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In-vitro Evidence for Carrier-mediated Uptake of Acidic Drugs by Isolated Bovine Brain Capillaries

TETSUYA TERASAKI, YOUNG SOOK KANG, TOSHIMASA OHNISHI AND AKIRA TSUJI

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan

Abstract—[^{3}H]Acetic acid was taken up into the isolated brain capillaries in a temperature and concentration dependent manner. The initial uptake rates were pH dependent over the range 5·0–7·4. An increase of the uptake rate with decrease in medium pH was not seen in the presence of unlabelled acetic acid (10 mM). The uptake of [^{3}H]acetic acid was significantly reduced in the presence of several drugs having a monocarboxylic group. The initial uptake of [^{14}C]salicylic acid was also pH dependent and was inhibited by acetic acid and acidic drugs. A mutually competitive inhibition was observed for the uptake of acetic acid and salicylic acid. The present study demonstrates that acidic drugs having a monocarboxylic group are transported by a common carrier-mediated system into the brain capillaries.

Recently, we have studied the mechanism of the blood-brain barrier (BBB) transport of basic (Kang et al 1990a) and acidic (Kang et al 1990c) drugs and have examined endogenous transport systems in BBB in normotensive rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) (Kang et al 1990b). In a previous study (Kang et al 1990c), using an in-vivo carotid artery injection technique, we demonstrated that acidic drugs such as salicylic acid are taken up in a carrier-mediated manner through the BBB via the monocarboxylic acid transport system. Monocarboxylic acids in the blood such as lactic acid, pyruvic acid and acetic acid are taken up by the brain and incorporated into phospholipids (Spitzer 1973), and are known to be transported via a carrier-mediated system at the BBB (Oldendorf 1973). However, the characteristics of the transport mechanism of monocarboxylic acids at the BBB and its relation to transport of acidic drugs through the BBB have not been elucidated.

The purpose of the present study was to elucidate the mechanism of acidic drug transport at the BBB using isolated capillaries from bovine brain.

Materials and Methods

Chemicals and radioisotopes

[^{3}H]Acetic acid sodium salt (3·3 Ci mmol^{-1}) and [^{14}C(U)]sucrose (4·6 mCi mmol^{-1}) were purchased from New England Nuclear, Boston, MA. [7-^{14}C]Salicylic acid (55 mCi mmol^{-1}) was purchased from American Radiolabeled Chemicals Inc., St. Louis, MO. All isotopes were stored at −20°C until use. Sodium valproate (Kyowa Hakko Co., Tokyo, Japan), benzylpenicillin (penicillin G), phenoxymethylpenicillin (penicillin V), propicillin (Meiji Seika Kaisha, Tokyo, Japan) and cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan) were kindly supplied from the cited companies. All other reagents were of reagent grade and commercially available.

Correspondence to: A. Tsuji, Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.

Preparations and uptake experiments

Capillaries were prepared from fresh bovine brains with a mechanical homogenization technique (Pardridge et al 1985; Terasaki et al 1989). The capillaries filtered through the mesh were suspended in buffer A (mM): NaCl 103, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES) 15, $NaHCO_3$ 25, D-glucose 10, pyruvic acid 1, and 0·5% bovine serum albumin, pH 7·4 and passed over a column of glass beads (450 μm) to remove erythrocytes and nucleus. The capillaries were resuspended in buffer B (mM): NaCl 103, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, HEPES 15, pH 7·4 and were used in the uptake study. Protein concentration of the brain capillaries was determined by the method of Lowry et al (1951) employing bovine serum albumin (Fraction V) as a standard.

