Review

Current Understanding of the Intestinal Absorption of Nucleobases and Analogs

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It has long been suggested that a Na+-dependent carrier-mediated transport system is involved in the absorption of nucleobases and analogs, including some drugs currently in therapeutic use, for their uptake at the brush border membrane of epithelial cells in the small intestine, mainly based on studies in non-primate experimental animals. The presence of this transport system was indeed proved by the recent identification of sodium-dependent nucleobase transporter 1 (SNBT1/Slc23a4) as its molecular entity in rats. However, this transporter has been found to be genetically deficient in humans and higher primates. Aware of this deficiency, we need to revisit the issue of the absorption of these compounds in the human small intestine so that we can understand the mechanisms and gain information to assure the more rational use and development of drugs analogous to nucleobases. Here, we review the current understanding of the intestinal absorption of nucleobases and analogs. This includes recent knowledge about the efflux transport of those compounds across the basolateral membrane when exiting epithelial cells, following brush border uptake, in order to complete the overall absorption process; the facilitative transporters of equilibrative nucleoside transporter 1 (ENT1/SLC29A1) and equilibrative nucleobase transporter 1 (ENBT1/SLC43A3) may be involved in that in many animal species, including human and rat, without any major species differences.

Key words nucleobase; uric acid; intestine; absorption; transporter; species difference

1. INTRODUCTION

Nucleobases are biologically indispensable as constituents of nucleic acids, which carry genetic information, and are also involved in various biological processes in derivatized forms, such as nucleosides and nucleotides.1,2) To maintain sufficient levels of these important nucleobases in the body, their intake from food has been suggested to be necessary, implicating their role as nutrients. Nucleobases are, however, hydrophilic compounds; it is difficult for them to permeate the cellular membrane by the mechanisms of partition into and diffusion inside the membrane’s lipoidal layer. Hence, as is generally recognized for nutrients with this delivery challenge, specialized carrier-mediated transport systems have been suggested to be in operation to enable their efficient and selective transport across the cellular membrane in absorption from the small intestine and also in their disposition for utilization in various organs.3–6) The presence of such a transport system for the intestinal absorption of nucleobases was initially suggested as early as 1960, in a study using rats,7) followed by a series of studies suggesting that the transport system is shared by synthetic nucleobase derivatives developed for cancer treatments,8–21) which drew attention to the transport system from a pharmaceutical viewpoint. This transport system was, in addition, indicated to be Na+-dependent and presumed to be located at the brush border membrane of epithelial cells for uptake operation.

In 2010, half a century after the initial suggestion of the presence of a putative Na+-dependent carrier-mediated nucleobase transport system in the rat small intestine, sodium-dependent nucleobase transporter 1 (SNBT1/Slc23a4) was identified as its molecular entity in rats.12,13) Importantly, SNBT1 was the first nucleobase-specific transporter identified in mammals at the time. However, the SNBT1 gene was, unexpectedly, found to be deficient in humans and higher primates. Therefore, we need to be aware that our current knowledge of the intestinal absorption of nucleobases and analogs, accumulated mainly from studies using non-primate experimental animals, is not applicable to humans (Fig. 1). The issue of the absorption of these compounds in the human small intestine must be revisited in order to understand the mechanisms involved and to gain information to guide the more rational use and development of drugs analogous to nucleobases. To that end, we review here the current understanding of the intestinal absorption of nucleobases and analogs, including recent knowledge about the efflux transport of those compounds across the basolateral membrane for exiting epithelial cells, following brush border uptake, in order to complete the overall absorption process. In this process, equilibrative nucleoside transporter 1 (ENT1/SLC29A1),14) a facilitative transporter that can operate for nucleobases in addition to nucleosides as its primary substrates,15) could be involved in many animal species, including human and rat.16–22) Equilibrative nucleobase transporter 1 (ENBT1/SLC43A3), a nucleobase-specific facilitative transporter, could also be involved in such transport in many animal species, although its expression in the small intestine has not yet been fully proven.19–25) Notably, ENT2/SLC29A2,26,27) a close homolog of ENT1, can transport nucleobases as well as nucleosides, but its expression in the small intestine has not been evident in recent studies.19–22)

