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Involvement of rBAT in Na^+-dependent and -independent transport of the neurotransmitter candidate L-DOPA in *Xenopus laevis* oocytes injected with rabbit small intestinal epithelium poly A^+ RNA

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Abstract

Although L-3,4-dihydroxyphenylalanine (L-DOPA) is claimed to be a neurotransmitter in the central nervous system (CNS), receptor or transporter molecules for L-DOPA have not been determined. In an attempt to identify a transporter for L-DOPA, we examined whether or not an active and high affinity L-DOPA transport system is expressed in *Xenopus laevis* oocytes injected with poly A^+ RNA prepared from several tissues. Among the poly A^+ RNAs tested, rabbit intestinal epithelium poly A^+ RNA gave the highest transport activity for L-[^{14}C]DOPA in the oocytes. The uptake was approximately five times higher than that of water-injected oocytes, and was partially Na^+-dependent. L-Tyrosine, L-phenylalanine, L-leucine and L-lysine inhibited this transport activity, whereas D-DOPA, dopamine, glutamate and L-DOPA cyclohexylester, an L-DOPA antagonist did not affect this transport. Coinjection of an antisense cRNA, as well as oligonucleotide complementary to rabbit rBAT (NBAT) cDNA almost completely inhibited the uptake of L-[^{14}C]DOPA in the oocytes. On the other hand, an antisense cRNA of rabbit 4F2hc barely affected this L-[^{14}C]DOPA uptake activity. rBAT was thus responsible for the L-[^{14}C]DOPA uptake activity expressed in *X. laevis* oocytes injected with poly A^+ RNA from rabbit intestinal epithelium. As rBAT is localized at the target regions of L-DOPA in the CNS, rBAT might be one of the components involved in L-DOPAergic neurotransmission.

Keywords: Levodopa; Neurotransmitter; 4F2hc; NBAT; Amino acid transporter; Parkinson's disease

1. Introduction

L-3,4-Dihydroxyphenylalanine (L-DOPA) has been believed to be only a precursor amino acid, that exerts its actions and effects in Parkinson's disease via its conversion to dopamine by aromatic L-amino acid decarboxylase (AADC). We previously proposed that L-DOPA itself is a neurotransmitter and/or modulator in the central nervous system (CNS) [1,2]. This 'L-DOPA transmitter hypothesis' is based on the following findings: (1) Immunohistochemical findings suggest the existence of tyrosine hydroxylase- and L-DOPA-positive, but AADC- and dopa-

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mine-negative neurons, which may contain L-DOPA as an end product in brain areas such as the hypothalamic nuclei and the nucleus tractus solitarii (NTS) [2]. (2) L-DOPA is released in a transmitter-like manner from striata and some nuclei in the lower brainstem. [2]. (3) L-DOPA produces pre- and postsynaptic responses, most of which are antagonized by L-DOPA antagonists such as L-DOPA methyl ester [3,4] and L-DOPA cyclohexylester (DOPA CHE) [5]. For example, microinjection of L-DOPA into the NTS and caudal ventrolateral medulla (CVLM) or rostral ventrolateral medulla (RVLM) of the lower brainstem, elicits blood-depressor or pressor responses [2]. (4) L-DOPAergic agonists and antagonists do not interact with catecholaminergic [3] and ionotropic glutamate receptors [6] in radiolabelled-ligand binding assays.

Although above findings provide criteria for L-DOPA being a neurotransmitter and/or neuromodulator, specific receptor(s) and transporter(s) for L-DOPA have not been identified [1,2]. L-DOPA has been characterized as a neutral and large amino acid. At present, candidate transport systems for L-DOPA may include system B, system $B^{0,+}$, system $b^{0,+}$, system L and system y^+L. Regarding neutral amino acid transport, system $B^{0,+}$, B and y^+L are Na^+-dependent, while system $b^{0,+}$ and L are Na^+-independent [7]. Recently, $hATB^{0+}$ which is responsible for system $B^{0,+}$ amino acid transport has been cloned due to the homology with the Na^+/Cl^--dependent transporter family of neurotransmitters [8]. System $b^{0,+}$, system L and system y^+L require unique molecules, related to $b^{0,+}$ amino acid transporter (rBAT) (NBAT/D2) [9–11] or 4F2 heavy chain (4F2hc) (CD98) [12,13], which are now clarified to be transporter-associated proteins, but not transporters themselves. 4F2hc forms heterodimers with transporter units of system L [14–19] and system y^+L [15,20,21]. On the other hand, rBAT cRNA-injected oocytes show system $b^{0,+}$ activity, which allows Na^+-independent transport of cystine and neutral and basic amino acids [22]. Most recently the rBAT-associated transporters, $b^{0,+}AT$ and BAT1 have been cloned [23,24]. In contrast to the ubiquitous expression of 4F2hc [25], rBAT is expressed in a definite subset of neuronal and/or non-neuronal cells in peripheral and central nervous tissues [26–29].

