Transport Mechanism of an H₁-Antagonist at the Blood-Brain Barrier: Transport Mechanism of Mepyramine Using the Carotid Injection Technique

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The blood-brain barrier (BBB) permeability of mepyramine was measured by the carotid injection technique to elucidate the transport mechanism of an H₁-antagonist in the central nervous system.

Mepyramine was found to enter the brain by saturable and carrier-mediated transport. The in vivo kinetic parameters were estimated as follows: the maximum uptake rate (Jmax) was 7.12 ± 1.37 μmol/min/g of brain, the Michaelis constant (Km) was 4.40 ± 2.00 mM, and the nonsaturable first order rate (Kp) was 0.28 ± 0.02 ml/min/g of brain. The mepyramine transport was not inhibited either by nutrients or by choline, hemicholinium-3, though it was inhibited by the classical H₁-antagonists such as diphenhydramine, diphenylephrine, and also by propranolol.

The above inhibitory effects suggest that a transport system different from the amine transport system exists for the BBB transport of mepyramine, and that this transporter is common not only for H₁-antagonists but also for basic drugs.

Keywords H₁-antagonist; blood-brain barrier; carrier-mediated transport; brain uptake index

H₁-Antagonists are useful drugs for the treatment of allergic disorders which have recently spread increasingly. Since classical H₁-antagonists have been known to exhibit a significant sedative effect, their usefulness is limited. It is reasonable to assume that such a sedative effect is caused by an H₁-receptor blockade in the central nervous system (CNS). But the transport mechanism of an H₁-antagonist has not yet been clarified.

In this report, we study the blood-brain barrier (BBB) transport mechanism of mepyramine as a model of H₁-antagonists, whose high permeability into the brain is well-known. We used the carotid injection technique.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 250–300 g were purchased from Sankyo Laboratory (Toyama, Japan). They had free access to food and water.

Chemicals and Radioisotopes Mepyramine ·[pyridine-5.3H] (24.8 Ci/mmol) and butanol: n-[1-14C] (1.7 mCi/mmol) were purchased from New England Nuclear: NEN (Boston, MA, U.S.A.). Mepyramine maleate, propranolol hydrochloride, (R)-(+)propranolol hydrochloride, (S)-(−)-propranolol hydrochloride, hemicholinium-3 and xylazine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); lactic acid from Nacalai Tesque, Inc. (Kyoto, Japan); diphenhydramine hydrochloride, arginine, glucose and HEPES from Wako Pure Chemical Industries (Osaka, Japan); phenylalanine from Kyowahakko Co. (Tokyo, Japan); choline solution from Tokyo Kasei Co. (Tokyo, Japan); chlorpheniramine maleate from Fujiwara Seiyaku Co. (Toyama, Japan); Ketalar 50 [ketamine hydrochloride injection] from Sankyo Co. (Tokyo, Japan). Diphenhydramine hydrochloride, diphenylephrine hydrochloride, homochlorcyclizine hydrochloride were synthesized at our laboratory.

All other reagents were of reagent grade and commercially available.

In Vivo Brain Uptake Study: Carotid Injection Technique The first pass brain extraction of [3H]mepyramine relative to [14C]butanol was measured using the carotid injection technique in ketamine–xylazine anesthetized (235, 2.3 mg/kg, respectively) rats.2) An approximate 200 μl bolus of buffered Ringer solution [pH 7.4; 10 mM HEPES] was rapidly injected (0.5 s) into the right common carotid artery via a 27-gauge needle. The injection solution contained 12.5 μCi/ml [3H]mepyramine and 0.25 μCi/ml [14C]butanol. In the uptake experiment at various pH values, HCl was used to adjust the solution to pH 5.0, 5.8, 6.6 and 7.4. At 15 s after the injection, the animal was decapitated. A sample of the injection solution and the hemisphere ipsilateral to the injection were solubilized in 1.5 ml of Protosol (NEN) at 50 °C for 12 h, and 200 μl of 30% hydrogen peroxide, 12 ml of a mixture of Aquasol-2 (NEN) and 1 N HCl (9:1) were added before double-isotope liquid scintillation counting.

Counts per minute were converted to disintegrations per minute by standard quench corrections, and the brain uptake index (BUI) was calculated as follows:

\[
BUI = \frac{E_M}{E_B} \times 100
\]  \hspace{1cm} (1)

\[
BUI = \frac{[^3H/14C]dpm \text{ in brain}}{[^3H/14C]dpm \text{ in injectate}} \times 100
\]  \hspace{1cm} (2)

Where \( E_M \) and \( E_B \) are fractional extractions of mepyramine and butanol, respectively, at 15 s after the injections. The value of \( E_M \) can be estimated when BUI and \( E_B \) are determined experimentally.