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SNBT1 is present in the rat small intestine for the brush border uptake of nucleobases and analogs. The substrates include, for example, uracil and guanine as the major nucleobases, 5-fluorouracil (5-FU) and 6-mercaptopurine (6-MP) as nucleobase analog drugs, and uric acid as a purine-derived metabolite. Humans lack the SNBT1-derived function for nucleobase absorption, as the SNBT1 gene is deficient, although there might be a transporter alternative to SNBT1. In the basolateral efflux transport of those compounds, ENT1, which can operate for both purine and pyrimidine nucleobases, and ENBT1, which can operate specifically for purine nucleobases, could be involved in both rats and humans. There could be a species difference in the intestinal handling of uric acid, which may be linked to the elimination of SNBT1 in humans. In the rat small intestine, uric acid may undergo a futile cycle of SNBT1-mediated uptake after secretion by secretory transporters, such as BCRP, repeatedly, only to retart its excretion. The elimination of SNBT1 may have been favored in evolutionary development, as it could have been of help in facilitating uric acid excretion by shutting down the futile cycle. In the basolateral transport of uric acid, GLUT9 has been suggested to be involved.

For the absorption of nucleosides as nutrients closely related to nucleobases, the Na\(^{+}\)-dependent uptake transporters of concentrative nucleoside transporter 1 (CNT1/SLC28A1), CNT2/SLC28A2, and CNT3/SLC28A3 are present at the brush border membrane to constitute the absorption pathway in combination with ENT1, but it is suggested that they do not operate for nucleobases.\(^{18,28,29}\)

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### 2. INTESTINAL ABSORPTION OF NUCLEOBASES AND ANALOGS IN NON-PRIMATE EXPERIMENTAL ANIMALS

In 2010, SNBT1 was cloned from the rat small intestine as the molecular entity of the putative Na\(^{+}\)-dependent carrier-mediated nucleobase transport system, as mentioned above.\(^{12,13}\) This transporter is the 4th identified member of the SLC23 family of transporters.\(^{30,31}\) The 1st and 2nd members of this family were identified to be Na\(^{+}\)-dependent ascorbic acid transporters, back in 1999,\(^{32–36}\) and were named sodium-dependent vitamin C transporter 1 (SVCT1/SLC23A1) and SVCT2/SLC23A2, respectively, leading to the recognition of this family as a Na\(^{+}\)-dependent ascorbic acid transporter family.\(^{30,31}\) However, it is suggested that they do not have affinities for nucleobases.\(^{36,37}\) Rat SNBT1 consists of 614 amino acids, and is 50% identical to the rat homologs of SVCT1 and SVCT2. Topologically, rat SNBT1 is predicted to have 12 transmembrane domains, with two N-glycosylation sites at Asn\(^{152}\) and Asn\(^{300}\).\(^{12}\) It is notable that, phylogenetically, the SLC23 family has been indicated to belong to the nucleobase-ascorbate transporter (NAT) family present in a variety of prokaryotic and eukaryotic organisms, suggesting that nucleobase transporters may be included among the SLC23 family members.\(^{12,23,30,31}\) Finding the nucleobase transport function for SNBT1 indeed suggests this possibility. However, it was revealed that humans lack the SNBT1-derived function for nucleobase absorption, since the SNBT1 gene, being a pseudogene, was found to be deficient in humans.\(^{12}\) The SNBT1 gene is also deficient in the chimpanzee, whereas intact SNBT1 genes are present in many non-primate mammals and non-mammalian vertebrates, such as the zebrafish, chicken, mouse, dog, and horse. Thus, it is likely that, in the evolutionary process, the SNBT1 gene was silenced in higher primates. This is unexpected, but understanding the function and physiological role of SNBT1 in non-primate experimental animals, in combination with addressing animal species differences in relevant issues, should help to elucidate the mechanism of nucleobase absorption in the human small intestine. On the other hand, SLC23A3, of which the rat ortholog is 31% identical to rat SNBT1 in its amino acid sequence, remains an orphan transporter, with no demonstrated transport function for either ascorbic acid or nucleobases.\(^{30,31}\) According to the initial study using rat SNBT1 expressed in human embryonic kidney 293 cells,\(^{12}\) SNBT1 is highly capable of transporting uracil, thymine, and guanine among the major nucleobases, whereas it cannot transport adenine. Cytosine has been indicated not to have an affinity for SNBT1, based on the absence of its inhibitory action on SNBT1-mediated uracil transport. In Table 1, the major nucleobases and analogs are categorized into groups of substrates and nonsubstrates. It has been suggested that an oxo group at the 4th position in the pyrimidine structure, or at the 6th position as the corresponding position in the purine structure, is likely to play an important role in substrate recognition by SNBT1, since substrate nucleobases characteristically have that oxo group in common, whereas nonsubstrate nucleobases have an amino group instead. Accordingly, hypoxanthine and xanthine, which have the required oxo group, are also SNBT1 substrates capable of being transported quite efficiently. Uric acid, which has recently been identified as an SNBT1 substrate, is also a purine derivative, having the required oxo group.\(^{38}\) 5-Fluorouracil and 6-mercaptopurine, which are synthetic nucleobase analogs used in cancer chemotherapy, have also been indicated to have an affinity for SNBT1, based on their inhibitory actions on SNBT1-mediated uracil transport. These have indeed recently been confirmed to be transported by SNBT1 (unpublished data). Notably, while 5-fluorouracil...
has the required oxo group, 6-mercaptopurine has sulfur in the place of oxygen, suggesting that sulfur, which is close to oxygen in atomic characteristics, may play a role in substrate recognition as a substitute for oxygen. On the other hand, the major nucleosides, such as adenosine, guanosine, cytidine, thymidine, and uridine, have been indicated to have either no or only minimal affinity for SNBT1, suggesting the highly nucleobase-specific characteristic of SNBT1. All these characteristics of SNBT1 are in agreement with those indicated for the Na$^{+}$-dependent carrier-mediated nucleobase transport system, which has been presumed to be in operation for brush border uptake in the rat small intestine.