Neurotransmitter-transporters in plasma membrane rapidly scavenge and reuptake substrates from the synaptic cleft with high affinity, thus terminating signal transmission. One of the most important aspects of these transporters is that they actively uptake neurotransmitters in a Na^+-dependent manner, by utilizing the transmembrane Na^+ gradient generated by Na^+/K^+-ATPase. Until now, systems for Na^+-dependent L-DOPA transport have not been well characterized. In the CNS, transports of L-DOPA in slices of rat cerebral cortex [30] and cultured rat or mouse astrocytes [31] have shown to be Na^+-independent, and show transport property of system L. We previously demonstrated Na^+-dependent L-DOPA uptake in slices of rat hypothalamus [3]. In an attempt to identify the transporter for L-DOPA, we investigated whether or not an active and high affinity L-DOPA transport system is functionally expressed in *Xenopus* oocytes by using poly A^+ RNA from various animal tissues, and further characterized the transport activity of L-DOPA in detail.

2. Materials and methods

2.1. Materials

Adult male Wistar rats and adult male New Zealand White rabbits were obtained from SLC (Shizuoka, Japan). ISOGEN, an acid guanidinium-phenol-chloroform reagent, was obtained from Nippongene (Tokyo, Japan). Oligo(dT)-cellulose type 7 was from Pharmacia (Uppsala, Sweden). Female *Xenopus laevis* was obtained from Hamamatsu Jikken Doubutsu (Shizuoka, Japan). Collagenase type I and gentamicin were from Sigma (St. Louis, MO, USA). A microdispensor was from Drumond (Broomall, PA, USA). RNA polymerases, RNase inhibitor and RNA cap analogue were from Stratagene (La Jolla, CA, USA), Promega (Madison, WI, USA) and Pharmacia (Uppsala, Sweden), respectively. L-[^{14}C]DOPA (10.0–11.4 mCi/mmol) was obtained from Amersham (Buckinghamshire, UK). To prevent possible oxidation of L-[^{14}C]DOPA, ascorbic acid was added to the solution at a final concentration of 0.28 mM. The stock solution was stored at −20°C. L-DOPA and D-DOPA were purchased from Nacalai tesque (Kyo-

to, Japan). L-DOPA CHE [5] was kindly supplied from Kyowa Hakkou Kogyo (Shizuoka, Japan).

2.2. RNA preparation and microinjection into oocytes

Tissues and organs of adult male Wistar rats and New Zealand White rabbits were dissected, and the rabbit intestinal epithelium was torn off using a slide glass. Total RNA was extracted from these tissues using ISOGEN. poly A^+ RNA was isolated with oligo(dT)-cellulose. *X. laevis* oocytes were treated with collagenase for 30–60 min at room temperature in 5 mM HEPES-buffered OR2 solution (pH 7.6) containing 82.5 mM NaCl, 2 mM KCl, and 1 mM $MgCl_2$. After additional manual defolliculation, the oocytes were maintained overnight at 18°C in 10 mM HEPES-buffered Barth's/gentamicin solution (pH 7.4) containing 88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, and 50 μg/ml gentamicin. Healthy stage V, VI oocytes were selected and injected with 50 nl of poly A^+ RNA (1.0 μg/μl) or water using a microdispensor. Following incubation at 18°C for 3 days, transport activity was measured.

2.3. L-DOPA uptake assay

The 4–10 injected oocytes were preincubated at room temperature for 10 min in 24-well plates filled with 3 ml of NaCl uptake solution (pH 7.5) containing 100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Tris, and 5 mM HEPES–KOH. The buffer was then replaced by 0.5 ml of uptake solution containing 30–50 μM L-[^{14}C]DOPA, and the oocytes were incubated at room temperature for 1 h. In Na^+-free uptake solution, choline chloride was substituted for NaCl. Cl^--free uptake solution (pH 7.5) contained 100 mM sodium gluconate, 2 mM potassium gluconate, 1 mM $MgSO_4$, 1 mM calcium gluconate, and 5 mM HEPES–KOH. Inhibition studies were performed by adding the L-DOPA-related compounds and various amino acids to the uptake solution at a concentration of 1 mM. Following incubation, the oocytes were washed five times with ice-cold Na^+-free solution. Each oocyte was then dissolved in 10% sodium dodecyl sulfate, and the radioactivity was measured using a liquid scintillation counter.

