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Inhibitory effects of 17-α-ethinyl-estradiol and 17-β-estradiol on transport via the intestinal proton-coupled amino acid transporter (PAT1) investigated in vitro and in vivo

Carsten Uhd Nielsen, Maria Pedersen, Stefanie Müller, Thea Kæstel, Maria Bjerg, Nithiya Ulaganathan, Salli Nielsen, Krestine Lundgaard Carlsen, Martha Kampp Nøhr, and René Holm.

Objective: To investigate the inhibitory effects of 17-α-ethinyl-estradiol and 17-β-estradiol on transport via the intestinal proton-coupled amino acid transporter (PAT1).

Methods: In vitro and in vivo experiments were conducted to assess the effects of the hormones on the transport of amino acids.

Results: The hormones showed significant inhibitory effects on the transport of amino acids via PAT1.

Conclusion: The findings suggest a potential role of 17-α-ethinyl-estradiol and 17-β-estradiol in regulating amino acid transport in the intestine.

Keywords: Proton-coupled amino acid transporter (PAT1), 17-α-ethinyl-estradiol, 17-β-estradiol, Intestinal transport, Amino acids.
Abstract

The proton-coupled amino acid transporter, PAT1, is known to be responsible for intestinal absorption drug substances such as gaboxadol and vigabatrin. The aim of the present study was to investigate, if 17-α-ethinyl-estradiol (E-E2) and 17-β-estradiol (E) inhibit PAT1-mediated intestinal absorption of proline and taurine in vitro in Caco-2 cells and in vivo using Sprague-Dawley rats to assess the potential for taurine-drug interactions. E and E-E2 inhibited the PAT1-mediated uptake of proline and taurine in Caco-2 cells with IC$_{50}$ values of 10.0 - 50.0 µM without major effect on other solute carriers such as the taurine transporter (TauT), di/tripeptide transporter (PEPT1), and serotonin transporter (SERT1). In PAT1-expressing oocytes E and E-E2 were non-translocated inhibitors. In Caco-2 cells, E and E-E2 lowered the maximal uptake capacity of PAT1 in a non-competitive manner. Likewise, the transepithelial permeability of proline and taurine was reduced in presence of E and E-E2. In male Sprague Dawley rats pre-dosed with E-E2 a decreased maximal plasma concentration (C$_{max}$) of taurine and increased the time (t$_{max}$) to reach this was indicated, suggesting the possibility for an in vivo effect on the absorption of PAT1 substrates. In conclusion, 17-α-ethinyl-estradiol and 17-β-estradiol were identified as non-translocated and non-competitive inhibitors of PAT1.
Introduction

Solute carriers (SLCs) are a group of membrane transport proteins encoded by 396 different human genes. The SLCs are expressed in a variety of tissues, where they facilitate the membrane transport of nutrients, vitamins, and minerals. Moreover, they are important for transporting drug substances across cell membranes, and thereby facilitating the oral absorption of some drug substances. One such solute carrier is the proton-coupled amino acid transporter, PAT1, that is expressed in the luminal membrane of the intestinal epithelium and Caco-2 cells, in intracellular vesicles of mouse renal tubular cells, in lysosomes of rat neurons, and in the nucleus of rat A7r5 smooth muscle cells. Human PAT1 and rat Pat1 proteins consist of 476 and 475 amino acids, respectively, and have 86% amino acid identity according to the current reference sequences. PAT1 transports neutral zwitterionic amino acids such as proline, alanine, glycine, and GABA, as well as drug substances such as gaboxadol, vigabatrin and δ-aminolevulinic acid with similar affinities as Pat1. Substrate transport via Pat1 is coupled to proton transport and is rheogenic resulting in transport activity under slightly acidic extracellular condition and limited activity under conditions lacking a transmembrane proton gradient. It has been suggested that the oral absorption of gaboxadol and vigabatrin in dogs and rats driven by the transport activity of Pat1, and that the absorption takes place in the small intestine, where PAT1 is predominantly expressed. Taurine is also a substrate of PAT1, and Anderson et al. demonstrated in vitro, in intestinal Caco-2 cells, that both PAT1 and the sodium/chloride-coupled taurine transporter, TauT, contributed to cellular uptake of taurine. In addition, two studies have shown that at taurine concentrations > 100 µM, the apical uptake of taurine under slightly acidic conditions is mediated by PAT1 rather than TauT. In vivo, it has been shown that PAT1 mediates the oral absorption of taurine in Sprague Dawley rats, and it was suggested that radiolabeled taurine can be used as a probe (victim substrate) for investigating physiological and pharmacological inhibitory (perpetrator) effects on PAT1-mediated intestinal absorption. In regulatory guidelines, one concern when drug substances are transported by a membrane transport protein is the possibility for drug-drug interactions in populations taken several medications, or that substrate exposure and/or Cmax is decreased by food or drugs. Such interactions can arise, when either substrates or inhibitors compete for binding to the transport protein. In the FDA guidance on “In Vitro Drug Interaction Studies” two transporters: P-glycoprotein (P-gp, ABCB1) and breast cancer resistance protein (BCRP, ABCG2) and seven solute carriers: organic anion transporter 1 (OAT1, SLC22A6) and 3 (OAT3, SLC22A8), organic cation transporter 2 (OCT2, SLC22A1), multidrug and toxin extrusion transporter 1 (MATE1, SLC47A1) and 2-K (MATE2-K, SLC47A2) and organic anion transporting polypeptide 1B1.
(OATP1B1, SLCO1B1) and 1B3 (OATP1B3, SLCO1B3) are mentioned, but there is no guidance for quantitatively evaluating interactions for intestinal solute carriers. Generally, one substrate may competitively inhibit the transport of another substrate, but a number of inhibitors for PAT1 have also been identified such as serotonin, L-tryptophan and 5-hydroxy-tryptophan (IC$_{50}$ of 5.9, 4.7 and 0.9 mM), dipeptides such as Gly-Tyr, Gly-Pro, and Gly-Phe (IC$_{50}$ of approx. 30-50 mM), and the anti-depressant sertraline (IC$_{50}$ of 177-241 µM), opening the possibility of both food components and drug substances having the potential to alter PAT1-mediated (victim) bioavailability. In oocytes injected with PAT1 cRNA it has been proposed that 17-α-estradiol and 17-β-estradiol decrease glycine induced inward currents at concentrations of 100 µM. Shan et al. suggested that these estrogens bind to PAT1 and thereby change the conformation of the PAT1 protein from an open to a closed state, thereby decreasing transport activity. Estradiol is an endogenous hormone, but it is also used as an oral hormone supplement to treat conditions related to menopause or prostate cancer. Depending on the disease, estradiol is given at oral doses between 1-10 mg daily, which results in a relatively low bioavailability of 2-10 %. A structurally related hormone is 17-α-ethinyl-estradiol, which is used as an oral contraceptive given at daily doses of 30 µg in combination with levonorgestrel. 17-α-ethinyl-estradiol is more resistant to metabolism than estradiol and has an oral bioavailability ranging from 43-74 % (see references in). Based on the observation that estradiol inhibits inward-directed PAT1 mediated H$^+$-currents in oocytes, we hypothesized that 17-α-ethinyl-estradiol could also interact with PAT1. The aim of the present study was thus to investigate, if 17-β-estradiol and 17-α-ethinyl-estradiol inhibit PAT1-mediated proline and taurine transport in vitro, and if there could be a potential oral absorption interaction with 17-β-estradiol or 17-α-ethinyl-estradiol taking place in vivo using Sprague Dawley rats as a preclinical model and taurine as the victim model drug substance.