As with the previous study, we checked the purity and viability of the isolated capillaries by the trypan blue exclusion test under light microscopy (Kang et al 1990a). No contamination by nerves of glial cells in the prepared capillary suspension was observed under light microscopy. Moreover, the initial uptake rate of [^{3}H]L-phenylalanine by the capillaries was determined to be 22·2 μL min^{-1} (mg protein)$^{-1}$. The value was similar to the initial uptake rates of [^{3}H]L-phenylalanine by the human and rat brain capillaries reported by Choi & Pardridge (1986). Therefore, the prepared bovine brain capillaries were considered to have sufficient viability to perform the in-vitro transport study, although the capillaries did not exclude trypan blue as reported previously (Choi & Pardridge 1986; Terasaki et al 1989).

Brain capillaries (200 μg protein) were preincubated in 20 μL of buffer B at 27°C (or 4°C) for 1 min. Then 180 μL of a solution containing (mM): NaCl 100, KCl 4, $CaCl_2$ 2·8, $MgSO_4$ 1, HEPES 50, D-glucose 10, 0·1% bovine serum albumin, pH 7·0, 300 mOsm, and [^{3}H]acetic acid (0·5 μCi) or [^{14}C]salicylic acid (0·3 μCi) or [^{14}C]sucrose (0·4 μCi) was incubated with the capillary suspension in the absence and presence of various compounds. In the uptake experiments at various pH values, 50 mM 2-(*N*-morpholino)ethanesulphonic acid, monohydrate (MES) and 50 mM HEPES were

used to achieve and maintain uptake medium pH 5·0 and 6·0, 7·0 and 7·4, respectively, after adjustment with 1 M NaOH.

Analytical procedures

At designated times after incubation, the mixture in each tube was poured over a Whatman glass microfibre filter on a filtration apparatus attached to a vacuum pump. The capillaries retained by the filter were then washed twice with 5 mL of cold incubation buffer. Each filter was put in 1 mL of 1 M NaOH in a glass scintillation vial and incubated for 1 h in a shaking incubator at 60°C. After neutralization with 0·2 mL of 5 M HCl and addition of 10 mL of Clear-sol I, the dissolved capillaries were used for isotope liquid scintillation counting in a liquid scintillation counter, LSC-700, Aloka Ltd, Tokyo, Japan. The uptake values were expressed as the cell-to-medium concentration (cell/medium) ratio, corrected for the adherent incubation medium by the apparent sucrose uptake. These calculations were as follows:

$$\text{cell/medium} = \frac{U_A}{S_M} - Ad_M \ \mu\text{L (mg prot)}^{-1}$$

$$U_A = \frac{\text{substrate count in capillary (d min}^{-1})}{\text{capillary amount (mg prot)}}$$

i.e. apparent uptake of substrate in capillary

$$S_M = \frac{\text{substrate count in medium (d min}^{-1})}{\text{volume of medium } (\mu\text{L})}$$

i.e. substrate concentration in medium

$$Ad_M = \frac{\text{sucrose count in capillary/capillary amount}}{\text{sucrose count in medium/volume of medium}}$$

i.e. adsorbed medium to capillary

mg protein represents the amount of capillary protein for the particular uptake study.

The transport data are presented as means ± s.e.m. Significant differences were assessed by Student's *t*-test.

The data were analysed and the kinetic parameters of the uptake were estimated by a non-linear-least squares regression analysis program MULTI (Yamaoka et al 1981).

Results

Effect of unlabelled acetic acid and temperature on the uptake of [^{3}H]acetic acid

Fig. 1a illustrates the time course of [^{3}H]acetic acid uptake by isolated capillaries at 27°C. The remarkable increase was observed for the cell/medium ratio of [^{3}H]acetic acid. As shown in Fig. 1b, a significant inhibition by 10 mM acetic acid was observed for the uptake of [^{3}H]acetic acid by the capillaries. The cell/medium ratio of [^{3}H]acetic acid at 15 s, 4°C was also determined to be 1·37 ± 0·10 μL min^{-1} (mg protein)$^{-1}$ which was significantly different from that determined at 27°C (2·79 ± 0·13 μL min^{-1} (mg prot)$^{-1}$) ($P < 0·01$). Thus, a significant temperature dependency was observed for the uptake of [^{3}H]acetic acid.