2.4. PCR amplification and cRNA synthesis

To obtain rabbit rBAT cRNA, rabbit intestinal poly A^+ RNA was reverse transcribed by Superscript Preamplification System (GIBCO BRL, Ont., Canada). Using this product as a template, PCR amplification was carried out with a forward primer containing an *Eco*RI site and the sequence upstream of the initiation codon of rabbit rBAT (5′-CAGAATTCGAAGACAGGAGGCTCTAGCAGAT-3′) and a reverse primer containing an *Xho*I site and the sequence downstream of the termination codon (5′-TGCTCGAGGTGCCGCGTCTCCGTCCTC-3′) [11]. After the PCR product was subcloned into pBluescript SK^-, the resulting construct was linearized with *Xho*I and the cDNA insert was transcribed using T3 RNA polymerase, in the presence of RNase inhibitor and RNA cap analogue as described previously [6]. The rabbit 4F2hc fragment was obtained by nested PCR using primers with reference to the rat 4F2hc sequence [32]. After first strand DNA was amplified by the 3′RACE System (GIBCO BRL) with the forward primer (5′-CGGAATTCCTGCTCTTCTGGCTCGGTTGGC-3′), this product was amplified again with the forward primer (5′-GGAATTCGTGGTTATCATCGTTCGGGCG-3′) and the reverse primer (5′-CCACTCGAGAACGCTCATTCTGGTCCCAGTG-3′). The rabbit 4F2hc RT-PCR product of 941 base pairs had 70% identity with the rat 4F2hc sequence. Capping antisense cRNAs of rabbit rBAT and 4F2hc were synthesized with T7 RNA polymerase by use of linearized rBAT/pBluescript SK^- lacking the 5′-region including the initiation codon, and linearized 4F2hc/pBluescript SK^- as a template, respectively.

2.5. Hybrid depletion

Rabbit intestinal poly A^+ RNA (1.0 μg/μl) with or without each antisense cRNA (0.1 μg/μl) was denatured at 65°C for 5 min and left at room temperature for 5 min before injection. Hybrid depletion by oligonucleotide was performed as described [33]. In brief, rabbit intestinal poly A^+ RNA (1.0 μg/μl) or rabbit rBAT cRNA (0.01 μg/μl) was denatured at 65°C for 5 min in a 50 mM NaCl solution containing 20 μM of a 20-mer sense (5′-TGCCCAAGGAGGTGCTGTTC-3′, corresponding to nucleotides 203–

222 downstream from the initiation codon of the rabbit rBAT mRNA sequence), or a 21-mer antisense oligonucleotide (5′-GAACAGCACCTCCTTGGG-CAT-3′, complementary to the nucleotide sequence position 202–222). These oligonucleotides were designed from conserved sequences among rabbit, human and rat. For annealing, the solution was further incubated at 42°C for 30 min prior to injection into oocytes.

2.6. Statistical analysis

Statistical analyses were performed by one-way ANOVA with the Tukey–Kramer multiple comparison test or Dunn's multiple comparison test. The statistical difference was accepted for $P < 0.05$.

3. Results

3.1. L-DOPA uptake activity of various tissues in Xenopus oocyte expression system

We comparatively assayed L-[^{14}C]DOPA uptake activity in *Xenopus* oocytes injected with poly A^+ RNA from several regions of the brain, the rat adrenal gland, and from the rabbit small intestinal epithelium (Table 1). Oocytes injected with each poly A^+ RNA of the brain regions tested showed a significant but very small increase in L-DOPA uptake activity, being approximately 40% higher than those injected with water. poly A^+ RNA from rabbit small intestinal epithelium gave the highest L-DOPA uptake among these RNAs. This uptake activity was five-fold higher than that of water-injected oocytes. In the following study, we characterized the L-DOPA transport activity expressed in *Xenopus* oocytes injected with poly A^+ RNA from rabbit small intestinal epithelium.

Table 1
L-DOPA uptake activity in *Xenopus* oocytes injected with water or poly A^+ RNA from various tissues

Injected poly A^+ RNA	L-DOPA uptake (pmol/h/oocyte)
Water	7.2 ± 0.2
Small intestinal epithelium	30.0 ± 2.1***
Cerebellum	9.9 ± 0.4*
Hypothalamus	10.0 ± 0.5*
Cerebral cortex	10.3 ± 0.4**
Brainstem	10.4 ± 0.3**
Adrenal gland	8.5 ± 0.2

poly A^+ RNAs were obtained from rat tissues except for rabbit small intestinal epithelium. Three days after injection with poly A^+ RNA or water, uptake activities were measured in NaCl uptake solution containing 50 μM L-[^{14}C]DOPA. Each value represents the mean ± S.E.M. ($n = 10$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, versus water-injected control.

