Physiological Characterization of the Transporter-Mediated Uptake of the Reversible Male Contraceptive H2-Gamendazole Across the Blood-Testis Barrier Supplementary Material

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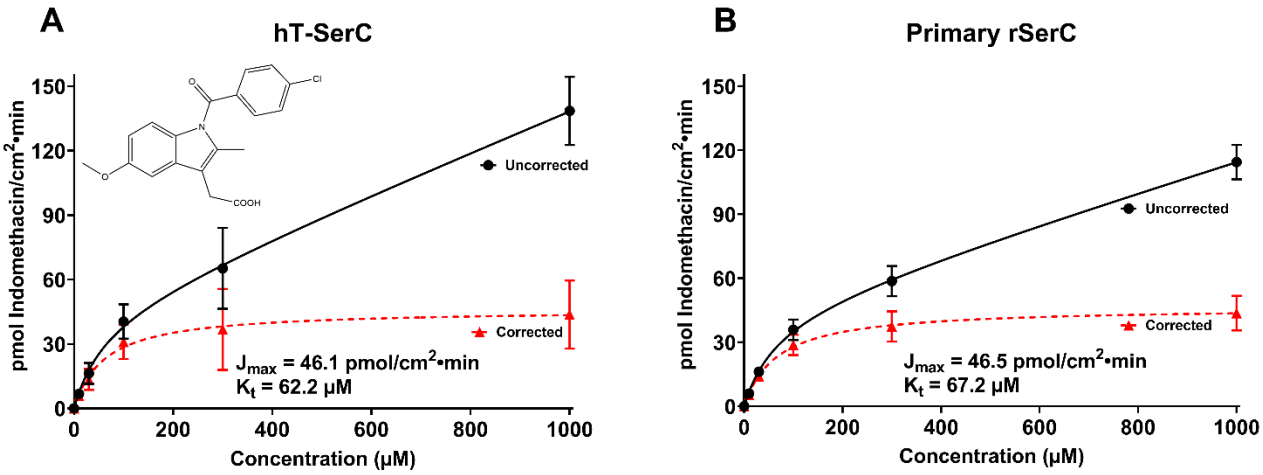
Supplementary Tables

Supplementary Table 1: Summary of kinetic parameters for indomethacin and diclofenac in hT-SerCs and primary rat SCs.

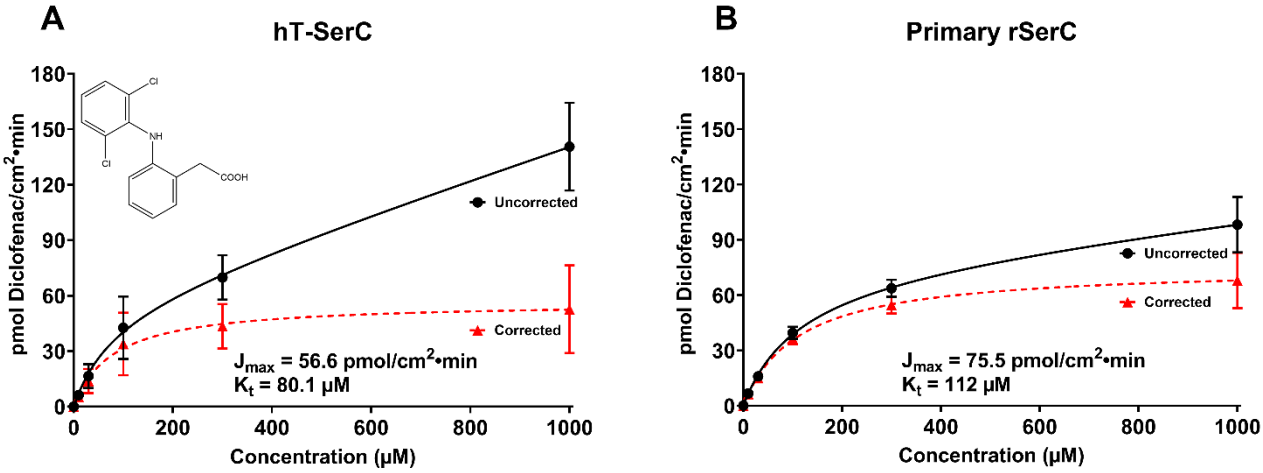
Compound	Cell	$J_{max\text{-}app}$ (pmol/cm^2•min)	$K_{t\text{-}app}$ (μM)
Indomethacin	hT-SerC	46.1 ± 17.4	62.2 ± 13.2
	Primary rat SC	46.5 ± 0.42	67.2 ± 13.9
Diclofenac	hT-SerC	56.6 ± 17.3	80.1 ± 56.3
	Primary rat SC	75.5 ± 7.50	112 ± 11.1

