Carrier-Mediated Transport of H_1-Antagonist at the Blood-Brain Barrier: A Common Transport System of H_1-Antagonists and Lipophilic Basic Drugs

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The blood-brain barrier (BBB) transport system for H_1-antagonists was studied using primary cultured bovine brain capillary endothelial cells (BCEC). The uptake of [^{3}H]mepyramine was inhibited by various H_1-antagonists. Ketotifen competitively inhibited [^{3}H]mepyramine uptake with an inhibition constant (K_i) of 46.8 μM. Lipophilic basic drugs such as propranolol, lidocaine and imipramine significantly inhibited [^{3}H]mepyramine uptake. In particular, propranolol inhibited [^{3}H]mepyramine uptake competitively at an inhibition constant (K_i) of 51.1 μM. Moreover, in ATP-depleted BCEC, [^{3}H]mepyramine uptake was stimulated by preloading with H_1-antagonists and lipophilic basic drugs. These results indicated that H_1-antagonists are transported across the BBB via a carrier-mediated transport system common to lipophilic basic drugs.

KEY WORDS: H_1-antagonist; blood-brain barrier (BBB); propranolol; lipophilic basic drugs; common transport system.

INTRODUCTION

Classical H_1-antagonists, which are used to treat allergic disorders, affect the central nervous system, causing sedation. We attempted to adopt zwitterionization to classical H_1-antagonists to obtain antiallergic drugs free from sedative effects (1). We studied the transport mechanism of H_1-antagonists into the brain.

We found that mepyramine as a model H_1-antagonist was transported into the brain via a carrier-mediated transport system *in vivo* and *in vitro* (2,3).

In this study, we examined whether some basic drugs pass through the BBB via a carrier system common to that of mepyramine, by using primary cultured bovine brain capillary endothelial cells (BCEC).

MATERIALS AND METHODS

Chemicals

[^{3}H]Mepyramine (28.0 Ci/mmol) was purchased from Amersham (Buckinghamshire, U.K.) and [^{14}C(U)]sucrose (5.0 mCi/mmol) from Dupont NEN (Boston, U.S.A.). All isotopes were stored at −20°C until use. Ketotifen fumarate was purchased from Orion Chemicals (Milan, Italy), Cyproheptadine hydrochloride from Co. Pharmaceutica (Milan, Italy). Azelastine hydrochloride, emedastine fumarate and cetirizine hydrochloride were synthesized in our laboratory. DL-Propranolol and all other chemicals were of reagent grade and commercially available.

Isolation and Culture of BCEC

Bovine capillary endothelial cells (BCEC) were isolated from bovine brain as described (3).

Cellular Uptake Experiments

The uptake of [^{3}H] or [^{14}C] labeled compounds (1.0 μCi) into cultured monolayers of BCEC was studied using the described methods reported previously (3).

RESULTS

Effects of H_1-Antagonists on the Uptake of [^{3}H]mepyramine

The effects of H_1-antagonists on the uptake of [^{3}H]mepyramine are summarized in Table I. H_1-Antagonists such as azelastine, ketotifen, cyproheptadine, emedastine and cetirizine at concentrations of 250 μM significantly diminished the uptake of [^{3}H]mepyramine. The inhibitory effect of cetirizine, which incorporates a zwitterionic carboxylic acid derivative of hydroxyzine, was the lowest among the compounds tested.

Effect of Ketotifen on the Uptake Rate of [^{3}H]mepyramine

Fig. 1 shows Lineweaver-Burk plots for the initial uptake rate of [^{3}H]mepyramine showing inhibition by ketotifen at a concentration of 50 μM. The results demonstrated that ketotifen competitively inhibited the initial uptake rate of [^{3}H]mepyramine. The inhibition constant of ketotifen, K_i, was estimated to be 46.8 μM, from the apparent K_t value of 110 μM, for the uptake of [^{3}H]mepyramine in the presence of 50 μM of ketotifen.