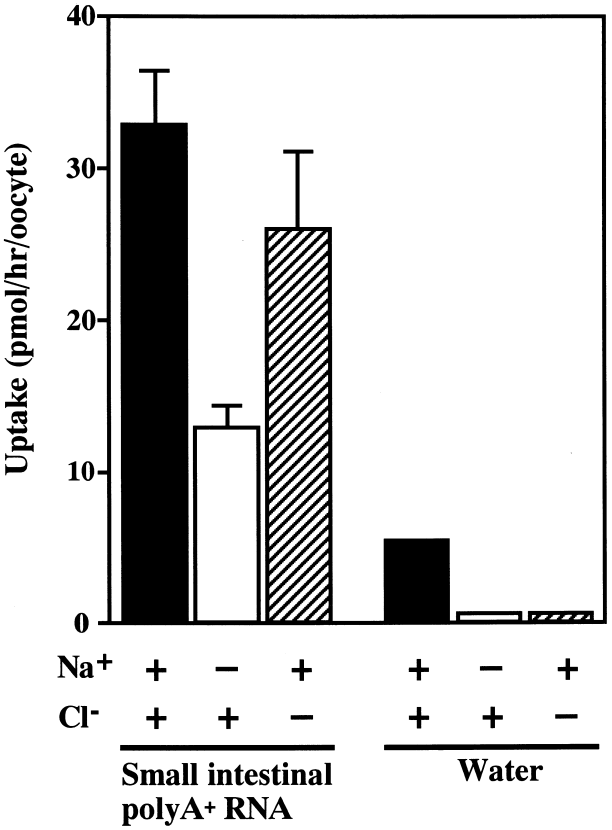

Fig. 1. Ion dependence of L-DOPA uptake into oocytes injected with rabbit small intestinal epithelium poly A^+ RNA or water. Oocytes were incubated for 1 h with NaCl (filled bars), Na^+-free (open bars) and Cl^--free uptake solution (hatched bars) containing 41.5 μM L-[^{14}C]DOPA. Data are mean ± S.E.M. ($n = 5$–7).

3.2. Ion dependence and kinetics of L-DOPA uptake in oocytes injected with poly A^+ RNA from rabbit small intestinal epithelium

L-[^{14}C]DOPA uptake in oocytes injected with poly A^+ RNA from rabbit small intestinal epithelium or with water was measured in NaCl containing, in

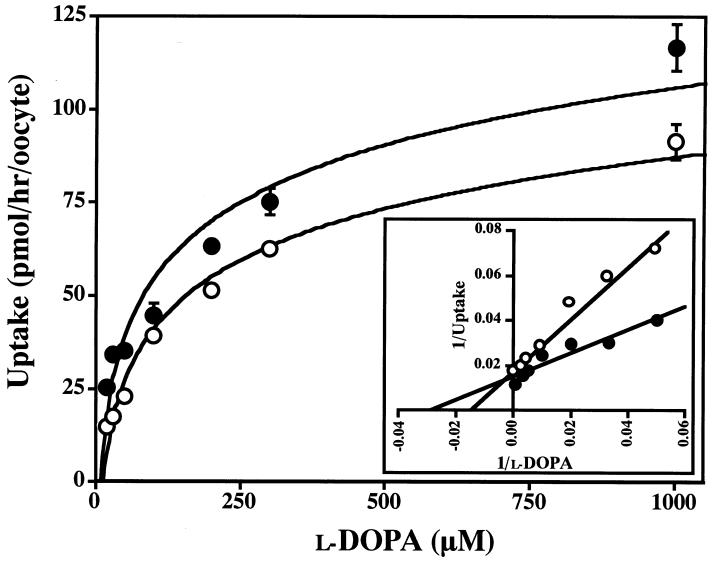

Fig. 2. Saturable uptake of L-DOPA into oocytes injected with rabbit intestinal epithelium poly A^+ RNA. Oocytes were incubated with various concentrations of L-[^{14}C]DOPA in the absence (open circles) or presence (closed circles) of Na^+. Inset shows Lineweaver–Burk plots for the experiments. Two independent experiments revealed that apparent K_m was 38.4 ± 3.5 μM or 60.4 ± 8.7 μM, and V_{max} was 73.6 ± 6.5 pmol/h/oocyte or 55.3 ± 3.5 pmol/h/oocyte, in the presence or absence of Na^+, respectively. Each point shows mean ± S.E.M. (n = 5–7).

