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Characterization of metoclopramide transport in human cerebral microvessel endothelial cells, hCMEC/D3

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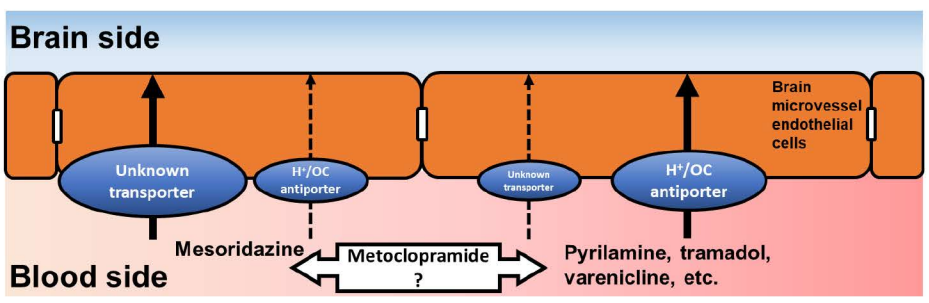
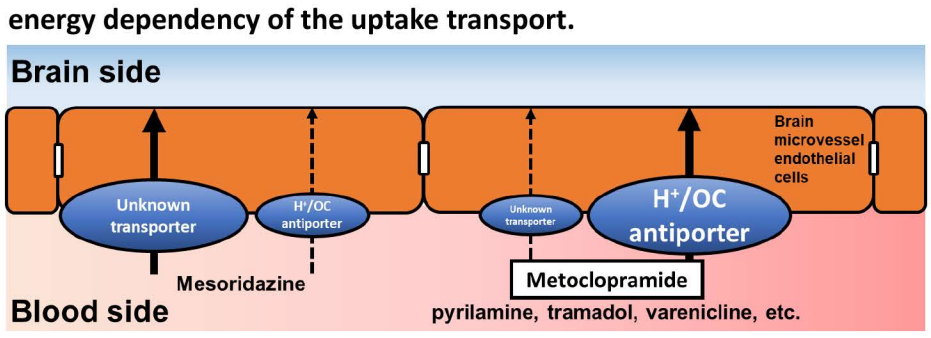
Purpose

In the last JSSX meeting, we have reported that not only proton-coupled organic cation antiporter (H^+/OC antiporter), but also unknown transporter(s) may be involved in the uptake process of mesoridazine into human cerebral microvessel endothelial cell line, hCMEC/D3 cells. Metoclopramide, which was reported as a substrate of OATP1A2 as well as mesoridazine (Cheng Z. et al., *Xenobiotica*, 2012; 42:880–90), is also a cationic drug, an antiemetic, and known to be distributed to brain ($K_{p,uu}$ = 1.1 in monkey) despite a substrate of MDR1. These suggest that some uptake transporters would be involved in the brain uptake process of metoclopramide in human. In the present study, we focused on the uptake process of metoclopramide into brain and aimed to elucidate the mechanism of its BBB transport using hCMEC/D3 cells.



Conclusion

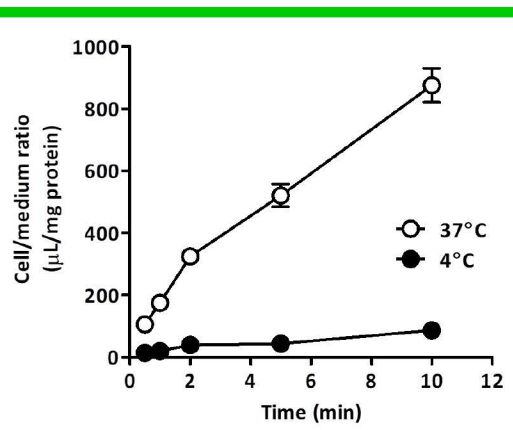
The present study suggested that H^+/OC antiporter was mainly involved in the uptake process of metoclopramide into hCMEC/D3 cells and the uptake mechanism was partially different from mesoridazine in the energy dependency of the uptake transport.