Material and methods

Materials

Caco-2 cells were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (Leibniz Institute, Braunschweig, Germany). Transwell inserts were from Corning Life Sciences (New York City, NY, USA) and all other cell culture plastic products from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Dulbecco’s Modified Eagle’s Medium (DMEM), taurine (Tau), L-proline (Pro), chenodeoxycholic acid (CDCA), cholesterol, dexamethasone (Dx), dimethyl sulfoxide ≥99.9 % (DMSO), diarylpropionitrile (DPN), 17-β-
estradiol (estradiol, E2), 17-α-ethinyl-estradiol (ethinyl-estradiol, E-E2), fulvestrant, glycocholic acid (GCA), genistein, L-glutamine (Gln), glycyrl-L-proline (Gly-Pro), non-essential amino acids (NEAA), 2-(4-morpholino)ethanesulfonic acid (MES), penicillin/streptomycin, L-tryptophan (Trp), (E/Z)-endoxifen hydrochloride hydrate, staurosporine, phorbol 12-myristate 13-acetate (PMA), sodium chloride, potassium chloride, sodium bicarbonate, calcium nitrate, magnesium sulphate heptahydrate, calcium chloride dihydrate and magnesium chloride was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Bovine serum albumin (BSA), sodium bicarbonate solution 7.5 %, fetal bovine serum (FBS) and Hank’s Balanced Salt Solution (HBSS) with calcium and magnesium without sodium bicarbonate and phenol red were obtained from Thermo Fisher Scientific (Waltham, MA, USA). 4-(2-hydroxyethyl)-1-piperazinoethansulfonic acid (HEPES) and Triton x-100 was obtained from AppliChem GmbH (Darmstadt, Germany). Ethanol >99.7 % (EtOH) was obtained from VWR (Radnor, PA, USA), and ultrapure water was obtained in house from Water Purification systems Milli-Q Gradient, Millipore (Merck, Darmstadt, Germany). L-[2,3,4,5-3H]-proline (75 Ci mmol⁻¹, radiochemical purity >97%), 5-[1,2-3H[N]]-hydroxytryptamine (5-HT) creatinine sulphate (28.3 Ci mmol⁻¹, radiochemical purity >95%), D-[1-14C]-Mannitol (57.2 mCi mmol⁻¹, radiochemical purity >97%), [2,2-3H(N)]-Taurine (19.1 Ci mmol⁻¹, radiochemical purity >97%) and Ultima Gold scintillation liquid were purchased from Perkin Elmer (Waltham, MA, USA). [14C]-Gly-Sar (56 mCi mmol⁻¹, radiochemical purity >95%) and DL-[4-3H]-propranolol hydrochloride (15.2 Ci mmol⁻¹, radiochemical purity >97%) was obtained from GE Healthcare (Little Chalfont, UK). Thapsigargin was a kind gift from Søren Brøgger Christensen, University of Copenhagen. All other chemicals were of reagent or laboratory grade.

Methods

Cell culture

Caco-2 cells were seeded on either Transwell® polycarbonate membrane inserts (1.12 cm², 0.4 μm pore size) or 24 wells cell culture plates (1.90 cm²) at a density of 8.9 ·10⁴ cells cm⁻². Experiments were performed either 6 days or 13-15 days post-seeding on 24 wells plates or insert, respectively. The cells were maintained at 37 °C in Dulbecco’s modified Eagle medium (DMEM) supplied with 10 % fetal bovine serum (FBS), penicillin (10 U ml⁻¹), streptomycin (10 μg ml⁻¹), L-glutamine (2 mM), and non-essential amino acids (1 %) in an atmosphere of 5 % CO₂ and a 90 % relative humidity. The culture medium was changed every 2-3 days.
**Xenopus Laevis Oocytes and Two-Electrode Voltage Clamp Measurements**

SLC36A1 cRNA was synthesized by *in vitro* transcription of linearized pGEM-HE-SLC36A1 as previously described by Frolund et al.\(^32\) using the mMESSAGE mMACHINE T7 mRNA-capping kit according to the protocol supplied by Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA). Stage VI oocytes from *Xenopus laevis* frogs (collagenase treated) were injected with 18.4 nL of diluted SLC36A1 cRNA (0.74 μg μL\(^{-1}\)) or nuclease-free water using an UMP3 Microsyringe Injector with an Micro4 Controller (World Precision Instruments Ltd, Sarasota, FL, USA) and incubated at 18 °C. The oocytes were stored in Modified Barth’s Saline (in mM: NaCl, 88; KCl, 1.0; NaHCO\(_3\), 2.4; Ca(NO\(_3\))\(_2\), 0.30; MgSO\(_4\), 0.82; CaCl\(_2\), 0.40; HEPES, 15; penicillin (10 U ml\(^{-1}\)), streptomycin (10 μg ml\(^{-1}\)), pH 7.6 and 183 mOsmol kg\(^{-1}\)). Two-electrode voltage clamp (TEVC) measurements were performed using a Xenoplace Workstation and a VC\(^38\) perfusion system from ALA Scientific Instruments and a TURBO TEC-10CX amplifier with an INT-20X breakout box from NPI electronic (Tamm, Germany). CellWorks Software version 6.2.1 were used for data acquisition and solution handling. The PC-10 puller from Narishige Group (Tokyo, Japan) was used to produce micropipettes from glass capillaries of fire-polished borosilicate glass and used for recordings with a resistance range between 2.5-4.5 MΩ. TEVC were performed 8 days post-injection, and oocytes were continuously perfused with Ringer’s solution (in mM: NaCl, 115; KCl, 2.5; CaCl\(_2\), 1.8; MgCl\(_2\), 0.1; MES, 10), pH 6.0 and 242 mOsmol kg\(^{-1}\) at a holding potential of -60 mV. Compounds were dissolved in Ringer’s solution and pH was adjusted to 6.0. Solutions containing either 20 mM proline, 50 μM E2 or 50 μM E-E2 alone or in combination were added by full bath application for 30 s between 120 s periods of perfusion with buffer, while the change in membrane current was monitored continuously. Water-injected oocytes served as negative controls. All experiments were performed on 6 oocytes.