Counter Transport on the Initial Uptake of [^{3}H]mepyramine by H_1-Antagonists

The counter transport effects on the initial uptake of [^{3}H]mepyramine by H_1-antagonists in the ATP-depleted condition with 25 μM of rotenone are summarized in Table II. The uptake rate of [^{3}H]mepyramine was significantly stimulated by preloading with 250 μM of H_1-antagonists such as cyproheptadine, ketotifen and azelastine for 20 min. In contrast, the uptake of [^{14}C]sucrose was not affected by preloading with any of H_1-antagonists examined.

Effects of Lipophilic Basic Drugs on the Uptake of [^{3}H]mepyramine

The inhibitory effects of lipophilic basic drugs on the uptake of [^{3}H]mepyramine are summarized in Table III. The

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Table I. Effects of H_1-Antagonists on the Uptake of [^{3}H]Mepyramine

Inhibitor	Relative Uptake[a]
	%
Control	100.0 ± 5.8[b]
Azelastine	8.6 ± 1.4*
Ketotifen	15.1 ± 1.4*
Cyproheptadine	15.8 ± 2.7*
Emedastine	28.5 ± 1.8*
Cetirizine	75.1 ± 2.9**

[a] Each value represents the mean ± S.E. of three experiments. The uptake of [^{3}H]mepyramine was measured at 37°C for 10 sec by incubating BCEC in 10 mM HEPES/NaOH buffer (pH 7.4) containing 0.25 mM of inhibitor. The concentration of [^{3}H]mepyramine was 0.14 μM.

[b] The uptake of [^{3}H]mepyramine was determined to be 11.2 ± 0.65 pmol/mg protein.

* Significantly different from the control value by Student's t test ($p < 0.001$).

** Significantly different from the control value by Student's t test ($p < 0.05$).

Table II. Counter Transport Effects on the Uptake of [^{3}H]Mepyramine by H_1-Antagonists and Lipophilic Basic Drugs

Compounds	Cell/Medium Ratio[a,b]	
	[^{3}H]Mepyramine	[^{14}C]Sucrose
Control	56.5 ± 2.7	2.6 ± 0.1
Mepyramine	91.7 ± 2.8*	2.9 ± 0.09
Cyproheptadine	85.4 ± 2.1*	2.8 ± 0.2
Ketotifen	82.3 ± 1.9*	2.9 ± 0.1
Azelastine	82.0 ± 1.9*	2.9 ± 0.06
Propranolol	71.4 ± 2.7*	2.9 ± 0.1
Imipramine	70.0 ± 2.0*	2.8 ± 0.2

[a] Each value represents the mean ± S.E. of four experiments. BCEC were preincubated for 20 min in the presence of each compound (250 μM) with rotenone (25 μM), and the control was in the absence of each compound. The uptake of [^{3}H]mepyramine and [^{14}C]sucrose were measured at 37°C for 10 sec by incubating BCEC in 10 mM HEPES/NaOH buffer (pH 7.4).

[b] Values are expressed as μl/mg protein.

* Significantly different from the control value by Student's t test ($p < 0.001$).

initial uptake of [^{3}H]mepyramine was decreased significantly by 1 mM of imipramine, propranolol and lidocaine. Moreover, the effects of preloading with 250 μM of propranolol and imipramine on the uptake of [^{3}H]mepyramine were also examined. As shown in Table II, the counter transport effects of propranolol and imipramine were also observed in ATP-depleted BCEC.

Effect of Propranolol on the Uptake Rate of [^{3}H]mepyramine

Fig. 2 shows the Lineweaver-Burk plots for the initial uptake rate of [^{3}H]mepyramine showing inhibition by propranolol at a concentration of 50 μM. The result demonstrates that propranolol competitively inhibited the initial uptake rate of [^{3}H]mepyramine. The inhibitory constant, K_i, was estimated to be 51 μM, from the apparent K_t value of 104.3 μM, for the uptake of [^{3}H]mepyramine in the presence of 50 μM of propranolol.