Materials and Methods

Metoclopramide uptake into hCMEC/D3 cells was measured under various conditions to evaluate the time-, temperature-, concentration-, energy- and ion-dependencies. Inhibition study was also performed with selected organic cations. Metoclopramide was quantified by LC-MS/MS system, Prominence (Shimadzu) and API5000 (Sciex). Multiple reaction monitoring transition was observed at Q1/Q3 300/227 for metoclopramide.

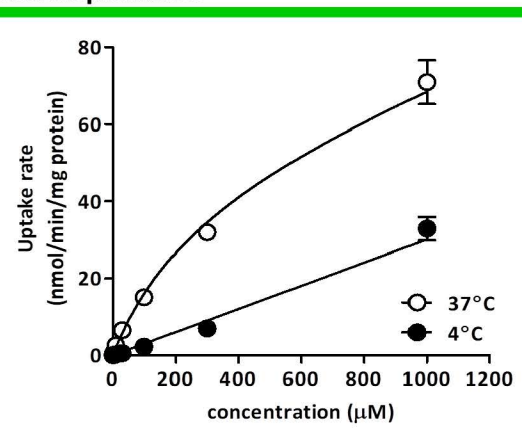
Results



Mean ± S.E. (n=4)

Fig. 1 Time profile of metoclopramide (1 μM) uptake in hCMEC/D3 cells

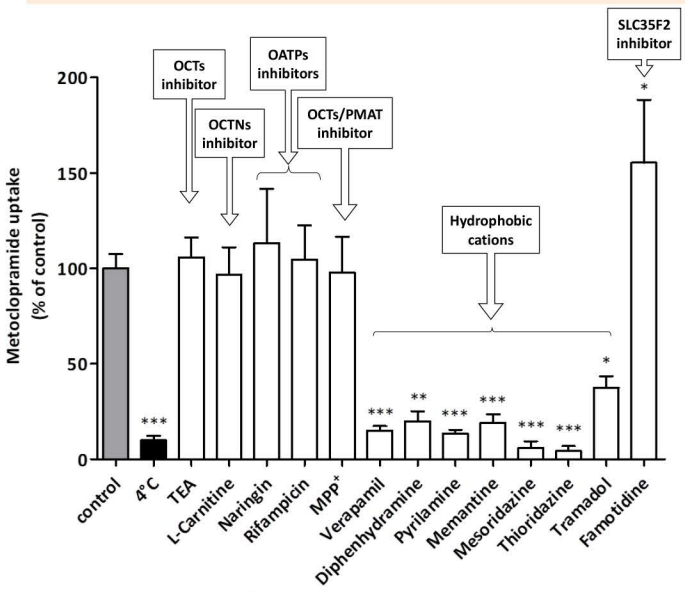
Uptake of metoclopramide was observed in time- and temperature-dependent manners.



Uptake was determined at various concentrations (0.1–1000 μM). The lines are fitted curves calculated by the nonlinear least-squares method based on $V=V_{max} \times S/(K_m+S)+P_{dif} \times S$. Mean ± S.E. (n=4)

Fig. 2 Concentration-dependent uptake of metoclopramide in hCMEC/D3 cells

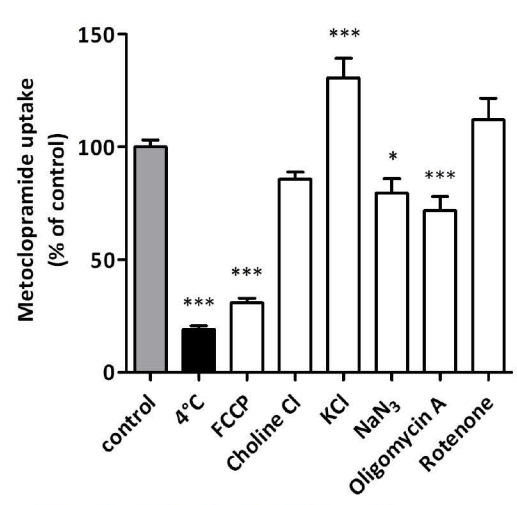
Carrier-mediated uptake of metoclopramide was observed in hCMEC/D3, with K_m, V_{max}, and P_{dif} values of 277 μM, 49 nmol/min/mg protein, and 30 μL/min/mg protein, respectively.