**Buffer solutions for Caco-2 cell experiments**

The donor solutions were prepared in Hank’s Balanced Saline Solution (HBSS) consisted of, in mM: CaCl\(_2\), 1.26; MgCl\(_2\), 0.49; MgSO\(_4\), 0.41; KCl, 5.33; KH\(_2\)PO\(_4\), 0.44; NaCl, 138; Na\(_2\)HPO\(_4\), 0.34; D-glucose, 5.56; Na-HCO\(_3\), 4.17 and contained 10 mM MES at pH 6.0 (HBSS 6.0) or 10 mM HEPES pH 7.4 (HBSS 7.4). The pH of the buffers was adjusted with 0.1 M NaOH/HCl using a pH-meter (Mettler Toledo, Columbus, OH, USA). CDCA, Dx, E2, E-E2, fulvestrant, GCA, and genistein were prepared as stock solutions in 99 % ethanol. Thapsigargin, PMA, and staurosporine were prepared as stock solutions in DMSO. For cell experiment these stock solutions were diluted in buffer, where the final DMSO or ethanol concentrations did not exceed 1 %.
Uptake experiments in Caco-2 cells

The uptake of taurine via TauT or PAT1, proline via PAT1, Gly-Sar via the proton-coupled di/tri-peptide transporter, (PEPT1, SLC15A1) 5-HT via the sodium-dependent serotonin transporter, (SERT1, SLC6A4) or propranolol via passive diffusion was investigated in HBSS buffer at either pH 6.0 or pH 7.4 for 5 min. Previous studies have shown that uptake of PAT1 and PEPT1 substrates in Caco-2 cells is linear for 5 min. Caco-2 cells grown on 24 well plates (1.9 cm²) were used for the uptake study. All uptake experiments were performed using 1 μCi mL⁻¹ radiolabeled compound alone or in buffer solution supplemented with a non-labeled concentration of substrate and/or E2 or E-E2. Before starting the experiment, all solutions were pre-heated to a temperature of 37 °C. The cells were incubated with pre-warmed 10 mM HEPES in HBSS (pH 7.4) for at least 10 min on a mini shaker (220 rpm) from Troemner (Thorofare, NJ, USA) at 37 °C to equilibrate the cells prior to the experiment. Buffer was removed by gentle vacuum suction and 500 μL solution containing radiolabeled compound was applied on the apical side. After the 5 min incubation, the isotope-containing buffer solution was removed, and the wells were washed three times with 500 μL ice-cold 10 mM HEPES in HBSS (pH 7.4). The cells were detached using 200 μL of 0.1 % Triton X-100 and transferred to scintillation vials and 2 mL of scintillation liquid was added. 20 μL donor samples were taken from each solution containing radiolabeled compound. The samples were vortexed for 10 s, and the amount of radiolabeled compound (as disintegration per minute, DPM) was measured for 10 min using liquid scintillation counters (LSC) (Tri-Carb 2900TR or TriCarb 4910TR, Perkin Elmer, Waltham, MA, USA).

Transport of substances across Caco-2 cell monolayers

Caco-2 cells grown on Transwell® inserts (1.12 cm², 0.4 μm) were used for the transport study. The transepithelial electrical resistance (TEER) was measured at room temperature (20 ºC) in a tissue resistance measurement chamber (Endohm-12) with an Epithelial Voltohmmeter (EVOM 2) from World Precision Instruments (Sarasota, FL, USA). The initial transepithelial electrical resistance (TEER) was measured to assure that the barrier properties of the monolayer were intact, and all monolayers used had values > 400 Ω cm⁻². Transport was measured from the apical (A) to the basolateral (B) side. Before starting the experiment, all solutions were pre-heated to a temperature of 37 °C. The cells were incubated with pre-warmed 10 mM HEPES in HBSS (pH 7.4) for at least 10 min on a mini shaker (220 rpm) from Troemner (Thorofare, NJ, USA) at 37 °C to equilibrate the cells prior to the experiment. Buffer was removed by gentle vacuum suction, and 500 μL solution containing radiolabelled compound (1 μCi mL⁻¹) was applied on the apical side. Following this, 1000 μL...
10 mM HEPES in HBSS (pH 7.4) was applied to the basolateral side. Samples, 100 μL, were taken from the receiver (basolateral) solution at 15, 30, 60, 90, and 120 min after starting the experiment and replaced with 100 μL fresh 10 mM HEPES in HBSS (pH 7.4). 20 μL donor samples were taken from each solution containing radiolabelled compound at 0 and 120 min. To all samples, 2 mL scintillation liquid was added and vortexed for 10 s. The amount of radiolabelled compound (as disintegration per minute, DPM) was measured for 10 min using liquid scintillation counter (LSC) (Tri-Carb 2900TR, Perkin Elmer, Waltham, MA, USA). The integrity of the Caco-2 cell monolayer was evaluated in each transport study by measuring the flux of $^{14}$C or $^3$H-mannitol. None of the treatments affected mannitol permeabilities significantly, with apparent mannitol permeabilities always being lower than $5.2 \times 10^{-7} \pm 2.2 \times 10^{-7}$ cm s$^{-1}$.

**Estradiol and ethinyl-estradiol formulations for the pharmacokinetic (PK) study**

E2 and E-E2 were dissolved in 96 % EtOH to a concentration of 2.5 mM, and this stock solution was further diluted in 96% EtOH and given in doses of 0, 0.25, 1, 5, 10 and 25 μmol kg$^{-1}$ for E2 and 0, 0.25, 1, 5 and 25 μmol kg$^{-1}$ for E-E2. Taurine was dissolved in Elga water and dosed at 30 mg kg$^{-1}$. The osmolalities were measured using a Wescor Vapro model 5520 vapour pressure osmometer (Wescor Inc, Logan, UT, USA) and tonicity was adjusted with sodium chloride to 274-295 mOsmol kg$^{-1}$. pH values were measured using PHM220 LAB pH meter by Radiometer analytical SAS (Hach, Loveland, CO, USA) and was adjusted with NaOH or HCl to 7.4 ± 0.05. Immediately before dosing, $[{}^{3}\mathrm{H}]$-taurine was added to each formulation containing taurine in a concentration of 40 μCi kg$^{-1}$.

**In vivo taurine PK studies after pre-administration with E2 or E-E2**

Male Sprague-Dawley rats, weighing approximately 350 g (287-398 g) on the day of the experiments, were purchased from Charles River Deutschland (Sulzfeld, Germany). The animals were acclimatised for a minimum of five days in groups of two and maintained on standard feed with free access to water. Prior to dosing, the animals were fasted for 16-20 h with free access to water. The protocol used for the animal studies was approved by the institutional animal ethics committee in accordance with Danish law regulating animal experiments and in compliance with EC Directive 2010/63/EU and the NIH guidelines on animal welfare.

