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Full paper

The activity of organic anion transporter-3: Role of dexamethasone

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ABSTRACT

Human organic anion transporter-3 (hOAT3) is richly expressed in the kidney, where it plays critical roles in the secretion, from the blood to urine, of clinically important drugs, such as anti-viral therapeutics, anti-cancer drugs, antibiotics, antihypertensives, and anti-inflammatories. In the current study, we examined the role of dexamethasone in hOAT3 transport activity in the kidney HEK293 cells. Cis-inhibition study showed that dexamethasone exhibited a concentration-dependent inhibition of hOAT3-mediated uptake of estrone sulfate, a prototypical substrate for the transporter, with IC_{50} value of 49.91 μM. Dixon plot analysis revealed that inhibition by dexamethasone was competitive with a Ki = 47.08 μM. In contrast to the cis-inhibition effect of dexamethasone, prolonged incubation (6 h) of hOAT3-expressing cells with dexamethasone resulted in an upregulation of hOAT3 expression and transport activity, kinetically revealed as an increase in the maximum transport velocity V_{max} without meaningful alteration in substrate-binding affinity K_m. Such upregulation was abrogated by GSK650394, a specific inhibitor for serum- and glucocorticoid-inducible kinases (sgk). Dexamethasone also enhanced sgk1 phosphorylation. Our study demonstrated that dexamethasone exhibits dual effects on hOAT3: it is a competitive inhibitor for hOAT3-mediated transport, and interestingly, when entering the cells, it stimulates hOAT3 expression and transport activity through sgk1.

1. Introduction

Organic anion transporter-3 (OAT3) belongs to a class of organic anion transporters (OATs) consisting of over 10 membrane proteins. OAT3 is expressed at the basolateral membrane of the renal proximal tubule cells and plays a critical role in the secretion of many clinical drugs, including anti-HIV therapeutics, anti-tumor drugs, antibiotics, antihypertension drugs, and anti-inflammatories.[1–6]

We previously established that OATs are subjected to the regulation by the serum- and glucocorticoid-inducible kinases (sgk).[7–9] Sgk consists of three serine/threonine kinase isoforms sgk1, sgk2 and sgk3. These kinases regulate many cellular processes such as Na^+ balance, osmoregulation, cell survival, and proliferation.[10–16] They also play important roles in oncology, diabetes, and obesity.[17] Sgk1 and sgk3 are expressed ubiquitously, whereas sgk2 is restricted to tissues such as liver, kidney, pancreas, and brain. We previously demonstrated that sgk1 and sgk2 stimulate OAT activity by weakening the association of OAT with a ubiquitin ligase Nedd4-2, which leads to a deceleration of ubiquitin-dependent OAT internalization from the plasma membrane to intracellular compartments. As a result, the amount of OAT at the cell surface is enhanced and OAT transport activity is increased.[7,8]

Glucocorticoids are hormones that regulate numerous physiological activities related with metabolic, cardiovascular, and inflammatory processes.[18] Excess of glucocorticoids contribute to obesity, hyperlipidemia, hypertension, and glucose intolerance.[19] Glucocorticoids have been used for the treatment of diarrhea related to inflammatory bowel diseases and nontropical sprue.[20] Several studies have shown that one of the signaling molecule downstream glucocorticoids is sgk.[21–23] In the current study, we investigated the role of dexamethasone, a synthetic glucocorticoid, in OAT3 expression and transport activity.

2. Materials and methods

2.1. Materials

The HEK293 cells were purchased from ATCC (Manassas, VA). [^{3}H]-labeled estrone sulfate ([^{3}H]-ES) was purchased from

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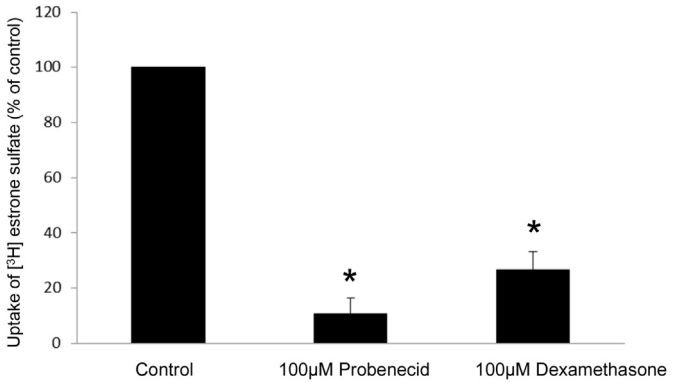

Fig. 1. Cis-inhibition of hOAT3-mediated [^{3}H]-ES uptake by dexamethasone. 4-min uptake of 300 nM [^{3}H]-ES in the presence of dexamethasone (100 μM) or probenecid (100 μM) was measured. Each data point represented only carrier-mediated transport after subtraction of values from parental cells. Uptake activity was expressed as percentage of uptake measured in control cells from three independent experiments. Results shown are means ± S.E. of three separate experiments. *P < 0.05.