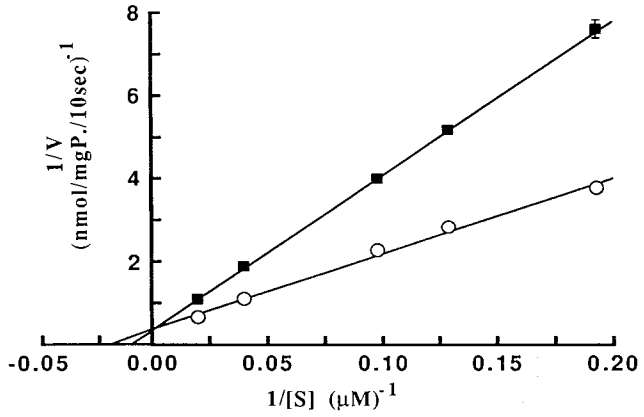

Fig. 1. Lineweaver-Burk plot of [^{3}H]mepyramine uptake rate by cultured monolayers of BCEC in the absence (open circles) and presence (filled squares) of 50 μM ketotifen. The initial uptake rate of [^{3}H]mepyramine was measured at 37°C for 10 sec by incubating BCEC in 10 mM HEPES/NaOH buffer (pH 7.4). Ketotifen was simultaneously added at the initiation of [^{3}H]mepyramine uptake. Each point represents the mean ± S.E. of three experiments. When the S.E. was small, it was included in the symbol. The inhibition constant, K_i of ketotifen was calculated to be 46.8 μM.

DISCUSSION

We previously found that mepyramine as a model of H_1-antagonists was transported into the brain via a carrier-mediated transport system. Since the classical H_1-antagonists are structurally similar, they may be transported into the brain via the same system as that suggested for mepyramine. If the characteristics of the system and the means of controlling the affinity to the carrier could be clarified, it would be possible to develop non-sedative H_1-antagonists derived from the classical structures.

To clarify the transport mechanism of H_1-antagonists, the inhibitory effects of several H_1-antagonists on [^{3}H]mepyramine uptake were examined using BCEC. As shown in Table I, H_1-antagonists significantly inhibited the uptake of [^{3}H]mepyramine, and ketotifen (K_i = 46.8 μM) was a competitive inhibitor. Moreover, the uptake of [^{3}H]mepyramine was significantly stimulated by preloading with H_1-antagonists (Table II). These results support the view that H_1-antagonists are transported into the BCEC by a common carrier system.

Table I shows that the inhibitory effect of cetirizine was much lower than that of other H_1-antagonists. This indicated that the affinity of cetirizine, which has a carboxylated side chain, for the transport system was lower than that of other H_1-antagonists. The transport mechanism of choline at the BBB has been elucidated (4). Choline is transported via carrier-mediated transport system of endogenous cationic substrates at the BBB. However, the carboxylated metabolite of choline, betaine does not inhibit the transport of choline. In a similar manner, cetirizine had low affinity for the transport system of H_1-antagonists. It is suggested that the control of

Table III. The Effects of Lipophilic Basic Drugs on the Uptake of [^{3}H]Mepyramine

Inhibitors	Relative Uptake[a]
	%
Control	100.0 ± 2.4[b]
Imipramine	7.7 ± 0.5*
Propranolol	8.3 ± 0.8*
Lidocaine	25.9 ± 2.1*

[a] Each value represents the mean ± S.E. of four experiments. The uptake of [^{3}H]mepyramine was measured at 37°C for 10 sec by incubating BCEC in 10 mM HEPES/NaOH buffer (pH 7.4) containing 1 mM of inhibitor. The concentration of [^{3}H]mepyramine used for the uptake was 0.14 μM.

[b] The uptake of [^{3}H]mepyramine was determined to be 10.8 ± 0.26 pmol/mg protein.

* Significantly different from the control value by Student's *t* test ($p < 0.001$).

carrier affinity is the key factor in the regulation of BBB permeability for H_1-antagonists.