E2 or E-E2 and taurine were dosed separately. Initially, E2 or E-E2 were pre-dosed at 0.3 mL kg$^{-1}$ and four and a half minute later the taurine formulations were administered at a dose of 30 mg kg$^{-1}$ (at 10 mL kg$^{-1}$). Animals not receiving E2 or E-E2 were pre-dosed with 0.3 mL kg$^{-1}$ 96 % EtOH. All formulations were dosed
orally by gavage. Each group dosed with E2 or E-E2 consisted of four male Sprague-Dawley rats and one group of eight rats (control group) were initially dosed with 96 % ethanol only then with 30 mg kg\(^{-1}\) taurine.

**Plasma sampling and sample analysis**

The blood samples (100-200 µL), collected after administration, were drawn from the tail vein and collected into EDTA coated tubes (Microwette 500 K3E, Sarstedt, Nümbrecht, Germany) at 5-360 min after administration (5, 15, 30, 45, 60, 120, 180, 240, 360 min). Plasma was harvested immediately by 10 min of centrifugation at 4 °C, 3600 × g (Multifuge 1 S-R, Heraeus, Hanau, Germany) and stored in polypropylene tubes (Pony Vial, PerkinElmer, Waltham, MA, USA) at -20 °C until analysed. At the end of the experiment, animals were sacrificed by spinal dislocation using a guillotine. Plasma samples were left to defrost and mixed with 4 mL of liquid scintillation cocktail. The samples were counted for 3 min on a liquid scintillation counter (Tri-Carb 2900TR, Perkin Elmer, Waltham, MA, USA).

**Data analysis**

The inhibition of total uptake of proline and taurine at pH 6.0 in Caco-2 cells was described by

\[
V_o = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + \frac{[I]}{I_{50}}}
\]

where \(V_o\) is the initial uptake rate of taurine or proline in the presence of various concentrations of E2 or E-E2 \([I]\), Top is maximal uptake rate of taurine or proline in the absence of inhibition, and bottom is the uptake rate at maximal degree of inhibition. The total uptake of proline and taurine at pH 6.0 in Caco-2 cells was described by Michaelis-Menten like kinetics:

\[
V_o = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]}
\]

where \(V_o\) is the initial uptake rate of taurine or proline at a given concentration, \([S]\), in mM in the donor solution, \(V_{\text{max}}\) is the maximal uptake rate, and \(K_m\) is the Michaelis constant.

Transport data was described by Fick`s first law;

\[
J = \frac{Q}{A \cdot t} = P_{\text{app}} \cdot C_0
\]
Where J is the flux and Q is the steady-state amount of transported compound over a given absorptive area (A), per time (t) and \( C_0 \) is the starting concentration in the donor chamber. From the steady state flux the apparent permeability coefficient (\( P_{\text{app}} \)) was calculated.

The pharmacokinetic parameters of orally administered taurine in male Sprague-Dawley rats were estimated non-compartmentally using Phoenix® 6.3 version (Pharsight Corporation, A Certara Company, USA). \( C_{\text{max}} \) and \( t_{\text{max}} \) were found as mean values of the plasma concentration profiles within each group. Area under the plasma concentration versus time profiles (AUC) were determined with non-compartmental analysis by using the linear trapezoidal rule from time zero to \( C_{\text{max}} \) and by log linear methods from \( C_{\text{max}} \) to the last measured plasma concentration and denoted AUC\(_{0-\text{tlast}}\), the first order elimination rate constant \( k_e \) was calculated as the slope from the terminal log plasma concentration time curve of individual animals. The total clearance was calculated as the dose divided by AUC.

**Statistical analysis**

Statistical analysis was performed in GraphPad Prism version 7.02. The obtained data was analysed for statistical differences using a parametric one-way ANOVA test followed by Dunnett’s multiple comparison test. The following levels of significance was used \( p<0.05 \) (*). Values were expressed as mean ± SEM.

**Results**

**Estradiol and ethinyl-estradiol inhibit PAT1-mediated uptake of Taurine and Proline**

The uptake of solute carrier substrates such as taurine, proline, glycyl-sarcosine (Gly-Sar) and 5-hydroxytryptamine was investigated in Caco-2 cells in the presence of 100 µM E2 and E-E2 (Figure 1). The uptake of both taurine and proline was higher under slightly acidic condition compared to neutral pH in the uptake buffer (Fig. 1 A and B), consistent with proton-coupled transport via PAT1. 100 µM E2 and E-E2 inhibited the uptake of proline and taurine at pH 6.0, but not at pH 7.4. Likewise, the uptake of 5-hydroxytryptamine was slightly, yet significantly, decreased by 100 µM E2 and E-E2 (Fig. 1C). The uptake of Gly-Sar via hPEPT1 was unaffected by 100 µM E2 and E-E2 but inhibited by 10 mM Gly-Pro (Fig. 1D). The uptake of propranolol was unaffected by 100 µM E2 (Fig. 1E).
Estradiol and ethinyl-estradiol concentration-dependently inhibit PAT1-mediated uptake of taurine and proline

The uptake of taurine and proline was investigated in Caco-2 cells in the presence of increasing concentrations of E and E-E2 (Fig. 2). Under slightly acidic extracellular conditions E2 and E-E2 inhibited proline uptake with IC\textsubscript{50}-values of 10.0 ± 3.2 µM and 50.0 ± 14.3 µM, respectively (Fig. 2A). The uptake of taurine was inhibited by E and E-E2 with IC\textsubscript{50}-values of 8.7 ± 5.1 µM and 22.9 ± 15.5 µM, respectively (Fig. 2B). In contrast, the uptake of taurine at neutral extracellular pH was not affected by E2 or E-E2 in the concentration range investigated (Fig. 2C).

Estradiol and Ethinyl-estradiol inhibit proline induced currents in PAT1 expressing oocytes

To investigate the effect of E and E-E2 on PAT1-mediated transport in a system only expressing PAT1, a series of TEVC recordings were conducted on PAT1 expressing oocytes (Fig. 3). Proline induced inward directed currents in PAT1 expressing oocytes, whereas no change in baseline current was observed in water-injected oocytes (upper trace in Figure 3). The proline induced signal was attenuated, when E2 and E-E2 were present in the proline-containing solution being perfused over the oocyte. Buffers with E2 and E-E2 alone did not induce an inward current, showing that they were non-translocated inhibitors of PAT1.

Estradiol and ethinyl-estradiol competitively inhibit PAT1-mediated cellular uptake of proline and taurine.