Na^+-free or in Cl^--free uptake solution (Fig. 1). L-DOPA uptake activity of water-injected oocytes under both Na^+-free and Cl^--free conditions was suppressed to 10–15% of the activity observed with NaCl uptake solution. This suggests that the endogenous L-DOPA transport in oocytes is Na^+- and Cl^--dependent. The uptake of L-DOPA induced by rabbit small intestinal epithelium poly A^+ RNA in Na^+-free medium was approximately 40% of that in NaCl containing medium. This indicates that the L-DOPA transport activity is composed of Na^+-dependent and -independent fractions. On the other hand, the L-DOPA uptake in Cl^--free medium was only slightly attenuated by 20%, compared to that in NaCl containing medium. This decrease is probably due to the Cl^--dependency of endogenous L-DOPA transport in native oocytes. It is thus likely that poly A^+ RNA-induced L-DOPA transport activity is Cl^--independent.

L-DOPA uptake activities of RNA-injected oocytes were measured in NaCl and Na^+-free uptake solution containing various concentrations of L-[^{14}C]DOPA (Fig. 2). In both NaCl and Na^+-free uptake solution, RNA-injected oocytes exhibited saturable L-DOPA transports. Lineweaver–Burk analysis revealed that the K_m was 38.4 ± 3.5 μM for NaCl uptake solution and 60.4 ± 8.7 μM for Na^+-free uptake solution, respectively (mean ± S.D.).

3.3. Inhibition study of L-DOPA uptake

To clarify the substrate specificity of the L-DOPA transport, we examined the effects of various compounds on L-DOPA uptake in the rabbit intestinal RNA-injected oocytes (Figs. 3 and 4). In the presence of an excess amount of unlabeled L-DOPA, L-[^{14}C]DOPA uptake was almost completely abolished (Fig. 3). This transport system was stereoselective, because D-DOPA did not significantly affect the uptake of L-[^{14}C]DOPA. Dopamine did not inhibit the L-DOPA transport, indicating that dopamine converted from L-DOPA was not a substrate for the L-DOPA transport system involved. L-DOPA CHE, a competitive L-DOPA antagonist [5], barely affected the L-DOPA uptake. This finding further confirms our previous finding that the recognition site for the competition between L-DOPA and L-DOPA antagonists such as L-DOPA CHE and L-DOPA methyl ester, differs from the site of transport of L-DOPA across the plasma membrane [3]. L-Tyrosine, L-phenylalanine, L-leucine and L-lysine at 1 mM almost completely inhibited the L-DOPA

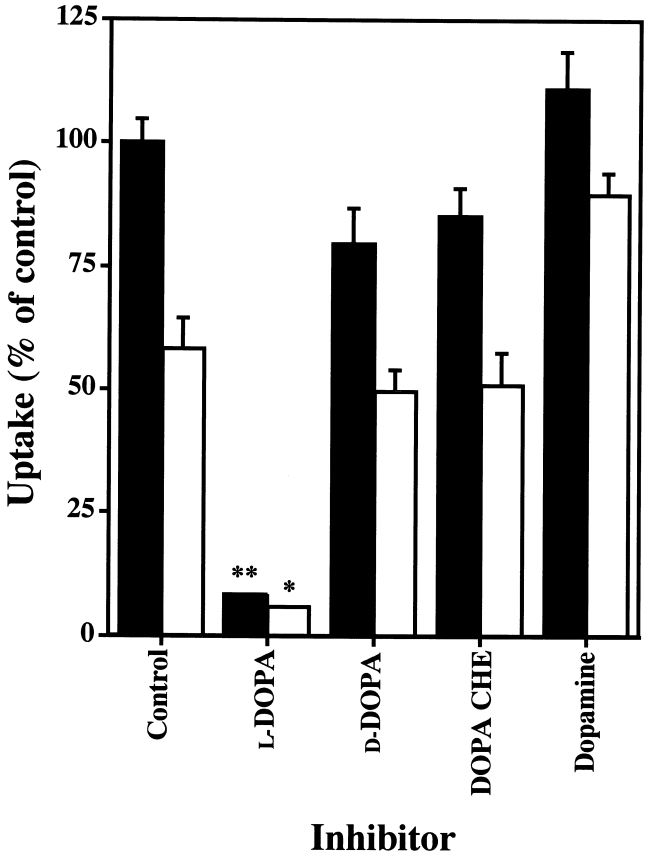

Fig. 3. Inhibition study of L-DOPA uptake by L-DOPA-related compounds. L-[^{14}C]DOPA (30 μM) uptake into oocytes injected with rabbit intestinal epithelium poly A^+ RNA was performed with 1 mM non-radioactive compounds in the presence (filled bars) or absence (open bars) of Na^+. L-DOPA uptake was expressed as a percentage of the control uptake without inhibitor in the presence of Na^+. Data are mean ± S.E.M. ($n = 6$–10). *$P < 0.05$, **$P < 0.01$, versus corresponding control uptake in the presence or absence of Na^+, respectively.

uptake with similar potencies, while L-alanine and L-tryptophan showed only partial inhibition of the uptake (Fig. 4). L-Proline and L-glutamate had no effect on the L-DOPA uptake. Methylamino isobutyric acid (MeAIB), a system A substrate [7], did not affect the L-DOPA uptake. 2-Amino-2-norbornanecarboxylic acid (BCH), a substrate for system L and $B^{0,+}$ [22] slightly inhibited the L-DOPA uptake by 20% (Fig. 4). These results indicate that the uptake of L-DOPA in oocytes injected with poly A^+ RNA from rabbit intestinal epithelium showed properties of neutral and basic amino acid transporter(s). The amino acid transporter systems that are fit for these characteristics include system $b^{0,+}$ and system y^+L.