Classical H_1-antagonists are cationic drugs with high lipophilicity, and are widely distributed in the brain. In this respect, H_1-antagonists resemble propranolol, which was reported to be transported by a specific carrier system at the BBB *in vivo* (5) and *in vitro* (6). In a previous study using isolated brain microvessels, the K_t of propranolol was determined to be 42.5 μM (6), which is close to the K_t (49.8 μM) of the high affinity [^{3}H]mepyramine uptake. Furthermore, the lack of effect by metabolic inhibitors suggests the energy independence of [^{3}H]mepyramine uptake. Similar energy independence has been observed in the uptake of propranolol in isolated brain microvessels. Therefore, it is possible that [^{3}H]mepyramine is transported via the same route as propranolol. As shown in table III, lipophilic basic drugs such as imipramine, propranolol and lidocaine significantly diminished the uptake of [^{3}H]mepyramine. The competitive inhibition of propranolol of [^{3}H]mepyramine uptake suggests the existence of a common transport system (Fig. 2), and the K_i of propranolol against [^{3}H]mepyramine uptake was 51.1 μM, which was very similar to the reported K_t of propranolol. Moreover, significant stimulation was evident after preloading with propranolol and imipramine. These results support the notion that H_1-antagonists and these lipophilic basic drugs are transported into the BCEC by common carrier systems.

In conclusion, the results obtained here indicated that the BBB transport system of H_1-antagonists is common to that responsible for the transport of lipophilic basic drugs such as propranolol, imipramine and lidocaine.

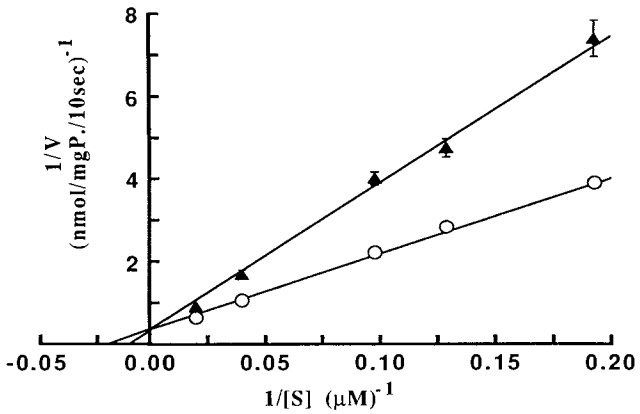

Fig. 2. Lineweaver-Burk plot of [^{3}H]mepyramine uptake rate by cultured monolayers of BCEC in the absence (open circles) and presence (filled triangles) of 50 μM propranolol. The initial uptake rate of [^{3}H]mepyramine was measured at 37°C for 10 sec. Propranolol was simultaneously added at the initiation of [^{3}H]mepyramine uptake. Each point represents the mean ± S.E. of three experiments. When the S.E. was small, it was included in the symbol. The inhibition constant, K_i of propranolol was calculated to be 51.1 μM.

REFERENCES

1. Y. Ito, H. Kato, E. Koshinaka, N. Ogawa, H. Nishino and J. Sakaguchi. Piperidine derivatives and pharmaceutical compositions comprising the same. *U.S. Patent.* 5,095,022 (1992).
2. M. Yamazaki, H. Fukuoka, O. Nagata, H. Kato, Y. Ito, T. Terasaki and A. Tsuji. Transport mechanism of an H_1-antagonist at the blood-brain barrier: Transport mechanism of mepyramine using the carotid injection technique. *Biol. Pharm. Bull.* 17:676–679 (1994).
3. M. Yamazaki, T. Terasaki, K. Yoshioka, O. Nagata, H. Kato, Y. Ito and A. Tsuji. Carrier-mediated transport of H_1-antagonist at the blood-brain barrier: mepyramine uptake into bovine brain capillary endothelial cells in primary monolayer cultures. *Pharm. Res.* 11:975–978 (1994).
4. Y. S. Kang, T. Terasaki, T. Ohnishi and A. Tsuji. *In vivo* and *in vitro* evidence for a common carrier mediated transport of choline and basic drugs through the blood-brain barrier. *J. PharmacobioDyn.* 13:353–360 (1990).
5. W. M. Pardridge, R. Sakiyama and G. Fidder. Blood-brain barrier transport and brain sequestration of propranolol and lidocaine. *Am. J. Physiol.* 247:R582–R588 (1984).
6. A. Kurihara, H. Suzuki, Y. Sawada, Y. Sugiyama, T. Iga and M. Hanano. Uptake of propranolol by microvessels isolated from bovine brain. *J. Pharm. Sci.* 76:759–764 (1987).