Since the \( E_B \) value of [14C]butanol is reported as 64% for the brain,3) the following equation is valid:

\[
E_M = \text{BUI} \times 0.64
\]  \hspace{1cm} (3)

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**Estimation of Kinetic Parameters** Tests for saturability of the mepyramine transport were performed by adding various concentrations of unlabeled mepyramine to the carotid injection solution and by determining the BU1 at 15 s.

In order to estimate the kinetic parameters, the brain uptake rate \( J, \mu\text{mol/min/g of brain} \) was fitted to the following equation, consisting of both a saturable term and a nonsaturable linear term, by using the nonlinear least-squares regression analysis program, NONLIN:

\[
J = \frac{J_{\text{max}} \times C}{K_t + C} + K_d \times C.
\]  

(4)

Where \( J_{\text{max}} (\mu\text{mol/min/g of brain}), K_t (\text{mM}), K_d (\text{mL/min/g of brain}) \) and \( C' (\text{mM}) \) represent the maximal rate of the saturable uptake, the half saturation concentration (Michaelis constant), the nonsaturable uptake rate constant, and the mean capillary concentration of the substrate, respectively. \( J \) and \( C' \) were calculated from the following equations:

\[
J = \left(\frac{E_M}{100}\right) \times F \times C_{\text{in}}
\]

(5)

\[
C' = C_{\text{in}} \times \left(\frac{-E_M/100}{\ln(1-E_M/100)}\right)
\]

(6)

Where \( F \) and \( C_{\text{in}} \) are the blood flow rate and the concentration in injection solution, respectively. In this calculation, we used the reported value for the carotid artery blood flow of 0.93 mL/min.\(^4\)

**RESULTS**

**Concentration Dependence of \(^{[3]}\text{H}\)Mepyramine Uptake**

The concentration dependence of the BU1 for \(^{[3]}\text{H}\)-mepyramine is illustrated in Fig. 1A. The BU1 value of \(^{[3]}\text{H}\)-mepyramine decreased with the increase of the unlabeled mepyramine concentration in the injection solution at pH 7.4 in a range of 1 to 75 mM, indicating the saturable uptake of mepyramine into the brain. The brain uptake rate \( J \) was calculated from the BU1 value by using Eqs. 3—5, and was plotted in Fig. 1B against mean capillary concentration \( C' \) calculated from Eq. 6. By a nonlinear least-squares analysis of these data to

![Fig. 2. Effect of pH on \(^{[3]}\text{H}\)Mepyramine Uptake](image)

Each point represents the mean ± S.E. of 3 to 4 determinations.

![Fig. 3. Concentration Dependence for BU1 of \(^{[3]}\text{H}\)Mepyramine at pH 5.8](image)

The BU1 of \(^{[3]}\text{H}\)-mepyramine relative to \(^{[14]}\text{C}\)-butanol is plotted vs. the concentration of mepyramine in the injection solution adjusted to pH 5.8. Insert: the brain uptake rate of \(^{[3]}\text{H}\)-mepyramine calculated by Eq. 3 was plotted vs. the mean concentration of mepyramine in the capillaries calculated by Eq. 6. The solid and dashed lines represent total and carrier-mediated uptake, respectively, which were generated from Eq. 4 by using parameters obtained from a nonlinear least-squares regression analysis. Each point represents the mean ± S.E. of 3 to 4 determinations.
be fitted to Eq. 4, the following kinetic parameters were estimated (mean ± S.D.): $J_{\text{max}}$ was $7.12 ± 1.37 \mu\text{mol/min/g}$ of brain, $K_i$ was $4.40 ± 2.00 \text{mM}$, and $K_d$ was $0.28 ± 0.02 \text{ml/min/g}$ of brain.

**Effect of Medium pH on [\textsuperscript{3}H]Mepyramine Uptake**  
The effect of medium pH on the BUI for [\textsuperscript{3}H]mepyramine is illustrated in Fig. 2. The concentration dependence of the BUI for [\textsuperscript{3}H]mepyramine in the medium pH at 5.8 is illustrated in Fig. 3. The following parameters were estimated in the same manner described in the foregoing paragraph. $J_{\text{max}}$, $K_i$, and $K_d$ were $7.19 ± 3.09 \mu\text{mol/min/g}$ of brain, $14.8 ± 9.29 \text{mM}$, and $0.02 ± 0.03 \text{ml/min/g}$ of brain, respectively.

**Effect of Various Compounds on [\textsuperscript{3}H]Mepyramine Uptake**  
Table I summarizes the effect of the various compounds at a concentration of 50 mM on [\textsuperscript{3}H]-
mepyramine uptake at pH 7.4 by the brain. H\textsubscript{1}-Antagonists and propranolol significantly inhibited [\textsuperscript{3}H]-
mepyramine uptake; however, nutrients including choline and hemicholinium-3 showed no inhibitory effect.

