Amiloride as a Probe Substrate for Investigation of Organic Cation Transport System

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[Introduction]

Disposition and elimination of cationic drugs are governed by drug transporters, organic cation transporter 1 (OCT1, *SLC22A1*), OCT2 (*SLC22A2)*, multidrug and toxin extrusion 1 (MATE1, *SLC47A1*), and MATE2-K (*SLC47A2*). Drug–drug interaction guidances from the regulatory agencies, Food and Drug Administration (USA) and Pharmaceuticals and Medical Devices Agency (Japan), have been updated that inhibition of OCT2, MATE1, and MATE2-K should be studied for all new investigational drugs. Amiloride is a potassium-sparing diuretic used for treatment of hypertension, and shows strong fluorescence in organic solvent or detergent solution. In this study, we investigated transport characteristics of amiloride by human OCT1, OCT2, MATE1, and MATE2-K.

[Methods]

Cellular accumulation of amiloride was evaluated with mock or hOCT1, hOCT2, hMATE1, or hMATE2-K overexpressed HEK293 cells. Cells were lysed with 1% SDS and fluorescence was measured with a microplate reader at 364 and 409 nm, excitation and emission wavelengths, respectively.

[Results and Discussion]

Amiloride was taken up by OCT1 and OCT2 in time- and concentration-dependent manner. These uptake was linear until 5 min and inhibited by the known substrates and inhibitors tetraethylammonium (TEA) and verapamil. High potassium induced-membrane depolarization also decreased the uptake. OCT1 showed higher affinity than OCT2 (*K*m of 42 and 253 μM; *V*max of 1.88 and 15.4 nmol/mg protein/3 min for OCT1 and OCT2, respectively). MATE1 and MATE2-K also transported amiloride. At extracellular pH 8.0, uptake was linear until 20 min. MATE1- and MATE2-Kmediated amiloride transport showed a bell-shaped pH profile and reached maximum at pH 8.0–8.5. Atextracellular pH 7.4 and ammonium prepulse-induced intracellular acidification, uptake showed an overshoot phenomenon. In this condition, MATE1 showed higher affinity than MATE2-K (*K*m of 21 and 40 μM; *V*max of 909 and 570 pmol/mg protein/min for MATE1 and MATE2-K, respectively). These transports were inhibited by TEA, metformin, verapamil, estrone-3-sulfate, and pyrimethamine.

[Conclusions]

This study demonstrates that amiloride is a suitable fluorescent substrate of OCT1, OCT2, MATE1, and MATE2-K, and useful for investigating organic cation transport system.

【感想】

2018 年 7 月、カナダ モントリオールで開催された 22nd North American Meeting に参加し、ポスター発表しました。海外での学会に参加するという貴重な経験をさせて頂きました。学会では、様々な国の研究者の発表を聴き、研究に対することだけではなく様々なことに関し、自分の視野を広げることができたと思います。援助してくださった愛知学院大学薬学会に感謝申し上げます。