Effect of pH of medium on acetic acid uptake

The effect of pH on [^{3}H]acetic acid uptake over the pH range 5·0–7·4 is shown in Fig. 2. The uptake was apparently pH

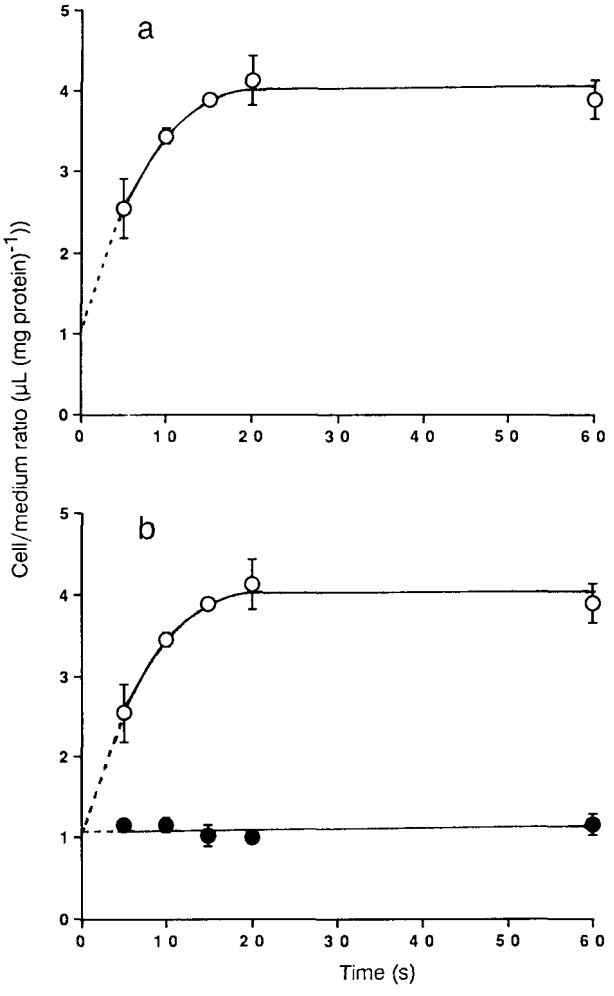

FIG. 1. (a) Time-course and (b) competition/saturation effects with unlabelled substrate. The uptake of [^{3}H]acetic acid into isolated bovine brain capillaries was determined at pH 7·0, 27°C. The cell/medium ratio was determined in the absence (○) and the presence (●) of 10 mM of unlabelled acetic acid in the uptake medium, respectively. Each point represents the mean ± s.e.m. of 3–4 experiments. When the s.e.m. was small, it was included in the symbol.

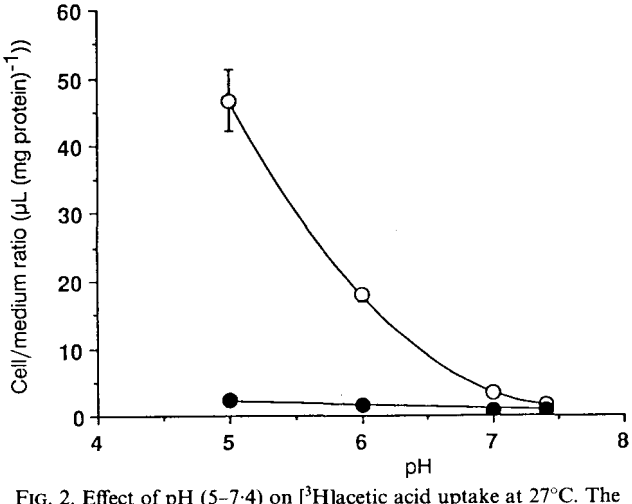

FIG. 2. Effect of pH (5–7·4) on [^{3}H]acetic acid uptake at 27°C. The initial uptake rate of [^{3}H]acetic acid was determined in the absence (○) and the presence (●) of 10 mM of unlabelled acetic acid. Each point represents the mean ± s.e.m. of 3 experiments. When the s.e.m. was small, it was included in the symbol.