**DISCUSSION**

It is generally accepted that BBB permeability depends on the lipophilicity and molecular weight of the drugs.\textsuperscript{5} On the other hand, it was proved that hydrophilic substances such as nutrients were transported into the brain through carrier-mediated systems. Furthermore, it has recently been elucidated that exogenous substances are transported into the brain through these carrier systems due to wrong recognition. From the view-point of drug design, it is necessary to elucidate the mechanism of drug BBB permeation, either by passive diffusion or carrier-mediated transport. Moreover, in the case of carrier-mediated transport, the control of the affinity of the drug to the carrier systems appears to be an important factor in regulating the BBB permeability of the drug.

The decreasing BUI of [\textsuperscript{3}H]mepyramine with the increasing concentration of the unlabeled mepyramine indicates the participation of a saturable uptake mechanism (Fig. 1). Significant inhibition of the BUI of [\textsuperscript{3}H]mepyramine by some basic drugs was also observed (Table I). These results suggest that the permeation of mepyramine into the brain is performed by the carrier-mediated system and by the passive diffusion caused by the lipophilicity of mepyramine. According to the kinetic parameters, it is reasonable to consider that mepyramine permeability into the brain is mainly caused by the carrier-mediated system, and partially by passive diffusion, because the value ($J_{\text{max}}/K_i$; 1.62) is 5.8 times greater than the value of $K_d$ (0.28). To confirm the characteristics of the uptake mechanism of mepyramine, we determined the effect of pH on the BUI of mepyramine. An apparent pH dependency was observed (Fig. 2). Acidosis resulted in a decrease in the uptake of mepyramine. In the medium pH at 5.8, $J_{\text{max}}/K_i$ and $K_d$ were 0.49 and 0.02, respectively. $J_{\text{max}}/K_i$ is 24 times greater than the value of $K_d$. With decreasing pH, from 7.4 to 5.8, $J_{\text{max}}/K_i$ and $K_d$ decreased 30% and 7%, respectively. The results of the pH effect on the BUI of mepyramine can be considered as follows: 1) decreasing pH might cause an allosteric effect to the carrier protein structure, e.g., an imidazolyl group of histidine residue (pK\textsubscript{a} 6) in the carrier protein, resulting in the decrease of transport rate, and 2) increasing the ionized form of the drug by decreasing pH produced a significant drop in the $K_d$ value.

It was found that the inhibitory effect of diphenhydramine and diphenylpyraline on the uptake of [\textsuperscript{3}H]mepyramine was larger than that of mepyramine alone, chlorpheniramine, or homochlorcyclizine. The inhibitory effect of propranolol was also larger than that of mepyramine. These results demonstrate that the affinities of the drugs to the carrier vary depending on the nature of their chemical structures. The drugs which showed the larger inhibitory effect contained an ether bond in their structures, suggesting that the affinity to the carrier is enhanced by having an ether bond.

We carried out the inhibitory study to characterize the carrier system of mepyramine in relation to endogeneous transport systems. Nutrients such as glucose, lactic acid, phenylalanine, and arginine, which are transported into the BBB through carrier systems, showed no inhibitory effect. In a recent paper, Kang reported that the choline transport system classified to amine transport system plays an important role in the transport of some lipophilic basic drugs.\textsuperscript{6} However, no inhibitory effect on mepyramine transport was observed by choline and hemicholinium-3, which are the most representative substrates of the amine transport system. Therefore, it is suggested that in the BBB, mepyramine might be transported via a different carrier system than that of choline. The transport of mepyramine was inhibited by classical H\textsubscript{1}-antagonists such as diphenhydramine, diphenylpyraline, chlorpheniramine, homochlorcyclizine, and also by propranolol, suggesting the presence of a common transport system not only for H\textsubscript{1}-antagonists but also for basic drugs.

In conclusion, our present study indicated that H\textsubscript{1}-antagonists, including mepyramine, are transported into the BBB through a carrier-mediated system which is different from that of choline. It is possible to assume that...
some basic drugs are transported via the carrier-mediated system which is common to that of mepyramine. In the drug design for developing H₁-antagonists, we have illustrated the idea that a strategy controlling the affinity to the carrier of the drug which is transported via some types of carrier-mediated transport systems may be a desirable approach in order to reduce adverse CNS reactions such as the sedative effect. In the processing study, it is necessary to clarify the characteristics of the carrier system for H₁-antagonists and to elucidate its usefulness for controlling the affinity to the carrier.

REFERENCES AND NOTES
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