Kinetically, the Michaelis constant ($K_m$) was determined to be 21.2 μM for uracil transport by rat SNBT1. This is comparable to that of uracil transport (40.3 μM) by the nucleobase transport system in the rat small intestine, supporting the suggested role of SNBT1 as its molecular entity. These $K_m$ values for uracil, together with the $K_m$ and IC$_{50}$ values for some other nucleobases and analogs, are listed in Table 2 to summarize the kinetic characteristics of rat SNBT1. In assessing the inhibition of SNBT1-mediated uracil transport by thymine, hypoxanthine, guanine, and 5-fluorouracil, IC$_{50}$ values were determined to be 12.7, 17.4, 80.7, and 69.0 μM, respectively, indicating their affinities comparable to or somewhat lower than that of uracil for SNBT1. The IC$_{50}$ of 5-fluorouracil was comparable to the reported $K_m$ values (25 and 74 μM) for 5-fluorouracil transport by the nucleobase transport system in

| Material          | Compound     | $K_m$ (μM) | IC$_{50}$ (μM) | References |
|-------------------|--------------|------------|----------------|------------|
| SNBT1             | Uracil       | 21.2       | —              | 12         |
|                   | Thymine      | —          | 12.7           | 12         |
|                   | Hypoxanthine | —          | 17.4           | 12         |
|                   | Guanine      | —          | 80.7           | 12         |
|                   | 5-Fluorouracil | —        | 69.0           | 12         |
|                   | Uric acid    | 433        | —              | 38         |
| Small intestine$^a$ | Uracil       | 40.3       | —              | 12         |
|                   | 5-Fluorouracil | 25        | —              | 39         |
|                   |              | 74         | —              | 40         |

$^a$ Excised tissue preparations.

Ellipses (dots) indicate the 4th and 6th positions, respectively, in pyrimidines and purines. An oxo group is present at those positions in substrates, whereas an amino group is in place of that in nonsubstrates. 6-Mercaptopurine, which has a sulfur in place of the oxygen, is also a substrate.