Supplementary Figures

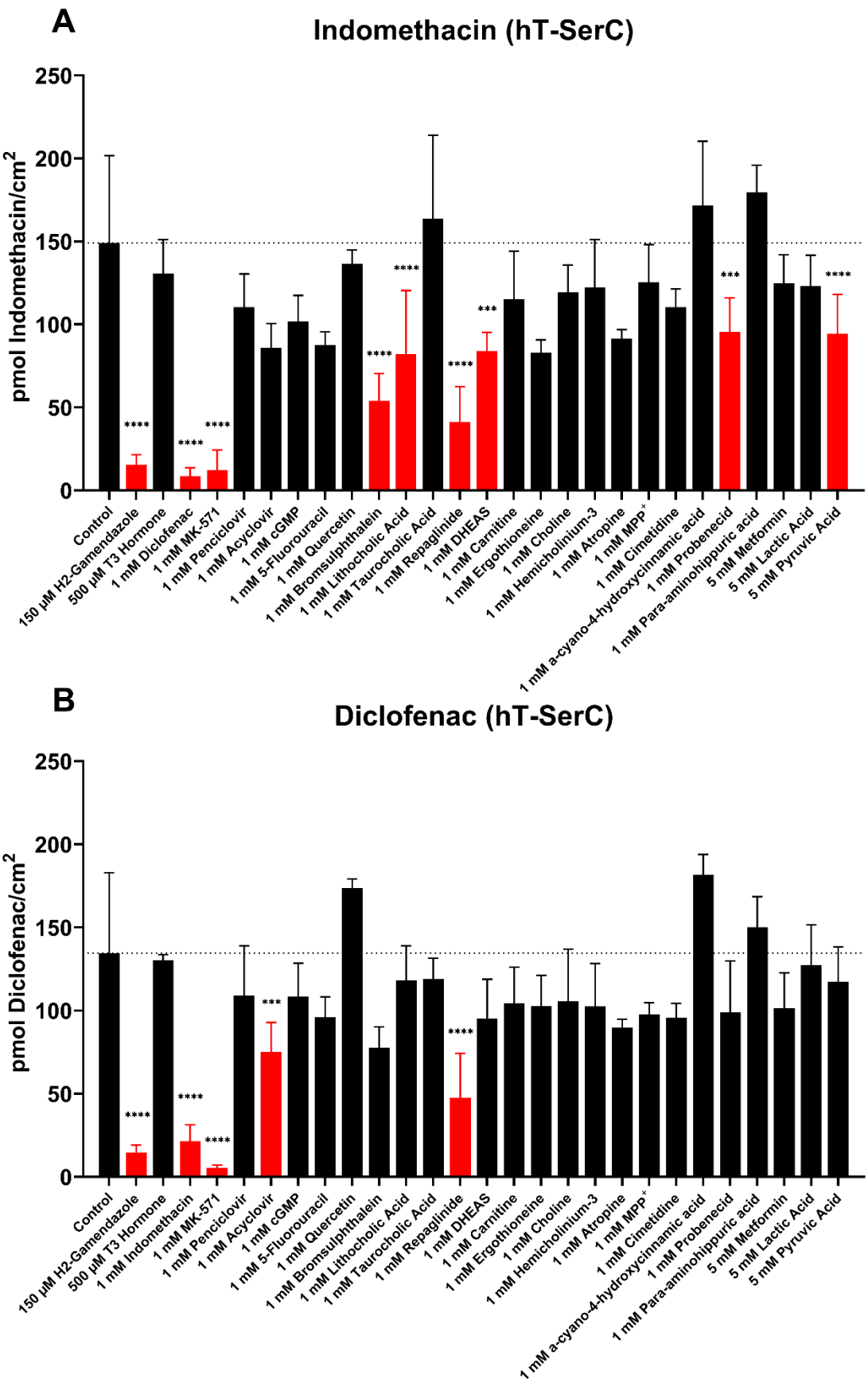
Supplementary Figure 1: Apparent kinetics of total transporter-mediated uptake of indomethacin in hT-SerCs and primary rat SCs. Michaelis-Menten kinetics of total transporter-mediated uptake of indomethacin in (A) hT-SerCs and (B) primary rat SCs. The solid line (uncorrected) was fitted to the raw data using equation 1 and reflects the combined effect of transporter-mediated uptake of indomethacin and a first-order component. The red dashed line (corrected) was fitted to the remaining data using equation 2. The chemical structure of indomethacin is shown in panel A. Data are represented as mean ± S.D.



