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Note

Specific inhibitory effects of myricetin on human proton-coupled folate transporter: Comparison with its effects on rat proton-coupled folate transporter and human riboflavin transporter 3

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ABSTRACT

Myricetin is a flavonoid that inhibits human proton-coupled folate transporter (hPCFT) in a transient manner, in which inhibition is manifested in its presence, and also in a sustained manner, in which inhibition induced in its presence persists after its removal. In an effort to elucidate the mechanisms involved in those, we examined if myricetin might or might not act similarly on some other transporters. Transporters examined for that, in comparison with hPCFT, were its rat ortholog (rPCFT) and human riboflavin transporter 3 (hRFVT3). Experiments were conducted, using human embryonic kidney 293 cells transiently expressing the transporter to be examined, to assess the effects of myricetin (100 μM) on the uptake of folate by the PCFTs and riboflavin by hRFVT3. For hPCFT, myricetin was confirmed to induce a transient inhibition and also a sustained inhibition. However, myricetin induced neither transient nor sustained type of rPCFT inhibition. hRFVT3 was inhibited by myricetin in a transient manner, but not in a sustained manner. These results suggest the involvement of a hPCFT-specific mechanism in the sustained inhibition. The transient inhibition may be induced by a mechanism specific to hPCFT and also hRFVT3.

1. Introduction

Flavonoids have been of increasing concern for their inhibitory effects on transporters and enzymes involved in the disposition of drugs and nutrients [1,2]. Myricetin, which is present in various fruits and vegetables, is one of such flavonoids. This flavonoid has recently been found to inhibit human proton-coupled folate transporter (hPCFT)/SLC46A1 [3–5], which is responsible for the intestinal uptake of folate (vitamin B_9) and antifolate drugs analogous to the vitamin [6–8]. This myricetin-induced inhibition of hPCFT could potentially lead to the problem of decreased absorption of folate and antifolate drugs.

An issue now arising from the finding of myricetin-induced hPCFT inhibition is a possibility that myricetin might or might not act similarly on some other transporters. As our initial attempt to address that issue, we examined in the present study the effects of myricetin on the rat ortholog of hPCFT (rPCFT) and human riboflavin transporter 3 (hRFVT3)/hRFT2/SLC52A3 [9], an intestinal uptake transporter for riboflavin (vitamin B_2). rPCFT was selected as a PCFT ortholog in rat as a widely used experimental animal, and hRFVT3 was selected as a transporter that is present in the human intestine and would be exposed to myricetin similarly to hPCFT. Since myricetin has been indicated to inhibit hPCFT in a transient manner [3], in which inhibition is manifested in its presence, and also in a sustained manner [4,5], in which inhibition induced in its presence persists after its removal, this study was designed to assess the both types of inhibitory effects, using human embryonic kidney 293 (HEK293) cells transiently transfected with each of those selected transporters.

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2. Materials and methods

2.1. Materials

[^{3}H]Folate (19.4 Ci/mmol) and [^{3}H]riboflavin (24 Ci/mmol) were obtained from Moravek Biochemicals (Brea, CA, USA). Unlabeled folate and riboflavin were from Wako Pure Chemical Industries (Osaka, Japan), and myricetin was from Tokyo Chemical Industry (Tokyo, Japan). All other reagents were of analytical grade and commercially obtained.

2.2. Transport study

Transport assays were conducted using HEK293 cells transiently transfected with hPCFT, rPCFT, or hRFVT3, based on our previous reports [3–5,7–9] and as described in detail in the Supplementary information. In brief, cells were cultured on 24-well plates and the initial uptake of [^{3}H]folate, a substrate for the PCFTs, or [^{3}H]riboflavin, a substrate for hRFVT3, into the cells was evaluated. To assess the transient effect, myricetin (100 μM) was added only to the test solution containing the substrate to be present only during the transport period. To assess the sustained effect, cells were preincubated for 60 min in the substrate-free test solution added with myricetin (100 μM) and subsequently, after replacing the solution with fresh one without myricetin, preincubated for 5 min for washout, before starting transport in the absence of myricetin. Cellular protein content was determined, using bovine serum albumin as the standard, for normalization of the substrate uptake.

The specific uptake of folate or riboflavin by the designated transporter was estimated by subtracting its uptake in mock cells from that in cells expressing the transporter. The uptake rate (v) by the transporter was normalized to the substrate concentration (s) to estimate the uptake clearance (CL_{up}): $CL_{up} = v/s$.

The studies were designed to assess the effect of myricetin on CL_{up} at a low s, which was smaller than the Michaelis constant (K_m) sufficiently to be in the linear phase of Michaelis-Menten transport kinetics, and at a high s, which was higher than the K_m sufficiently to achieve an uptake rate that can approximately represent the maximum transport rate (V_{max}). Based on the previously determined K_m values of 1.67 μM and 2.4 μM, respectively, for folate transports by hPCFT [7] and rPCFT [8], the low and high concentrations of folate were set to be 5 nM and 5 μM, respectively, in studies for those PCFTs. In studies for hRFVT3, of which the K_m was 0.77 μM for riboflavin [9], the low and high concentrations of riboflavin were set to be 5 nM and 1 μM, respectively.