Table 1. Substrate Recognition Characteristics of Rat SNBT1

| Type       | Substrates | Nonsubstrates |
|------------|------------|---------------|
| Pyrimidines| ![Pyrimidine Structures](image) | ![Pyrimidine Structures](image) |
| Purines    | ![Purine Structures](image) | ![Purine Structures](image) |
| Others     | ![Other Structures](image) | ![Other Structures](image) |
| 6-Mercaptopurine | ![Structure](image) | ![Structure](image) |
the rat small intestine.\textsuperscript{39,40} The $K_m$ of SNBT1-mediated uric acid transport was an order of magnitude higher (433\,\mu M), suggesting a lower affinity of this purine derivative for SNBT1.\textsuperscript{38}

SNBT1 is highly expressed in the small intestine in rats.\textsuperscript{12} In addition, an assessment using polarized Madin–Darby canine kidney II cells stably transfected with rat SNBT1 tagged with green fluorescent protein (GFP) for fluorescent microscopic observation indicated its localization to the apical membrane.\textsuperscript{12} These expression and localization characteristics suggest its specific role in the uptake of nucleobases at the brush border (apical) membrane in the small intestine, as expected for a Na\textsuperscript{+}-dependent carrier-mediated nucleobase transport system (Fig. 1).

The Na\textsuperscript{+}-dependent operation of SNBT1 is apparently in agreement with the Na\textsuperscript{+}-dependent characteristics of the carrier-mediated nucleobase transport system in the rat small intestine.\textsuperscript{12} However, there remains a possibility that its operation mechanism might be more complicated than ordinary Na\textsuperscript{+}/substrate cotransport as a typical secondary active transport mechanism, since a study using brush border membrane vesicles prepared from the rat small intestine could not demonstrate Na\textsuperscript{+} dependence.\textsuperscript{39} In this study, the transport of 5-fluorouracil was demonstrated to be highly Na\textsuperscript{+}-dependent and efficient, similarly to that of D-glucose, in the everted tissue sacs of the rat small intestine. However, in the brush border membrane vesicles, 5-fluorouracil transport was not observed to be dependent on Na\textsuperscript{+}, whereas D-glucose transport was highly Na\textsuperscript{+}-dependent and efficient. These results apparently indicate that, whereas sodium/glucose cotransporter 1 (SGLT1/Sle5a1) for D-glucose\textsuperscript{45} was in operation in the brush border membrane vesicles, as well as in the tissue sacs, as expected, SNBT1 for 5-fluorouracil was in operation in the tissue sacs, but not in the brush border membrane vesicles. Since the vesicle preparation of the brush border membrane isolated from epithelial cells lacks intracellular components, there may be some additional intracellular components required for the Na\textsuperscript{+}-dependent operation of SNBT1. Although one study has demonstrated Na\textsuperscript{+}-dependent hypoxanthine transport in the brush border membrane vesicles of the calf small intestine,\textsuperscript{5}\textsuperscript{5} the detailed mechanism of the Na\textsuperscript{+}-dependent operation of SNBT1 remains inconclusive. The Na\textsuperscript{+} dependence of 5-fluorouracil transport has also been indicated in the excised tissue of rabbit small intestine,\textsuperscript{43} but its transport has not been examined in brush border membrane vesicles.