Table 1. Effect of compounds on the [^{3}H]acetic acid uptake by bovine brain capillaries at pH 7·0.

Addition	Concn (mM)	% of Control
None		100±1·71 (4)
Acetic acid	10	26·8±1·61 (4)*
Salicylic acid	10	19·4±1·06 (4)*
Valproic acid	10	25·4±4·17 (4)*
Nicotinic acid	10	45·9±0·91 (4)*
Penicillin G	10	57·6±4·19 (4)*
Penicillin V	10	72·5±6·64 (4)*
Propicillin	10	51·5±4·73 (4)*
Cefazolin	10	61·1±4·08 (4)*
Succinic acid	10	112·6±9·77 (4)
Citric acid	10	90·9±8·31 (4)
Phenylalanine	10	84·1±7·38 (4)
DIDS	1	110·2±2·38 (4)
FCCP	0·05	64·2±3·83 (4)*

Data are presented as the mean±s.e.m. with the number of experiments in the parentheses. The concentration of [^{3}H]acetic acid used for the uptake was 1·5 μM. * Level of significance was set at $P<0·01$.

dependent. Below pH 7·0, the cell/medium ratio of [^{3}H]acetic acid at 5 s increased markedly with decreasing pH of the medium.

Fig. 2 also compares the initial uptake of [^{3}H]acetic acid in the absence and presence of 10 mM of unlabelled acetic acid and shows the uptake of [^{3}H]acetic acid to be inhibited and apparently independent of pH of the medium.

Inhibitory effect of compounds on the initial uptake of acetic acid

Table 1 shows the inhibitory effect of various compounds on the [^{3}H]acetic acid uptake by the capillaries. The acidic compounds, acetic acid, salicylic acid, valproic acid, nicotinic acid and β-lactam antibiotics (penicillin G, penicillin V, propicillin and cefazolin), inhibited significantly [^{3}H]acetic acid uptake by the capillaries ($P<0·01$), whereas, a dicarboxylic acid (succinic acid), a tricarboxylic acid (citric acid) and a neutral amino acid (phenylalanine) did not change the uptake of [^{3}H]acetic acid.

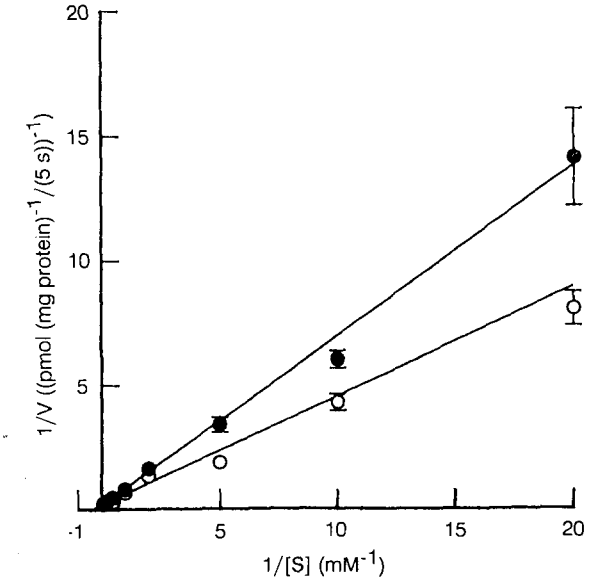

FIG. 3. Lineweaver-Burk plots of [^{3}H]acetic acid uptake rate by isolated bovine brain capillaries in the absence (○) and presence (●) of unlabelled salicylic acid (2 mM). Salicylic acid was simultaneously added at the initiation of [^{3}H]acetic acid uptake. Each point represents the mean±s.e.m. of 3–4 experiments. The inhibition constant, K_i, of salicylic acid was calculated to be 3·63±0·74 mM.