3.4. Hybrid depletion of rabbit intestinal poly A^+ RNA-induced L-DOPA uptake

rBAT or 4F2hc, when solely expressed in the *X. laevis* oocyte system, is known to induce the transport of neutral and basic amino acids via system $b^{0,+}$ or system y^+L, respectively [22]. We therefore examined whether or not these molecules are involved in the L-DOPA uptake activity in oocytes after injection of rabbit intestinal poly A^+ RNA. Hybrid depletion of the poly A^+ RNA with an antisense cRNA complementary to rabbit rBAT or 4F2hc was carried out

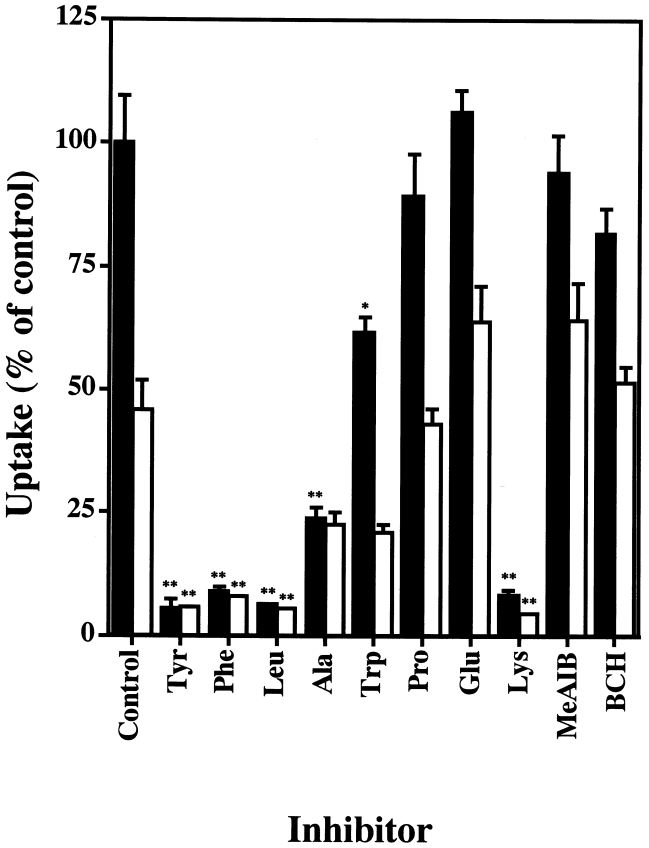

Fig. 4. Inhibition study of L-DOPA uptake by amino acids. L-[^{14}C]DOPA (30 μM) uptake into oocytes injected with intestinal epithelium poly A^+ RNA was performed with 1 mM non-radioactive compounds in the presence (filled bars) or absence (open bars) of Na^+. L-DOPA uptake was expressed as in Fig. 3. Data are mean ± S.E.M. ($n = 4$–10). *$P < 0.01$, **$P < 0.001$, versus corresponding control uptake in the presence or absence of Na^+, respectively.

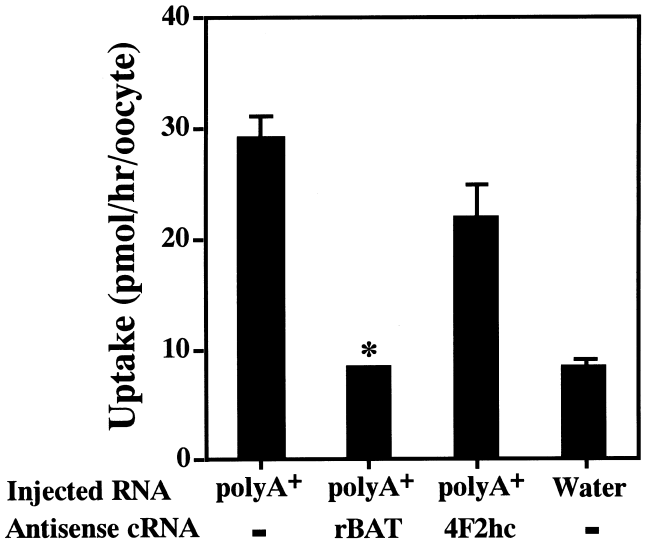

Fig. 5. Hybrid depletion of L-DOPA uptake by antisense cRNA of rabbit rBAT or 4F2hc. Oocytes were injected with rabbit intestinal poly A^+ RNA (poly A^+) or water, with or without an antisense cRNA (rBAT, 4F2hc). Three days after injection, uptake of L-[^{14}C]DOPA (50 μM) was determined in the presence of Na^+. Data are mean ± S.E.M. ($n = 7$–10). *$P < 0.001$, versus poly A^+ RNA alone.