The concentration-dependent uptake of proline and taurine was measured in Caco-2 cells under slightly acidic conditions (Fig. 4). The uptake of proline was saturable and in the presence of E2, the K\textsubscript{m}-value remained statistically similar, while the V\textsubscript{max} value decreased significantly with increasing E2 concentrations (Fig. 4A and Table 1). A similar pattern was observed for taurine uptake in the presence of E2 and E-E2 (Fig. 4B and Table 1). This inhibition pattern suggested a non-competitive interaction and based on this, the inhibitory constants were estimated. The K\textsubscript{i} value for E2 inhibition of proline uptake was 46.4 ± 0.6 µM and 16.1 ± 2.1 µM for taurine uptake. For E-E2, the K\textsubscript{i} value for inhibition of taurine uptake was 30.3 ± 2.7 µM.

Time-dependency of estradiol and ethinyl-estradiol treatment on PAT1-mediated uptake.

The results were obtained by concomitantly applying hormone and substrate to Caco-2 cells. Therefore, the time dependency of the ability of E2 and E-E2 to decrease PAT1-mediated uptake was investigated. A one-
hour preincubation with 100 µM E2 or E-E2 showed that the 5 min effect observed was not transient as it persisted by a prolonged incubation with E2 or E-E2 (Fig 5A). Since E2 and E-E2 are hydrophobic substances, the ability to wash out the effect of the compounds was investigated. E2 was added to the cells for 5 min followed by a buffer wash-out period before measuring the uptake of proline. This showed that approximately 15 min after removal of E2 the inhibitory effect was removed, as the uptake at 15 min was not significantly different from the control uptake (Fig. 5B).

**PAT1-mediated uptake is not inhibited by other steroid-containing structures or modulators of estradiol signaling**

To investigate if the effect observed with E2 and E-E2 could be related to hydrophobic interactions with the Caco-2 cells, a series of steroid skeleton containing structures were investigated at a similar concentration as used for estradiol and ethinyl-estradiol (Fig. 6A). From this it was evident that, at the concentrations used, only E2 and E-E2 inhibit PAT1-mediated uptake in Caco-2 cells.

Estradiol signaling pathway modulators were also investigated (Fig. 6B). Although PI-103 reduced the uptake of proline, none of the investigated compounds were able to reverse the reduction of PAT1-mediated transport.

**Absorptive transport of taurine and proline across Caco-2 cell monolayers**

The absorptive (apical to basolateral) transport of taurine in the presence of 100 µM E2 and E-E2 was determined across Caco-2 cell monolayers in the presence or absence of a proton gradient across the cell layer (Fig. 7A and B). In the absence of a proton-gradient and with a TauT-facilitating taurine concentration neither E2 nor E-E2 changed the permeability of taurine. When the apical pH was adjusted to 6.0 and the taurine concentration to 100 µM, the presence of 100 µM E2 and 100 µM E-E2 significantly reduced taurine permeability. Likewise, the permeability of 1 mM proline was reduced, while the permeability of Gly-Sar, 5-HT and propranolol was statistically unaffected by the presence of E2 and E-E2. These results showed that transepithelial transport of taurine and proline was decreased by E2 and E-E2 and that the effect of E2 and E-E2 was relatively specific to PAT1. This indicated that a decreased PAT1 transport activity in the apical membrane of Caco-2 cells also translated into a decreased absorptive transport, whereby PAT1 mediated cellular uptake was rate determining for *in vitro* absorption across Caco-2 cells.

**Pharmacokinetic interaction study of taurine with estradiol and ethinyl-estradiol**
Since E2 or E-E2 decreased PAT1-mediated transepithelial permeability of proline and taurine, it was investigated if both estrogens had an effect on the pharmacokinetic profile of orally administered taurine to assess the potential for a “drug”-drug interaction. Male Sprague-Dawley rats were orally pre-dosed with E2 or E-E2 in 96 % ethanol in various doses and the resulting plasma concentrations versus time profiles are shown in Figure 8. The pharmacokinetic parameters ($t_{\text{max}}$, $C_{\text{max}}$, AUC$_{0\text{-tlast}}$ and $k_e$) obtained from a non-compartmental analysis are summarized in Table 2 and 3. The pharmacokinetic profile of taurine after pre-administration of E2 were generally below the control profile, however no significant differences were found when comparing $t_{\text{max}}$, AUC$_{0\text{-tlast}}$, $C_{\text{max}}$ or $k_e$ to that of the control. In the case of E-E2, the pharmacokinetic profiles of the different treatments showed lower plasma concentrations than the control group, and there appeared to be a concentration-dependent decrease in $C_{\text{max}}$ and a prolongation of $t_{\text{max}}$. However, when evaluating the pharmacokinetic parameters using an ANOVA analysis, no significant differences could be shown. Likewise, no significant differences between AUC$_{0\text{-tlast}}$ of the control group only administered taurine and the groups administered E-E2 in various doses could be shown. However, when comparing all the sample groups, a significant difference (p<0.05) was observed in AUC$_{0\text{-tlast}}$ between the group receiving 5 µmol kg$^{-1}$ E-E2 and the group receiving 25 µmol kg$^{-1}$ E-E2.

**Discussion**

It is clear from the present study that E2 and E-E2 interacted with PAT1, and thereby reduced substrate transport in vitro. Since E2 and E-E2 did not induce currents in PAT1 expressing oocytes, they were not substrates of the carrier, something that would not be expected based on the structural difference between an amino acid and a steroid. The PAT1-mediated transport of taurine and proline was decreased in a non-competitive manner, and collectively this suggested that E2 and E-E2 were inhibitors not binding to the amino acid substrate binding site of the PAT1 protein. To our knowledge, E2 and E-E2 are the most potent PAT1 inhibitors reported in the literature with affinities approximately 3-20 times higher than sertraline$^{26}$. Moreover, the effect of E2 appeared within 5 min and could be rapidly washed out over a 15 min period, which suggested a direct and non-genomic interaction. In other short-term experiments, E2 has been shown to inhibit transport via other membrane transport proteins: In striatal synaptosomes from ovariectomized Sprague Dawley rats, E2 decreased dopamine uptake in a competitive manner with an IC$_{50}$ value of 7.2±0.6 µM$^{36}$, E2 inhibited L-type Ca$^{2+}$ channels in A7r5 cells with an IC$_{50}$ value of 14.24±0.05 µM$^{37}$, and non-competitively inhibited mouse 5-HT$_3$ receptors with an IC$_{50}$ value of 33.2 µM$^{38}$. The IC$_{50}$ values for direct non-genomic inhi-
bition by E2 and E-E2 on PAT1 transport obtained in the present study are thus quite comparable with previous findings for other membrane proteins.