Uptake (1 μM) was determined in the absence and presence of each inhibitor (1 mM) except for rifampicin (0.3 mM) . Mean + S.E. (n=3-4)
* $p < 0.05$; ** $p < 0.01$; *** $p<0.001$ vs control by Dunnett's test

Fig. 3 Effect of various compounds on metoclopramide uptake in hCMEC/D3 cells

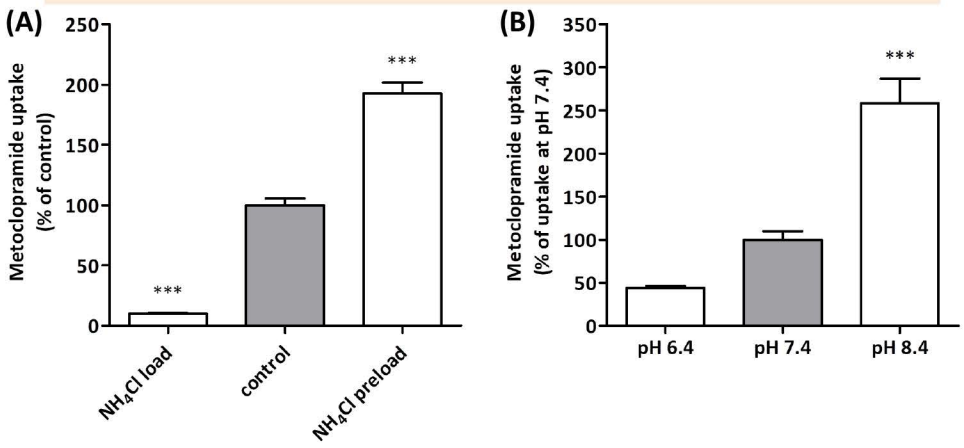
Metoclopramide uptake was significantly inhibited by typical cations. This result suggests that transporter(s) different from the known cation transporters are involved in the uptake of metoclopramide in hCMEC/D3 cells.



Uptake (1 μM) was determined in the absence and presence of 10 μM FCCP, choline Cl, KCl, 0.1% NaN_3, 25 μM rotenone, and 10 μM oligomycin A at 37°C. Na^+ in transport buffer was replaced with choline and potassium to induce loss of Na^+-gradient and membrane potential, respectively. Mean + S.E. (n=6-12).
* $p < 0.05$; *** $p<0.001$ vs control by Dunnett's test

Fig. 4 Effect of driving force on metoclopramide uptake in hCMEC/D3 cells

Metoclopramide uptake was significantly decreased by FCCP, a protonophore, and NaN_3 and oligomycin A, ATP depleters, but not by replacement of extracellular sodium ion with choline and potassium. This result suggests that H^+-dependent, Na^+-independent, and membrane potential-independent transporter is involved in the uptake of metoclopramide.



(A)In condition of NH_4Cl load, uptake (1 μM) was determined in the absence (control) and presence of 30 mM NH_4Cl at 37°C and pH7.4 to increase intracellular pH (pH_i). In condition of NH_4Cl preload, hCMEC/D3 cells were preincubated with 30 mM NH_4Cl for 20 min at 37°C and subsequently replaced with NH_4Cl-free buffer to decrease pH_i. (B)Uptake (1 μM) was measured in medium at pH values of 6.4, 7.4, and 8.4.
Mean + S.E. (n=6)
*** $p < 0.001$ vs control or pH7.4 by Dunnett's test

Fig. 5 Effects of intracellular (A) and extracellular (B) pH on metoclopramide uptake in hCMEC/D3 cells

(A)Metoclopramide uptake was significantly decreased by NH_4Cl load (pH_i: ↑), whereas the uptake was significantly increased by NH_4Cl preload (pH_i: ↓). (B)The uptake was decreased at pH 6.4 and significantly increased at pH 8.4, compared with that at pH 7.4. These results were well consistent with the transport characteristics of H^+/OC antiporter.

COI disclosure information

We have no financial relationship to disclose for our presentation contents.