3. Results

3.1. Transient effect of myricetin

We first confirmed the transient effect of myricetin, which was added in the test solution to be present only for the transport period, on folate transport by hPCFT in the transient transfectant HEK293 cell system employed in the present study. As shown in Fig. 1A, the CL_{up} for hPCFT-specific folate uptake in the absence of myricetin was smaller at the high concentration (5 μM) of folate than at its low concentration (5 nM), manifesting the saturable characteristic of hPCFT-mediated folate transport. The CL_{up} was significantly reduced in the presence of myricetin (100 μM), compared with that in its absence for control, by 44% at the low concentration and by 43% at the high concentration. The reduction in the CL_{up} at the high concentration, where CL_{up} represents V_{max}/s, was attributable to a decrease in V_{max}. Since the reduction in the CL_{up} at the low concentration, where CL_{up} represents V_{max}/K_m, was

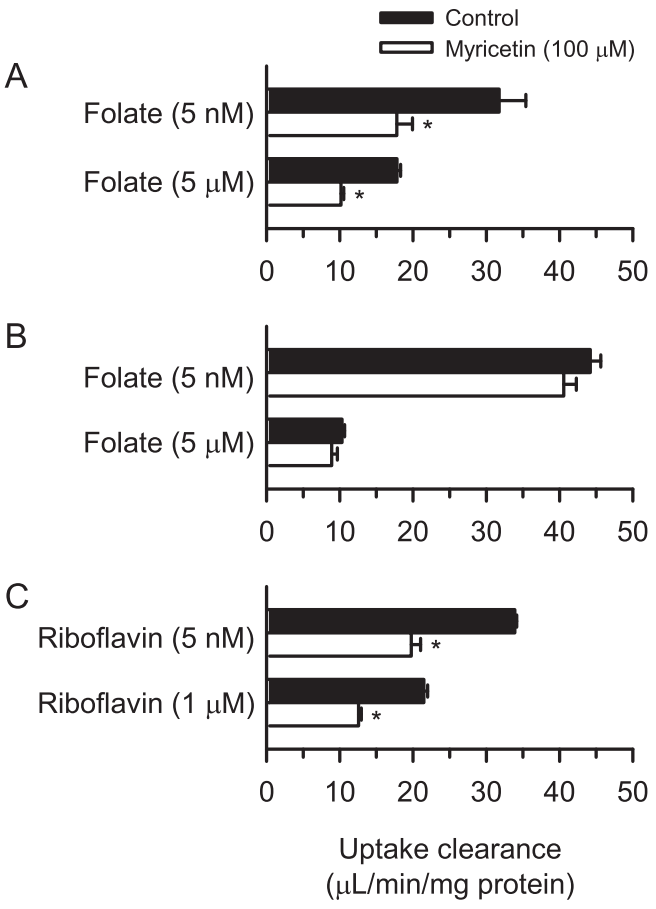

Fig. 1. Transient effect of myricetin on the transport function of hPCFT (A), rPCFT (B), and hRFVT3 (C) in HEK293 cells transiently expressing the designated transporter. (A) The specific uptake of folate by hPCFT was evaluated in hPCFT-expressing cells for the initial 2-min period at pH 5.5 and 37 °C in the presence of myricetin (100 μM) or in its absence for control. (B) The specific uptake of folate by rPCFT was similarly evaluated in rPCFT-expressing cells. (C) The specific uptake of riboflavin by hRFVT3 was evaluated in hRFVT3-expressing cells for the initial 1-min period at pH 6.0 and 37 °C in the presence of myricetin (100 μM) or in its absence for control. Data are presented as the means ± SE ($n = 4$). *$p < 0.05$ compared with control, as assessed by Student's t-test.

to almost the same extent, the reduction could be accounted for by the decrease in V_{max} and, hence, K_m was suggested to be unchanged. These results suggest that myricetin induced a transient inhibition of hPCFT in a manner consistent with the noncompetitive one observed in the stable transfectant Madin-Darby canine kidney II (MDCKII) cell system and the Caco-2 cell line [3]. The inhibition constants of myricetin for hPCFT were 61 μM in the former and 130 μM in the latter, which predict inhibitions by 62% and 43%, respectively, at 100 μM. These predicted extents of inhibition were comparable with those observed in the present study (43% and 44%), suggesting consistency in inhibitory potency as well.

For each of the uptakes of folate by rPCFT (Fig. 1B) and riboflavin by hRFVT3 (Fig. 1C), the saturable characteristic was also confirmed in the absence of myricetin by a reduction in CL_{up} with an increase in the substrate concentration. In contrast to the results for hPCFT (Fig. 1A), however, folate uptake by rPCFT was not altered by myricetin either at the low or high folate concentration (Fig. 1B), indicating that this rat ortholog of hPCFT is insensitive to the flavonoid in terms of the transient effect. Riboflavin uptake by hRFVT3 was, on the other hand, reduced by myricetin at both the low and high riboflavin concentrations of 5 nM and 1 μM, respectively (Fig. 1C). Since the extents of reduction in CL_{up} were almost the same at the two concentrations, being 42% and 41%, respectively, it is likely that