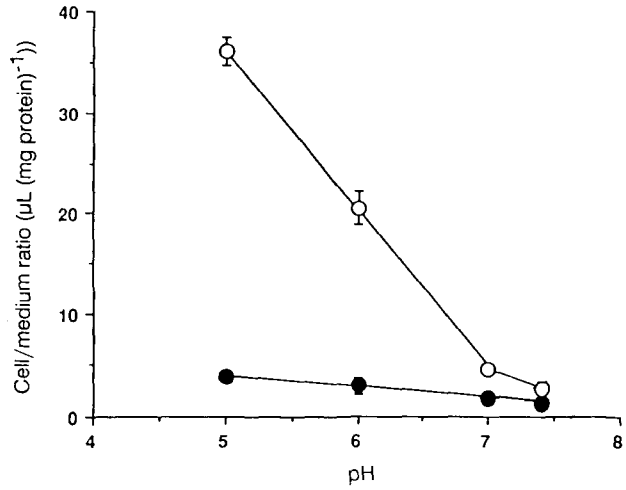

FIG. 4. pH dependency of [^{14}C]salicylic acid uptake by isolated bovine brain capillaries in the absence (○) and presence (●) of the unlabelled salicylic acid (10 mM). Each point represents the mean±s.e.m. of 3 experiments. When the s.e.m. was small, it was included in the symbol.

Fig. 3 shows the Lineweaver-Burk plots for [^{3}H]acetic acid uptake rate showing competitive inhibition by 2 mM salicylic acid. The apparent Michaelis-Menten constant, K_t, and the maximum uptake rate, V_{max}, for [^{3}H]acetic acid uptake were 2·20±0·28 mM and 5·00±0·48 pmol/5 s (mg protein)$^{-1}$, respectively. The inhibition constant, K_i, of salicylic acid for the uptake of [^{3}H]acetic acid was 3·63±0·74 mM.

Effect of pH of medium on salicylic acid uptake

Fig. 4 illustrates the effect of pH on the [^{14}C]salicylic acid uptake into the capillaries. Like the result for acetic acid, the uptake of [^{14}C]salicylic acid was greatest at pH 5·0 and fell significantly at pH 6·0, 7·0 and 7·4. Also, the cell/medium ratio of [^{14}C]salicylic acid was significantly diminished by the presence of 10 mM of salicylic acid at pH 5·0, 6·0 and 7·0.

Table 2. Effect of acidic and basic compounds on the [^{14}C]salicylic acid uptake by bovine brain capillaries at pH 7·0.

Addition	Concn (mM)	% of Control
None		100±10·1 (4)
Acetic acid	10	40·8±2·38 (4)*
Salicylic acid	10	37·1±2·85 (3)*
Valproic acid	10	57·9±3·42 (4)*
Propicillin	10	69·0±4·47 (4)*
Cefazolin	10	66·0±3·23 (4)*
Succinic acid	10	95·9±8·46 (4)
Citric acid	10	98·5±6·88 (4)
Choline	10	92·1±8·89 (4)
Phenylalanine	10	88·7±3·20 (4)
DIDS	1	120·4±6·84 (4)
FCCP	0·05	74·8±4·03 (4)*

Data are presented as the mean±s.e.m. with the number of experiments in the parentheses. The concentration of [^{14}C]salicylic acid used for the uptake was 20 μM. * Level of significance was set at $P<0·05$.