(Fig. 5). The antisense cRNA of rBAT decreased the poly A^+ RNA-induced L-DOPA uptake to the endogenous L-DOPA uptake level, while that of 4F2hc did not have a significant effect. We also observed that an antisense oligonucleotide of rBAT suppressed L-DOPA uptake activities induced by the injection of rabbit intestinal poly A^+ RNA, but the sense oligonucleotide had no effect (Fig. 6). We confirmed that rBAT-expressing oocytes exhibited a 14-fold higher L-DOPA uptake activity in comparison with water-injected oocytes, and that the antisense but not sense oligonucleotide of rBAT completely abolished L-DOPA uptake induced by rBAT cRNA-injection (Fig. 6). These findings indicate that rBAT rather than 4F2hc is an essential component for L-DOPA transport activity observed in oocytes injected with poly A^+ RNA from rabbit small intestinal epithelium.

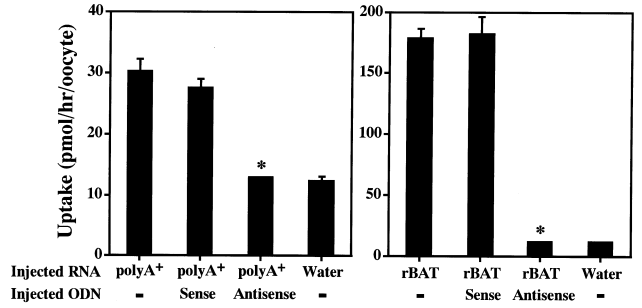

Fig. 6. Hybrid depletion of L-DOPA uptake by a sense or an antisense oligonucleotide (ODN) of the rabbit rBAT cDNA sequence in oocytes injected with poly A^+ RNA (poly A^+, left) or rBAT cRNA (rBAT, right). Three days after injection, uptake of L-[^{14}C]DOPA (50 μM) was determined in the presence of Na^+. Data are mean ± S.E.M. ($n = 10$). *$P < 0.001$, versus corresponding RNA alone.

4. Discussion

In this study, we demonstrated for the first time the Na^+-dependent and -independent L-DOPA uptake in *X. laevis* oocytes injected with poly A^+ RNA from rabbit intestinal epithelium. Furthermore, rBAT was essentially involved in both the Na^+-dependent and -independent L-DOPA uptake, because hybrid depletion of rBAT almost completely inhibited the uptake activities expressed in the oocytes.

We examined the L-DOPA uptake activities expressed by injection of various poly A^+ RNA from the CNS. Microinjection of rat hypothalamus, cerebral cortex, cerebellum and brainstem poly A^+ RNA into oocytes significantly increased the uptake of L-DOPA by approximately 40% of that due to the endogenous activity. However, these levels of increase in L-DOPA uptake were quite low, and hence we were unable to characterize the uptake properties in detail. We then tested rabbit small intestinal epithelium, since amino acid uptake activity is known to be relatively high in the intestinal epithelium. As expected, oocytes injected with rabbit intestinal epithelium poly A^+ RNA showed the highest L-DOPA uptake activity of approximately five-fold higher than that of water-injected oocytes, among the tissue poly A^+ RNAs tested. The L-DOPA transport in the oocytes showed saturable uptake with a high affinity. The apparent K_m value was comparable to that of the transporters for the neurotransmitter glutamate [34]. These data suggest that uptake carrier molecule(s), but not diffusion across the membrane, were involved in the L-DOPA transport. The uptake experiments in the presence or absence of Na^+ re-

vealed that the L-DOPA transport was composed of Na^+-dependent and Na^+-independent fractions. In the absence of Cl^-, the uptake showed a slight decrease. This is probably due to a decrease in endogenous L-DOPA transport, as we confirmed that L-DOPA transport in native *Xenopus* oocytes depended upon both Na^+ and Cl^- [7]. The property of L-DOPA transport expressed by rabbit intestinal poly A^+ RNA was thus different from that of endogenous *Xenopus* oocyte L-DOPA transport, and different from that of Na^+/Cl^--dependent amine transporters including the dopamine transporter in plasma membrane. This again suggests that the L-DOPA transport activity represents uptake of L-DOPA itself, but not that of dopamine converted from L-DOPA. Consistently, an excess amount of dopamine did not inhibit this L-DOPA uptake.