Supplementary Figure 2: Apparent kinetics of total transporter-mediated uptake of diclofenac in hT-SerCs and primary rat SCs. Michaelis-Menten kinetics of total transporter-mediated uptake of diclofenac in (A) hT-SerCs and (B) primary rat SCs. The solid line (uncorrected) was fitted to the raw data using equation 1 and reflects the combined effect of transporter-mediated uptake of diclofenac and a first-order component. The red dashed line (corrected) was fitted to the remaining data using equation 2. The chemical structure of diclofenac is shown in panel A. Data are represented as mean ± S.D.



Supplementary Figure 3: Inhibition of total transporter-mediated uptake of indomethacin or diclofenac by transporter substrates and inhibitors in hT-SerCs. Inhibitory effect of transporter substrates or inhibitors co-incubated with 30 μM (A) indomethacin or (B) diclofenac on monolayers of hT-SerCs. Intracellular accumulation of indomethacin or diclofenac was measured after five minutes in all experiments. Data are represented as mean ± S.D. An ordinary one-way ANOVA with Bonferroni's multiple comparison correction was used to determine statistical significance between the control and inhibitor groups. Significance is indicated as *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, and ****: $p \leq 0.0001$.


A
Indomethacin (hT-SerC)
pmol Indomethacin/cm2
250
200
150
100
50
0
****
****
****
****
****
***
****
***
****
Control
150 µM H2-Gamendazole
500 µM T3 Hormone
1 mM Diclofenac
1 mM MK-571
1 mM Penciclovir
1 mM Acyclovir
1 mM cGMP
1 mM 5-Fluorouracil
1 mM Quercetin
1 mM Bromsulphthalein
1 mM Lithocholic Acid
1 mM Taurocholic Acid
1 mM Repaglinide
1 mM DHEAS
1 mM Carnitine
1 mM Ergothioneine
1 mM Choline
1 mM Hemicholinium-3
1 mM Atropine
1 mM MPP+
1 mM Cimetidine
1 mM a-cyano-4-hydroxycinnamic acid
1 mM Probenecid
1 mM Para-aminohippuric acid
5 mM Metformin
5 mM Lactic Acid
5 mM Pyruvic Acid
B
Diclofenac (hT-SerC)
pmol Diclofenac/cm2
250
200
150
100
50
0
****
****
****
***
****
Control
150 µM H2-Gamendazole
500 µM T3 Hormone
1 mM Indomethacin
1 mM MK-571
1 mM Penciclovir
1 mM Acyclovir
1 mM cGMP
1 mM 5-Fluorouracil
1 mM Quercetin
1 mM Bromsulphthalein
1 mM Lithocholic Acid
1 mM Taurocholic Acid
1 mM Repaglinide
1 mM DHEAS
1 mM Carnitine
1 mM Ergothioneine
1 mM Choline
1 mM Hemicholinium-3
1 mM Atropine
1 mM MPP+
1 mM Cimetidine
1 mM a-cyano-4-hydroxycinnamic acid
1 mM Probenecid
1 mM Para-aminohippuric acid
5 mM Metformin
5 mM Lactic Acid
5 mM Pyruvic Acid

Supplementary Figure 4: Immunocytofluorescence analysis for Flag-tagged human OATP1A2 or V5-tagged human OATP2B1 in HEK-293 or CHO cells. Control or transporter-overexpressing HEK-293 or CHO cells were probed with an (A, B) anti-Flag or (C, D) anti-V5 antibody to confirm plasma membrane expression. Intense positive staining for Flag or V5 was observed at the plasma membrane and cytoplasm of (B, D) transporter-overexpressing cell lines but not in the (A, C) respective control cells. Functional plasma membrane expression of each transporter was validated by measuring uptake of [^{3}H]E3S. Nuclei of all cells were counterstained with DAPI in blue. Images were captured at 40× magnification with a laser confocal microscope.