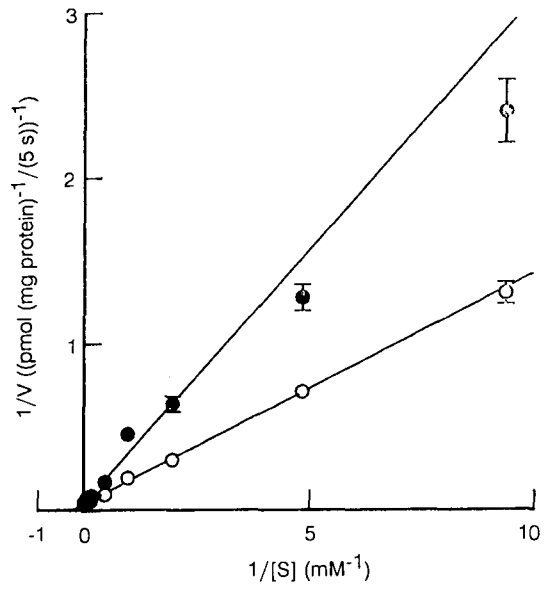

FIG. 5. Lineweaver–Burk plots of [^{14}C]salicylic acid uptake rate by isolated bovine brain capillaries in the absence (○) and presence (●) of the unlabelled acetic acid (3 mM). Acetic acid was simultaneously added at the initiation of [^{14}C]salicylic acid uptake. Each point represents the mean ± s.e.m. of 3–4 experiments. The inhibition constant, K_i, of acetic acid was calculated to be 2·50 ± 0·54 mM. When the s.e.m was small, it was included in the symbol.

Inhibitory effect of compounds on the uptake of salicylic acid

As shown in Table 2, the inhibitory effect of various compounds on the uptake of [^{14}C]salicylic acid was similar to the results obtained in Table 1. The uptake was significantly diminished by the presence of a monocarboxylic acid (acetic acid), acidic drugs (salicylic acid and valproic acid) and β-lactam antibiotics (propicillin and cefazolin) but not affected by a dicarboxylic acid (succinic acid), a tricarboxylic acid (citric acid), choline and phenylalanine. Also, 4,4′-diisothiocyano-2,2′-disulphonic acid stilbene disodium salt (DIDS), an inhibitor of anion exchanger, did not change the uptake of [^{14}C]salicylic acid, whereas carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), a protonophore, decreased significantly the cell/medium ratio of [^{14}C]salicylic acid.

Effect of acetic acid on the initial uptake rate of salicylic acid

Fig. 5 represents the Lineweaver–Burk plot of [^{14}C]salicylic acid uptake showing inhibition by 3 mM acetic acid. The apparent K_t and V_{max} for [^{14}C]salicylic acid uptake were 3·54 ± 0·48 mM and 25·4 ± 1·6 pmol/5 s (mg protein)$^{-1}$, respectively. The K_i of acetic acid for the uptake of [^{14}C]salicylic acid was 2·50 ± 0·54 mM.

Discussion

Recently, we have shown that basic drugs are transported by a common system for a basic endogenous compound, choline at the BBB (Kang et al 1990a). Whether the transport of acidic drugs having a central nervous effect across the BBB is mediated by a carrier system for an acidic endogenous compound, a monocarboxylic acid, is an interesting problem. A previous study concerning the in-vivo BBB transport of an acidic drug suggested that acidic drugs such as salicylic acid, valproic acid and some β-lactam antibiotics are transported by a carrier-mediated transport system for monocarboxylic acids at the BBB (Kang et al 1990c). To prove that there is a common transport system between monocarboxylic acids and acidic drugs, an in-vitro uptake technique using isolated bovine brain capillaries has been used.

To confirm the nature of the carrier-mediated transport system of acetic acid, we determined the time course of acetic acid uptake by isolated capillaries. A significant time dependency was observed for the uptake of [^{3}H]acetic acid in the absence of unlabelled acetic acid (Fig. 1a), while, on the contrary, no time dependency was observed in the presence of 10 mM of unlabelled acetic acid (Fig. 1b). A significant temperature dependency was also observed for the uptake of [^{3}H]acetic acid. These time, concentration and temperature dependent uptakes suggest that [^{3}H]acetic acid is taken up by the capillaries via a carrier-mediated transport system.