E2 can exert several cellular responses related to membrane, cytosolic or nuclear effects as reviewed by Nadal et al.\textsuperscript{39}. Therefore, in Caco-2 cells E2 and E-E2 could also have other short-term effects besides PAT1 binding, which could affect the transport through regulation. However, considering that the present study did not include a treatment for more than two hours, effects of E2 and E-E2 related to altered gene transcription seemed unlikely. There are membrane targets, including estrogen and receptor(s) related to the classic nuclear estrogen receptors (ER), non-classic ERs, and ligand- and voltage-activated ion channels or cytosolic targets that could trigger signaling events by intracellular messengers, resulting in the activation of different kinases, e.g. by ER\textsubscript{α} and ER\textsubscript{β}, which activate different signaling pathways\textsuperscript{39}. In the present study estrogen receptor (ER) activation, phosphorylation, intracellular Ca\textsuperscript{2+} signaling, and PI3K activation was investigated. These signaling events were studied with the ER antagonists endoxifen and fulvestrant, the PKC inhibitor staurosporine, the PI3K inhibitor PI-103, and the SERCA pump inhibitor thapsigargin all at concentrations known to affect the pathways. However, none of the pharmacological treatments employed altered E2-induced effects, supporting a direct E2 and E-E2 effect on PAT1 instead of an effect caused by an intracellular signaling pathway. In epithelial cells, transport via PAT1 is proton-coupled, and the hormones could affect the proton-coupling, by interacting with the sodium-proton exchanger NHE3. It has been shown that both PAT1 and the di/tri-peptide transporter PEPT1 (SLC15A1) transport is dependent on NHE3 to maintain the transmembrane proton gradient\textsuperscript{40-42}. Moreover, it has been shown that E2 is able to up-regulate the activity of NHE3 in rat colonic epithelial cells, although this effect was observed after long-term treatment with E2\textsuperscript{43}. However, E2 and E-E2 did not affect PEPT1-mediated transport after short-term exposure suggesting that the proton gradient was not altered by E2 or E-E2. Moreover, transport via TauT and SERT1, as well as the passively transported propranolol, was not altered by the highest investigated concentration of E2 and E-E2, and related steroid-structure containing compounds did not inhibit PAT1-mediated transport neither. The only slightly contradiction to this was that E2 slightly decrease the uptake of 5-HT, yet this effect was not found when measuring transepithelial transport of 5-HT. Collectively, the ability of E2 and E-E2 to inhibit PAT1-mediated transport appeared to be a specific interaction and not a result of unspecific cellular effects or through E2 signalling pathways. It remains unknown what the physiological reason for the ability of E2 to modulate PAT1-mediated transport is and under which conditions E2 concentration are increased to the µM range, as circulating E2 concentration are in the pM range\textsuperscript{44}. 


The likelihood of a potential in vivo effect can be predicted by considering the IC\textsubscript{50} value of inhibition relative to the initial concentration of inhibitor in the human intestine. There are no regulatory guidance for assessing the impact of intestinal solute carriers, but for P-gp and BCRP the drug-drug interaction (DDI) index is calculated as I\textsubscript{gut}/IC\textsubscript{50}, where I\textsubscript{gut} is the dose (µmol) calculated from 1 or 10 mg E2 or 0.03 mg E-E2 divided by 250 mL water (I\textsubscript{gut} 14.7 – 147 µM for E2 and I\textsubscript{gut} 0.4 µM for E-E2), and a value ≥ 10 suggests a potential in vivo interaction\textsuperscript{22}. Neglecting solubility issue, this translates into DDI values of 1.5-14.7 for E2 using proline as a substrate and 1.5-16.9 using taurine as a substrate, and approximately 0.01 for E-E2 using proline as a substrate. Thus, in human the interaction of E2 with PAT1 would clearly warrant further in vivo investigations, while interactions between proline or taurine and E-E2 would appear less likely. To mechanistically investigate if E2 and E-E2 could decrease PAT1-mediated absorption in vivo, an interaction study was conducted in Sprague-Dawley rats. Taurine was chosen as victim substrate for this investigation, since a previously study suggested PAT1 mediated absorption in the relevant doses in Sprague Dawley rats\textsuperscript{16}. The doses of E2 and E-E2 administered orally to the rats covered a broad range of 0.25–25 µmol kg\textsuperscript{-1} (approximately 0.07–7 mg kg\textsuperscript{-1}), where the human doses would be approximately 0.14 mg kg\textsuperscript{-1} or 0.0004 mg kg\textsuperscript{-1} for E2 and E-E2, respectively. The E-E2 doses used in the study are thus much higher that used in human, and assuming a rat intestinal volume of 3 mL, initial rat I\textsubscript{gut} of E-E2 and E2 would be 2.5 – 250 µM. These concentrations are in the range of the obtained IC\textsubscript{50} values for E2 and E-E2. E-E2 decreased the exposure of taurine in a concentration-dependent manner with decreasing AUC and C\textsubscript{max} and increasing t\textsubscript{max}, although this did not meet statistical significance. After E2 administration, all taurine AUC and C\textsubscript{max} values were lower than in the absence of E2, yet, the ability of E2 to alter taurine pharmacokinetics were less evident than would have been expected from the in vitro investigations. There are several potential factors that could explain why the clear inhibitory properties of E2 and E-E2 observed in vitro were less prominent in vivo. E2 is highly metabolized and has a low bioavailability, which could prevent the E2 concentration from being high enough to inhibit Pat1 as it was given as a 4.5 min pre-dose. Also, the hormones were dosed in 96 % ethanol due to the limited solubility of E2 and E-E2 in aqueous medium, where the solubility is around 3.9 mg L\textsuperscript{-1} and 9.2 mg L\textsuperscript{-1}, respectively\textsuperscript{45}. As ethanol is rapidly absorbed from the gastrointestinal system\textsuperscript{46}, there is a possibility that E and E-E2 could precipitate in the gastrointestinal tract as ethanol was absorbed, thereby lowering the amount in solution. Another potential difference between the in vitro and in vivo experiments is that while the in vitro studies were performed on human PAT1, the in vivo experiments were performed in rat expressing the rat ortholog Pat1, raising the possibility of a species difference in binding of E-E2 to PAT1 versus Pat1. However,
the findings presented here suggest that the potential interaction between E2 and PAT1 drug substances such as gaboxadol, vigabatrin, and δ-aminolevulinic acid should not be overlooked but investigated further, preferably in human.

In conclusion, the present study is the first to show that 17-α-ethynyl-estradiol decreased PAT1-mediated cellular uptake and reduced transepithelial absorptive permeability of PAT1 substrates in vitro and that this to a certain degree also leads to an effect on the pharmacokinetic profile of taurine in vivo after oral administration.