With regard to the basolateral efflux for exiting the epithelial cells following brush border uptake (Fig. 1), ENT1 could be in operation, as this transporter, which is primarily for nucleosides, is also capable of transporting both purine and pyrimidine nucleobases,\textsuperscript{13} is expressed in the small intestine, and is localized to the basolateral membrane in epithelial type cells.\textsuperscript{14,16–22} Notably, the operation of ENT1 is evident in the absorption of ribavirin, an antiviral ENT1 substrate drug analogous to nucleosides, in the mouse small intestine; its absorption has been demonstrated to be reduced by knocking out the Ent1 gene.\textsuperscript{44} In addition to ENT1, ENBT1, a purine-specific nucleobase transporter identified more recently, could also potentially be involved in the basolateral efflux of purine nucleobases.\textsuperscript{23–29} Although the ENT1 and ENBT1 of rat have not been evaluated for their nucleobase transport functions, they could be expected to be capable of transporting nucleobases, as demonstrated for their human counterparts. These transporters are facilitative transporters that can operate for both influx and efflux across the cellular membrane, depending on the concentration gradient of the substrate. For example, although ENBT1 has been suggested to operate for nucleobase uptake in cooperation with salvage enzymes, as is its physiologically important function when the concentrations of nucleobases are lower in the cell than the outside,\textsuperscript{29} it could operate for nucleobase efflux when the concentrations are higher in the cell, and downward concentration gradients are formed toward the blood side, as in the intestinal absorption process. The characteristics of these transporters have been reviewed in more detail elsewhere.\textsuperscript{39} In addition to those transporters, the organic anion transporter 1 (OAT1/SLC22A6) and OAT3/SLC22A8 can operate for 6-mercaptopurine, as reported for their rat orthologs.\textsuperscript{45} OAT3 can also operate for 5-fluorouracil, as reported for its mouse ortholog.\textsuperscript{46} OAT2/SLC22A7, of which the human ortholog can operate for 5-fluorouracil,\textsuperscript{47} could be expected to similarly operate for 5-fluorouracil in non-primates and other animals. However, all these OATs, which localize to the basolateral membrane, are not significantly expressed only poorly or at negligible levels in small intestine, as reported in rats,\textsuperscript{48,49} and hence are unlikely to be involved in the absorption of those drugs.

The absorption characteristics of cytosine and adenine, which cannot be transported by SNBT1, are unclear, with little information available regarding rats or any other animals. According to our assessment, in conjunction with the evaluation of SNBT1 function in the rat small intestine (unpublished data), the uptakes of cytosine and adenine in the everted tissue sacs were only about 3 and 15\%, respectively, of uracil uptake, and were not dependent on Na\textsuperscript{+}, in agreement with the suggested absence of SNBT1-mediated transport. However, the uptake of adenine was saturable, suggesting the involvement of a Na\textsuperscript{+}-independent transporter with weak activity. On the other hand, the uptake of cytosine was not saturable, suggesting that no carrier-mediated mechanism was involved. Thus, the rat small intestine seems to lack a mechanism for the efficient absorption of these nucleobases, although an unidentified transporter might be modestly involved in adenine transport. Their supply in the body may rely mainly on endogenous production from relevant sources, rather than intake of the nucleobases themselves from dietary sources.

3. INTESTINAL ABSORPTION OF NUCLEOBASES AND ANALOGS IN HUMANS

The identification of SNBT1 led to the perception that humans lack it and, hence, its derived function of brush border uptake of nucleobases and analogs for their absorption in the small intestine. Coinciding with that, the Caco-2 cell line, a widely used human intestinal epithelial cell model, has been suggested to lack this function as well.\textsuperscript{51} In the Caco-2 cell model, the uptake of uracil is low and not dependent on Na\textsuperscript{+}, indicating the absence of Na\textsuperscript{+}-dependent SNBT1-like transporter activity for uracil, a major SNBT1 substrate. This cell model, however, is suggested to lack the activity of Na\textsuperscript{+}-dependent nucleoside uptake by CNT1 and CNT2, which are present in the human small intestine.\textsuperscript{52} Therefore, the Caco-2 cell line may not be suitable as a model for the evaluation of the transport of nucleobases and related compounds, being
poor in the expression of relevant transporters; and we cannot exclude the possibility that a transporter alternative to SNBT1 might exist in the human small intestine for nucleobase absorption (Fig. 1).