The apparent pH-dependencies for the uptakes of acetic acid and salicylic acid by bovine brain capillaries (Figs 2, 4) could be ascribed to either the pH-partition hypothesis or some specialized transport mechanism. Since the pH dependencies of [^{3}H]acetic acid and [^{14}C]salicylic acid uptake disappeared in the presence of 10 mM acetic acid or salicylic acid, respectively, it is suggested that an H^+ or OH^- related carrier-mediated transport system contributes to the acetic acid uptake that is dependent on pH. The results showing increased uptake of acetic acid with decreasing pH are similar to those for the uptake of L-lactate at the BBB (Oldendorf et al 1979), and 3-hydroxy[3-^{14}C]butyrate by dissociated cells from rat brain (Tildon & Roeder 1988), and the uptake of [^{3}H]acetic acid by the intestinal brush-border membrane (Tsuji et al 1990).

The [^{3}H]acetic acid uptake by bovine brain capillaries was significantly inhibited by acidic compounds with a monocarboxylic group, and some β-lactam antibiotics, whereas the dicarboxylic acid and tricarboxylic acid did not inhibit the uptake of [^{3}H]acetic acid (Table 1). These results suggest that acidic drugs such as salicylic acid and valproic acid exert a selective inhibitory effect upon the uptake of [^{3}H]acetic acid. The result showing that the uptake of [^{3}H]acetic acid is not influenced by a neutral amino acid, phenylalanine, is consistent with the finding that independent transport systems for nutrients exist at the BBB (Pardridge 1983). According to the apparent pH dependent uptake, at least three possibilities are suggested: (i) pH dependent conformational change of the transport carrier, (ii) H^+ co-transport system, (iii) OH^- exchange system. To clarify these points, the inhibition studies of DIDS and FCCP were performed. No inhibition effect of DIDS on the uptake of [^{3}H]acetic acid or of [^{14}C]salicylic acid suggests that the OH^- exchange system was not involved (Tables 1, 2). Moreover, the inhibition effect of FCCP suggests that the transport of [^{3}H]acetic acid and [^{14}C]salicylic acid would be accelerated by a proton gradient between the uptake medium and the intracellular space of endothelial cells (Tables 1, 2). Accordingly, like the transport of monocarboxylic acids at the brush-border membrane of the small intestine (Tsuji et al 1990), it is

possible to postulate that a monocarboxylic acid is transported via an H^+ co-transport system at the BBB.

To further elucidate the effect of acidic drugs on acetic acid transport, the acetic acid uptake by isolated bovine brain capillaries was analysed kinetically. The fact that salicylic acid, 2 mM, competitively inhibited the uptake of [^{3}H]acetic acid (Fig. 3) suggests that the uptake of [^{14}C] salicylic acid proceeds via a common carrier system for acetic acid uptake in the capillaries.

The study of inhibition effects by various compounds was also carried out to clarify the effect of monocarboxylic acids on the [^{14}C]salicylic acid uptake by isolated bovine brain capillaries (Table 2). The significant inhibition by unlabelled salicylic acid of the uptake of [^{14}C]salicylic acid demonstrates that the salicylic acid is transported by the capillaries via a carrier-mediated transport system. As shown in Table 2, and similar to the results in Table 1, the uptake of [^{14}C]salicylic acid was significantly decreased by a monocarboxylic acid (acetic acid), acidic drug (valproic acid) and β-lactam antibiotics (propicillin and cefazolin) but not affected by a dicarboxylic acid, a tricarboxylic acid, choline or phenylalanine. These results indicate that salicylic acid is taken up by a monocarboxylic acid carrier mediated transport system independent of that for di- or tricarboxylic acids. Moreover, that the choline used in the present study did not inhibit the uptake of acidic drugs such as [^{14}C]salicylic acid, supports the contribution of choline to transport of basic drugs at the BBB.