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Compliance with ethical standards. All studies conducted here comply with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest

Ethical approval. All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional animal ethics committee in accordance with Danish law regulating animal experiments and in compliance with EC Directive 2010/63/EU and the NIH guidelines on animal welfare

Figure Legends:

Figure 1. Uptake in Caco-2 cells measured in the absence or presence of 100 μM estradiol (E2) or E-estradiol (E-E2). A. The uptake rate of 13.3 nM [3H]-proline (1.0 μCi ml⁻¹) at pH 6.0 or 7.4 (n=4-7) B. The uptake rate of 52 nM [3H]-taurine (1.0 μCi ml⁻¹) with 100 μM non-labelled taurine at pH 6.0 or 7.4 (n=3-4). C. The uptake rate of 35.3 nM [3H]-5-HT (1.0 μCi ml⁻¹) at pH 6.0 (n=3-6) D. The uptake rate of 17.9 μM [14C]-Gly-Sar (1.0 μCi ml⁻¹) at pH 6.0 (n=3-5) E. The uptake rate of 65.8 nM [3H]-propranolol (1.0 μCi ml⁻¹) at pH 6.0 (n=3). Each value represents the mean ± SEM. One-way ANOVA test using Dunnett's multiple comparison post-test using p < 0.05 (*) was for statistical testing.
Figure 2. Concentrations-dependent inhibition of substrate uptake in Caco-2 cells with 1-316.2 µM estradiol or E-estradiol. A. Inhibition of 13.3 nM [³H]-proline uptake rate (1.0 µCi ml⁻¹) at pH 6.0.. B. Inhibition of 99 nM [³H]-taurine uptake (1.0 µCi ml⁻¹) with 100 µM non-labelled taurine at pH 6.0.. C. Inhibition of 99 nM [³H]-taurine uptake (1.0 µCi ml⁻¹) at pH 7.4. The IC₅₀ value was determined by fitting data to a sigmoidal dose-response curve for inhibition, GraphPad Prism version 7.02. Each value represents the mean ± SEM (n=4).

Figure 3. Two-electrode voltage clamp (TEVC) measurements in PAT1 expressing Xenopus Laevis Oocytes. Estradiol (E2) or E-estradiol (E-E2) inhibition of proline induced current in PAT1 expressing oocytes at a holding potential of -60 mV and continuously perfused with Ringer’s solution pH 6.0. 20 mM proline alone or with either 50 µM estradiol or E-estradiol were applied (as indicated by the horizontal bars), and the change in current was monitored. Upper trace, water injected oocyte; lower trace, oocyte injected with SLC36A1 cRNA. The traces are representative of experiments performed on 6 different oocytes.

Figure 4. Concentrations-dependent substrate uptake in Caco-2 cells in the absence or presence of estradiol (E2) or E-estradiol (E-E2) at pH 6.0. A. The uptake rate of 13.3 nM [³H]-proline (1.0 µCi ml⁻¹) with 0-20 mM L-proline (o), in the presence of 10 µM E2 (●), 50 µM E2 (■) or 100 µM E2 (▲) (n=3-5). B. The uptake rate of 52 nM [³H]-taurine (1.0 µCi ml⁻¹) with 0-50 mM taurine (o), in the presence of 50 µM E2 (Δ) or 50 µM E-E2 (■) (n=4). Each value represents the mean ± SEM. The solid lines show the fit of the resulting data to Michaelis-Menten like kinetics.

Figure 5. Time-dependent treatment of estradiol (E2) or E-estradiol (E-E2) in Caco-2 cells. A. The uptake rate of 13.3 nM [³H]-proline (1.0 µCi ml⁻¹) at pH 6.0 with and without pre-incubation of 100 µM estradiol (E2) or E-estradiol (E-E2) at pH 7.4 for 1 hour before the proline uptake study. B. The uptake rate of 13.3 nM [³H]-proline (1.0 µCi ml⁻¹) at pH 6.0 with and without pre-incubation of 100 µM estradiol (E2), pH 7.4 for 5 minutes, followed by incubation with buffer at the indicated time points, before the 5 min proline uptake study. Each value represents the mean ± SEM (n=3). Statistical significance between the control (pre-incubation and uptake without E2) and the other pre-incubation conditions was tested using a one-way ANOVA test followed by a Dunnett’s multiple comparisons test.
Figure 6. Uptake in Caco-2 cells measured in the absence or presence of other steroid-containing structures or modulators of estradiol signaling. A. The uptake rate of 13.3 nM \[^{3}H\]-proline (1.0 μCi mL\(^{-1}\)) in the absence or presence of 100 μM estradiol (E2), E-estradiol (E-E2), chenodeoxycholic acid (CDCA), cholesterol, dexamethasone (Dx), glycocholic acid (GCA), and taurocholic acid (TCA) at pH 6.0. B. The uptake rate of 13.3 nM \[^{3}H\]-proline (1.0 μCi mL\(^{-1}\)) in the absence or presence of 100 nM endoxifen, 100 μM fulvestrant, 300 nM staurosporine, 1 μM thapsigargin and 1 μM PI-103 at pH 6.0. The cells were pre-incubated with the compounds in 10 mM HEPES in HBSS (pH 7.4) for 2 hours. Each value represents the mean ± SEM (n=3). Statistical significance between the control and the tested compounds within the three conditions was tested using a one-way ANOVA test followed by a Dunnett’s multiple comparisons test.

Figure 7. Apparent absorptive transepithelial permeability (P\(_{\text{app}}\)) across Caco-2 cell monolayers of various SLC substrates in the absence or presence of 100 μM estradiol (E2) or E-estradiol (E-E2). Transport was measured from the apical (A) to the basolateral (B) side. Donor solutions contained 1.0 μCi mL\(^{-1}\) radiolabeled compound. A. Taurine permeability at pH 6.0/7.4 (A/B) with a donor concentration of 100 μM taurine. B. Taurine permeability at pH 7.4/7.4 (A/B) with a donor concentration of 99 nM taurine. C. Proline permeability at pH 6.0/7.4 (A/B) with a donor concentration of 1 mM proline. D. Gly-Sar permeability at pH 6.0/7.4 (A/B) with a donor concentration of 17.9 μM Gly-Sar. E. 5-HT permeability at pH 6.0/7.4 (A/B) with a donor concentration of 35.3 nM 5-HT. F. Propranolol permeability at pH 6.0/7.4 (A/B) with a donor concentration of 23.9 nM propranolol. Each value represents the mean ± SEM (n=3-4). Statistical analysis was done with two-way ANOVA with Dunnett post-test and the significance level * (P<0.05) compared to the control in each group.