The amino acid transport systems which may be responsible for L-DOPA transport include system B, system $B^{0,+}$, system $b^{0,+}$, system y^+L and system L. The uptake of L-DOPA was inhibited by neutral amino acids such as L-tyrosine, L-phenylalanine and L-leucine. The uptake was also inhibited by the basic amino acid L-lysine, but was not attenuated by the acidic amino acid glutamate. These data suggest that the uptake of L-DOPA is mediated via basic as well as neutral amino acid transporter(s). This property appears to be different from that of system B which catalyzes Na^+-dependent uptake only for neutral amino acids [7]. In the present study, BCH, a substrate for system L and system $B^{0,+}$ [22], slightly inhibited the Na^+-dependent L-DOPA uptake with intestinal poly A^+ RNA by 20%. This inhibition seems to be attributed to the inhibitory effect of BCH on system $B^{0,+}$ amino acid transport activity in native *Xenopus* oocytes [7]. In accordance with this, the L-DOPA uptake in water-injected oocytes showed both Na^+- and Cl^--dependency. These findings exclude the possibility that system L or system $B^{0,+}$ was responsible for the intestinal L-DOPA uptake expressed.

In the hybrid depletion study, an antisense cRNA and antisense oligonucleotide of rBAT almost completely suppressed the L-DOPA uptake activities to the levels of the uptake in water-injected oocytes. An antisense cRNA of 4F2hc, however, had little, if any, effect on the L-DOPA transport. These findings indicate that rBAT, rather than 4F2hc, was involved in the L-DOPA uptake in oocytes injected with rabbit intestinal poly A^+ RNA. In addition, we confirmed that both rat 4F2hc/y^+LAT1- and human 4F2hc/y^+LAT2-expressing oocytes barely transported L-DOPA (unpublished observation). It is therefore unlikely that system y^+L was responsible for the L-DOPA transport in this experiment. An essential role of rBAT in the L-DOPA uptake suggests that system $b^{0,+}$ was responsible for the L-DOPA transport. However, $b^{0,+}$ amino acid transport has been characterized as the system for Na^+-independent neutral and basic amino acid transport, and this property appears to be different from that of L-DOPA uptake in the oocytes injected with rabbit poly A^+ RNA. In this regard, it is noteworthy that in rBAT-expressing *Xenopus* oocytes, a partially Na^+-dependent transport activity for L-histidine is observed [35]. It is thus possible that rBAT and rBAT-associated transporter(s) may also be involved in the Na^+-dependent transport of amino acids such as L-DOPA. Recently, the first rBAT-associated transporter molecules, termed $b^{0,+}$AT and BAT1, have been identified [23,24]. Heterodimerization of rBAT and BAT1 results in expression of system $b^{0,+}$ activity. Further studies are required to investigate whether or not rBAT-associated transporters other than $b^{0,+}$AT and BAT1 are involved in the L-DOPA transport in oocytes injected with rabbit intestinal poly A^+ RNA. Do rBAT and rBAT-associated transporters play a role in L-DOPAergic systems? Interestingly, it appears that rBAT expression [26–28] is localized at the target regions of L-DOPA in the CNS such as the hypothalamus and the lower brainstem [2–4].

The highest level of L-DOPA uptake being found in the small intestinal epithelium may suggest a large capacity of this tissue for L-DOPA uptake. A recent clinical study provides evidence that intestinal absorption of L-DOPA may be an important factor for therapeutic efficacy and the untoward actions of L-DOPA in Parkinson's disease [36]. In fact, repeated administration of L-DOPA in rats or patients with Parkinson's disease significantly increases the peak plasma concentration of L-DOPA, which reflect acceleration of L-DOPA absorption [36]. As the 'wearing-off' phenomenon is a motor fluctuation that is closely related to the timing of L-DOPA dose intake, the peripheral pharmacokinetics of L-DOPA has

been regarded as an important contributory factor. Clarifying whether or not the rBAT-involved L-DOPA transport system in the intestinal epithelium can be up-regulated with repeated L-DOPA administration may provide an insight into molecular mechanisms of the untoward actions encountered with long term L-DOPA therapy in Parkinson's disease.

In conclusion, using *Xenopus* oocytes as an expression system, we have demonstrated that rBAT is responsible for the high affinity Na^+-dependent and -independent L-DOPA uptake shared with basic and neutral amino acids in rabbit small intestinal epithelium. To identify rBAT-associated transporter(s) involved in this L-DOPA uptake is a subject for ongoing study.

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