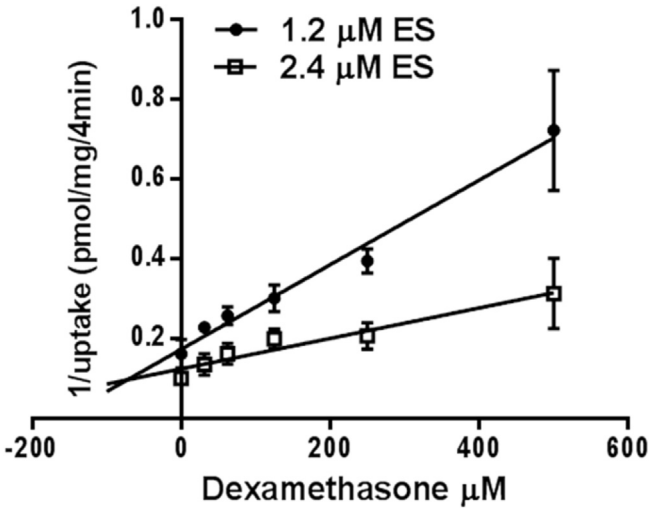

Fig. 3. Dixon plot analysis of the inhibitory effects of dexamethasone on hOAT3-mediated transport. [^{3}H]-ES uptake (1.2 μM and 2.4 μM) was determined at 4 min in the presence of varying concentrations of dexamethasone. Each data point represented only carrier-mediated transport after subtraction of values from parental cells. Results shown are means ± SE percentage of uptake measured in control cells. The data were fitted by linear regression and K_i was calculated.

PerkinElmer (Waltham, MA). cDNA for mouse Flag-tagged sgk1 was generously provided by Dr. Alan C. Pao from the Department of Medicine, Stanford University, (Stanford, CA). Dexamethasone and all other reagents were purchased from Sigma–Aldrich (St Louis, MO).

2.2. Cell culture and transient transfection

Parental HEK293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Corning, Corning, NY) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY) at 37 °C in 5% CO_2. Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used for transfection of cDNAs following the manufacturer's instructions. 48 h after transfection, the cells were used for further experiments. Cells stably expressing hOAT3 were maintained in DMEM medium supplemented with 0.2 mg/ml G418 (Invitrogen, Carlsbad, CA), and 10% fetal bovine serum.

2.3. Transport measurement

The transport activity of hOAT3 was determined by measuring [^{3}H]-ES uptake into hOAT3-expressing cells. The uptake solution consists of phosphate-buffered saline (PBS) with 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS/CM) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.4) and [^{3}H]-ES (300 nM). At the time points indicated, uptake was terminated by removing the uptake solution followed by washing with ice-cold PBS twice. The cells were then lysed in 0.2 N NaOH, neutralized with 0.2 N HCl and transferred into scintillation vials for liquid scintillation counting.

2.4. Cell surface biotinylation

The expression level of hOAT3 at the cell surface was examined by using a biotinylation strategy. The cells in monolayer culture were washed with ice-cold PBS and then incubated with 1 ml of NHS-SS-biotin (0.5 mg/ml in PBS/CM) on ice for two consecutive 20 min periods under gentle shaking. Biotinylation was stopped by rinsing with 100 mM glycine in PBS/CM. Afterwards, the cell extracts were prepared in lysis buffer (10 mM Tris/HCl, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100 with 1/100 protease inhibitor cocktail) for 30 min at 4 °C and cleared by centrifugation at 16,000×*g* at 4 °C. The supernatant was mixed with streptavidin-agarose beads (Pierce, Rockford, IL) to isolate cell surface proteins. Membrane-expressed hOAT3 was detected by SDS-PAGE and

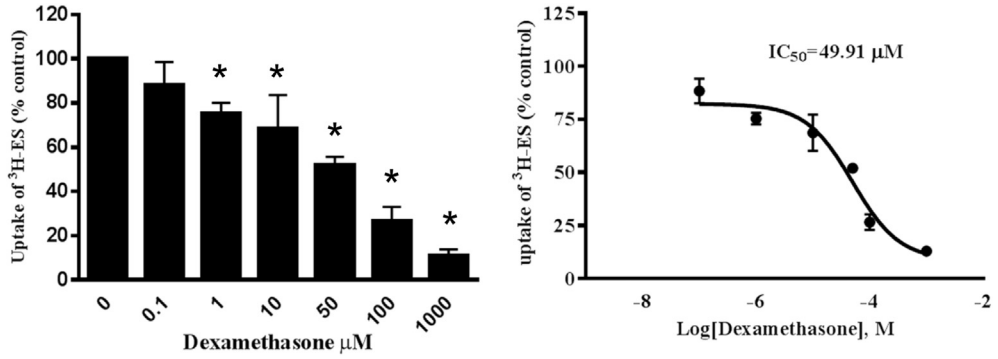

Fig. 2. Concentration dependence of dexamethasone inhibition on hOAT3-mediated uptake. hOAT3-expressing cells were incubated for 4 min with 300 nM [^{3}H]-ES in the presence of various concentrations of dexamethasone. Each data point represented only carrier-mediated transport after subtraction of values from parental cells. Uptake activity was expressed as percentage of uptake measured in control cells from three independent experiments. Results shown are means ± S.E. of three separate experiments. *P < 0.05. The line represents a best fit of data using nonlinear regression analysis.