Clinically, the bioavailability of orally administered 5-fluorouracil is known to be low and highly variable among subjects.\(^53\) It has been suggested that this variable is mainly originated from its first-pass metabolism by dihydropyrimidine dehydrogenase (DPD),\(^53–55\) with bioavailability as high as nearly 100% in some subjects.\(^50\) The occurrence of such high bioavailability suggests that 5-fluorouracil may be almost completely absorbed from the small intestine, but its bioavailability is lowered to a varied extent depending on the activity of DPD. This implicates the fairly high intestinal membrane permeability of 5-fluorouracil, for almost complete absorption. Based on a correlation between the fraction absorbed \((F_a)\) after an oral dose and the effective membrane permeability coefficient \((P_{eff})\) in the perfused human intestine, the \(P_{eff}\) required for an \(F_a\) of 85%, above which complete absorption is considered to be practically achieved, is indicated to be \(1.5 \times 10^{-4}\) cm/s.\(^57\) This \(P_{eff}\) is only a fraction of that of \(\alpha\)-glucose (3.9–13.9 \times 10^{-4}\) cm/s at 10 mM),\(^58\) which is highly permeable by transport mediated by SGLT1,\(^59\) but greater than that of atenolol (0.2 \times 10^{-4}\) cm/s at 0.038 mM),\(^58\) the permeation of which has recently been suggested to be mediated by plasma membrane monoamine transporter (PMAT/SLC29A4).\(^60\) Notably, the \(n\)-octanol/water partition coefficient \((P_{ow})\) of 5-fluorouracil (0.11)\(^61\) is smaller than that of atenolol (1.45),\(^62\) indicating that 5-fluorouracil is more hydrophilic than atenolol. Taking into account their relatively close molecular weights of 130 and 266, respectively, 5-fluorouracil would likely be less permeable by simple diffusion than atenolol, based on the large difference in \(P_{ow}\). Therefore, it is possible that a transporter is involved in the absorption of 5-fluorouracil to achieve the suggested \(P_{eff}\) greater than that of atenolol for its almost complete absorption, although it would not necessarily be a highly efficient transporter like SGLT1. This issue remains to be addressed.

With regard to basolateral efflux for exiting the epithelial cells following brush border uptake (Fig. 1), the facilitative transporters ENT1\(^14–22\) and ENBT1\(^23–25\) could be in operation, as mentioned in the preceding section. On the other hand, OAT1, OAT2, and OAT3, which could potentially operate for 5-fluorouracil and/or 6-mercaptopurine, are not likely to be involved in the basolateral efflux of those drugs, as also mentioned above, since these OATs are expressed only poorly or at negligible levels in the human small intestine.\(^63\) However, although ENT1 has been shown to be expressed in the human intestinal epithelial cell model of Caco-2, and in operation for nucleoside transport,\(^52\) ENT1-like nucleobase transporter activity has not been confirmed so far. A modest Na\(^+\)-independent carrier-mediated transport activity for uracil has been suggested to be present at the basolateral membrane,\(^51\) but its high affinity characteristic, with a \(K_m\) of 1.31 \mu M, is different from its low affinity characteristics for nucleobases, as suggested by \(K_m\) values three orders of magnitude higher for several nucleobases.\(^15\) ENBT1 could not be the transporter involved, as ENBT1 has little affinity for uracil. Thus, the basolateral nucleobase transport in this cell model remains inconclusive.

4. IMPLICATION OF THE GENETIC DEFFICIENCY OF SNBT1 IN HUMANS IN THE INTESTINAL HANDLING OF URIC ACID

Uric acid was found to be transported by rat SNBT1 as a purine-derived substrate that has the required oxo group at the 6th position.\(^38\) However, since it is a metabolite produced from purine nucleobases, and needs to be eliminated, there seems to be no good reason to have an efficient carrier-mediated mechanism for its intestinal absorption. Indeed, it has been indicated that uric acid absorption cannot be observed in the rat small intestine.\(^64\) Rather, the small intestine has been suggested to play an important role in uric acid excretion,\(^65–67\) in which secretory transporters, for example, breast cancer resistance protein (BCRP/ABCG2) as the major one, are involved to facilitate this excretion.\(^68\) Although non-primate experimental animals, such as rats, have uricase for the metabolic elimination of uric acid, uric acid metabolism has been suggested to be insignificant in the small intestine, since this organ has poor uricase activity, uricase expression being much lower than that in the liver.\(^69\) Therefore, one possibility is that uric acid undergoes a futile cycle, in which uric acid secreted into the intestinal lumen by secretory transporters is taken up by SNBT1, only to be immediately secreted back again (Fig. 1). Thus, uric acid seems to be recognized by SNBT1 as a substrate, inevitably because of its structural characteristics, but the suggested futile cycle may occur as a consequence, only to retard its excretion by retaining it in the cycle without any physiological significance. Therefore, shutting down this futile cycle by eliminating SNBT1 would make sense for the purpose of excreting uric acid more efficiently, perhaps in combination with an evolutionary adaptation to the reduced supply of dietary nucleobases. This may be a reason for the elimination of the SNBT1 gene in humans and higher primates.

