2 ml fresh phenol red-

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