To further clarify the role of monocarboxylic acid in the uptake of salicylic acid, we also analysed kinetically salicylic acid uptake by isolated capillaries. The uptake of [^{14}C]salicylic acid was competitively inhibited by 3 mM acetic acid (Fig. 5), demonstrating that salicylic acid could be transported via a common carrier-mediated system for monocarboxylic acids at the BBB. The K_i value of acetic acid ($2{\cdot}50 \pm 0{\cdot}54$ mM) was similar to the K_t value of acetic acid ($2{\cdot}20 \pm 0{\cdot}28$ mM). The K_i value of salicylic acid ($3{\cdot}63 \pm 0{\cdot}74$ mM) was also similar to the K_t value of salicylic acid ($3{\cdot}54 \pm 0{\cdot}48$ mM). These results suggest that salicylic acid is transported via a carrier-mediated system common to that of acetic acid at the BBB.

With regard to the polarity of the transport activity of the endothelial cell membrane, it would be difficult to separate the uptake rate into the capillaries at the luminal and abluminal plasma membrane and to conclude whether the transport mechanism of a monocarboxylic acid is ascribable to the luminal plasma membrane or abluminal plasma membrane.

In conclusion, the present study demonstrates a carrier-mediated transport of acidic drugs having a monocarboxyl group, such as salicylic acid and β-lactam antibiotics, at the BBB. A carrier-mediated H^+ co-transport system is suggested for the uptake of the endogenous monocarboxylic acid and monocarboxylic acid drug at the BBB.

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References

Choi, T. B., Pardridge, W. M. (1986) Phenylalanine transport at the human blood-brain barrier. J. Biol. Chem. 261: 6536–6541

Kang, Y. S., Terasaki, T., Ohnishi, T., Tsuji, A. (1990a) In vivo and in vitro evidence for a common carrier mediated transport of choline and basic drugs through the blood-brain barrier. J. Pharmacobio-Dyn. 13: 353–360

Kang, Y. S., Terasaki, T., Tsuji, A. (1990b) Dysfunction of choline transport system through blood-brain barrier in stroke-prone spontaneously hypertensive rats. Ibid. 13: 10–19

Kang, Y. S., Terasaki, T., Tsuji, A. (1990c) Acidic drug transport in vivo through the blood-brain barrier. A role of the transport carrier for monocarboxylic acids. Ibid. 13: 158–163

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Oldendorf, W. H. (1973) Carrier-mediated blood-brain barrier transport of short chain monocarboxylic organic acids. Am. J. Physiol. 224: 1450–1453

Oldendorf, W. H., Braun, L., Cornford, E. (1979) pH dependence of blood-brain barrier permeability to lactate and nicotine. Stroke 10: 577–581

Pardridge, W. M. (1983) Brain metabolism: a perspective from the blood-brain barrier. Physiol. Rev. 63: 1481–1535

Pardridge, W. M., Eisenberg, J., Yamada, T. (1985) Rapid sequestration and degradation of somatostatin analogues by isolated brain microvessels. J. Neurochem. 44: 1178–1184

Spitzer, J. J. (1973) CNS and fatty acid metabolism. Physiologist 16: 55–68

Terasaki, T., Hirai, K., Sato, H., Kang, Y. S., Tsuji, A. (1989) Absorptive-mediated endocytosis of a dynorphin-like analgesic peptide, E-2078, into the blood-brain barrier. J. Pharmacol. Exp. Ther. 251: 351–357

Tildon, J. T., Roeder, L. M. (1988) Transport of 3-hydroxy[3-^{14}C]butyrate by dissociated cells from rat brain. Am. J. Physiol. 255: C133–C139

Tsuji, A., Simanjuntak, M. T., Tamai, I., Terasaki, T. (1990) pH dependent intestinal transport of monocarboxylic acids: carrier-mediated and H^+-co transport mechanism versus pH-partition hypothesis.

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4: 879–885