The elimination of SNBT1 in humans and higher primates could also help to avoid the unwanted perturbation of uric acid concentration in blood. It is known that humans and higher primates are less capable than other animals of eliminating uric acid, as they lack uricase, which prompts the metabolic elimination of uric acid by converting it to allantoin; accordingly, uric acid concentration in blood is maintained at a higher level in the former (humans and higher primates) than in the latter.\(^71\) This may be an evolutionary adaptation in humans and higher primates to take advantage of the beneficial effects of uric acid, such as its antioxidant effect. However, because of the low capacity of an elimination pathway, uric acid concentration in blood, which is homeostatically regulated by the balance between production and elimination, could be easily disturbed if there is a massive and transient input of uric acid. This could be caused by uric acid production in the small intestine when nucleobases are supplied dietarily and taken up rapidly into the epithelial cells by a highly efficient transporter like SNBT1, since the epithelial cells are rich with enzymes for the conversion of nucleobases to uric acid.\(^69\) Uric acid produced in this way could be excreted into the intestinal lumen by secretory transporters, but in part supplied to the blood as well, via basolateral uric acid transporters such as the recently identified glucose transporter 9 (GLUT9/SLC2A9).\(^72\) Although this could be an additional source of uric acid supply, it may be more harmful than beneficial. Because of
this, suppressing nucleobase supply by eliminating SNBT1 may have been favored in evolutionary development. Thus, the elimination of SNBT1 may have coincided with that of uricase. The function of nucleobase metabolism in the small intestine may be, in part, to eliminate nucleobases to manage their oversupply. Under this hypothesis, a reduction in the supply of nucleobases by the elimination of SNBT1 may not have been a critical problem for humans physiologically, although a different, as yet unknown, adaptation might have been associated.

Regardless, the intestinal handling of uric acid in humans and higher primates is presumed to be different from that in the other animals, due to the genetic elimination of SNBT1 in the former in the evolutionary process, although information about this is scarce, and the evolutionary and biological implications underlying this species difference remain unclear. It may be linked to differences in the systemic disposition of uric acid, in which the genetic elimination of uricase in humans and higher primates is a major factor.

5. CONCLUSION

Knowledge of the intestinal absorption of nucleobases and analogs has accumulated mainly from studies using rats and some other non-primate experimental animals. Particularly, the Na\(^+\)-dependent carrier-mediated nucleobase transport system has been of major interest for its role as the pathway for those compounds to enter the epithelial cells at the brush border membrane in the small intestine. However, while SNBT1, as its molecular entity, is present in non-primate experimental animals, it is genetically deficient in humans and higher primates. Accordingly, SNBT1-like transporter activity is absent in the human intestinal epithelial cell model of Caco-2, although there is a possibility that a transporter alternative to SNBT1 might be present in the human small intestine, as the fairly high absorption of 5-fluorouracil has been suggested clinically. This remains an issue for future study. It is also notable that SNBT1 operates specifically for uracil/guanine type nucleobases and analogs. The characteristics and mechanisms of the brush border uptake of cytosine/adename type nucleobases remain unclear in non-primate experimental animals, as well as in humans.

With regard to the basolateral efflux for exiting epithelial cells following brush border uptake, the transport of nucleobases and analogs is likely to be mediated by ENT1, which is a facilitative transporter for purine and pyrimidine nucleobases, and by ENBT1, which is a facilitative transporter specific for purine nucleobases. These transporters have been suggested to exist in many animal species, including human and non-primate experimental animals, without any major species differences in their functions and expression characteristics.

Uric acid is also a substrate of SNBT1 as a purine derivative. The elimination of the SNBT1 gene in humans and higher primates may have occurred in relation to an adaptive alteration in the handling of uric acid in the evolutionary process. Since uric acid and nucleobases are closely related, chemically and biologically, they could also be mutually related in some other disposition processes and species differences. We need to be aware of such possibilities in addressing the issue of the intestinal absorption of nucleobases and analogs in order to understand the mechanism, and to gain information for the more rational use and development of drugs analogous to nucleobases.

Conflict of Interest The authors declare no conflict of interest.

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