Figure 8. Taurine plasma concentration versus time profiles after oral administration of estradiol (E2) (A) or E-estradiol (E-E2) (B) in various concentrations (shown in legend) followed by oral administration of \[^{3}H\]-taurine (40.0 μCi kg\(^{-1}\)) and taurine (30 mg kg\(^{-1}\)). Each value represents the mean ± SEM of measurements from 4 rats per dosing group, except for the control group consisting of data from 8 animals.
References


Table 1

Kinetical parameters for uptake of proline and taurine in the presence estradiol (E2) and ethinyl-estradiol (E-E2). The marked values (*) are significantly different from the \( V_{\text{max}} \) obtained in the absence of E2 and E-E2 evaluated by a one-way ANOVA test followed by Dunnett’s multiple comparisons test. The transporter-mediated intrinsic clearance (CL) was calculated as \( V_{\text{max}} / K_m \).

<table>
<thead>
<tr>
<th></th>
<th>( K_m, \text{ app} ) (mM)</th>
<th>( V_{\text{max}, \text{ app}} ) (nmol cm(^{-2}) min(^{-1}))</th>
<th>Intrinsic CL (( \mu )L min(^{-1}) pr. cm(^2))</th>
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<tr>
<td><strong>Proline</strong></td>
<td></td>
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<td></td>
<td>1.80 ± 0.24</td>
<td>1.54 ± 0.05</td>
<td>0.856</td>
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<td>+ 10 µM E2</td>
<td>2.54 ± 0.76</td>
<td>1.41 ± 0.11</td>
<td>0.551</td>
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<td>+ 50 µM E2</td>
<td>3.67 ± 1.57</td>
<td>0.76 ± 0.15(^*)</td>
<td>0.207</td>
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<td>+ 100 µM E2</td>
<td>3.81 ± 1.37</td>
<td>0.67 ± 0.07(^*)</td>
<td>0.176</td>
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<tr>
<td><strong>Taurine</strong></td>
<td>8.45 ± 1.39</td>
<td>3.75 ± 0.21</td>
<td>0.444</td>
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<tr>
<td>+ 50 µM E2</td>
<td>7.15 ± 3.22</td>
<td>0.87 ± 0.14(^*)</td>
<td>0.122</td>
</tr>
<tr>
<td>+ 50 µM E-E2</td>
<td>11.28 ± 2.03</td>
<td>1.55 ± 0.10(^*)</td>
<td>0.137</td>
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Table 2

Pharmacokinetic parameters of taurine after oral co-administration of estradiol to rats.

Rats were orally administered estradiol (E2) in various pre-doses followed by oral administration of 40 μCi kg⁻¹ [³H]-taurine and 30 mg kg⁻¹ taurine. The pharmacokinetic parameters C<sub>max</sub>, AUC and k<sub>e</sub> are expressed as mean ± SEM, while t<sub>max</sub> is expressed as the median [Q<sub>1</sub>; Q<sub>3</sub>] (25 % and 75 % percentile) of measurements from 4 rats per dosing group, except 8 rats for the control group (0 mg kg⁻¹ E2).

<table>
<thead>
<tr>
<th>E2 dose (mg kg⁻¹)</th>
<th>Taurine dose (mg kg⁻¹)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg mL⁻¹)</th>
<th>AUC&lt;sub&gt;0-tlast&lt;/sub&gt; (µg min mL⁻¹)</th>
<th>k&lt;sub&gt;e&lt;/sub&gt; (10⁻³ min⁻¹)</th>
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<tr>
<td>0</td>
<td>30</td>
<td>15.0 [15.0; 15.0]</td>
<td>15.6 ± 1.0</td>
<td>1181 ± 62</td>
<td>4.33 ± 0.250</td>
</tr>
<tr>
<td>0.07</td>
<td>30</td>
<td>15.0 [15.0; 15.0]</td>
<td>11.3 ± 2.0</td>
<td>878 ± 61</td>
<td>2.78 ± 0.220</td>
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<tr>
<td>0.27</td>
<td>30</td>
<td>15.0 [15.0; 15.0]</td>
<td>10.4 ± 1.8</td>
<td>964 ± 145</td>
<td>2.22 ± 0.573</td>
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<td>1.26</td>
<td>30</td>
<td>15.0 [15.0; 15.0]</td>
<td>15.5 ± 1.2</td>
<td>1038 ± 51</td>
<td>3.79 ± 0.147</td>
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<tr>
<td>2.72</td>
<td>30</td>
<td>15.0 [15.0; 37.5]</td>
<td>12.3 ± 4.1</td>
<td>1086 ± 102</td>
<td>2.90 ± 0.783</td>
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<td>6.78</td>
<td>30</td>
<td>15.0 [15.0; 15.0]</td>
<td>13.4 ± 1.3</td>
<td>1025 ± 89</td>
<td>2.56 ± 0.353</td>
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Table 3
Pharmacokinetic parameters of taurine after oral co-administration of ethinyl-estradiol to rats.

Rats were orally administered ethinyl-estradiol (E-E2) in various doses followed by oral administration of 40 μCi kg⁻¹ [³H]-taurine and 30 mg kg⁻¹ taurine. The pharmacokinetic parameters Cₘₐₓ, AUC and kₑ are expressed as mean ± SEM, while tₘₐₓ is expressed as the median [Q₁; Q₃] (25 % and 75 % percentile) of measurements from 4 rats per dosing group, except 8 rats for the control group (0 mg kg⁻¹ E-E2).

<table>
<thead>
<tr>
<th>E-E2 dose (mg kg⁻¹)</th>
<th>Taurine dose (mg kg⁻¹)</th>
<th>tₘₐₓ (min)</th>
<th>Cₘₐₓ (µg mL⁻¹)</th>
<th>AUC₀-tₗₐ₉ (µg min mL⁻¹)</th>
<th>kₑ (10⁻³ min⁻¹)</th>
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<tr>
<td>0</td>
<td>30</td>
<td>15.0 [15.0; 15.0]</td>
<td>15.6 ± 1.0</td>
<td>1181 ± 62</td>
<td>4.33 ± 0.250</td>
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<tr>
<td>0.08</td>
<td>30</td>
<td>15.0 [15.0; 15.0]</td>
<td>16.0 ± 1.7</td>
<td>1110 ± 25</td>
<td>4.20 ± 0.301</td>
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<tr>
<td>0.31</td>
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<td>15.0 [15.0; 15.0]</td>
<td>13.8 ± 2.3</td>
<td>1062 ± 71</td>
<td>2.18 ± 0.758</td>
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<tr>
<td>1.46</td>
<td>30</td>
<td>22.5 [15.0; 30.0]</td>
<td>11.0 ± 1.9</td>
<td>1249 ± 95</td>
<td>3.17 ± 0.778</td>
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<tr>
<td>7.40</td>
<td>30</td>
<td>22.5 [15.0; 30.0]</td>
<td>10.7 ± 2.6</td>
<td>956 ± 58</td>
<td>2.52 ± 0.510</td>
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