ACIDIC DRUG TRANSPORT *IN VIVO* THROUGH THE BLOOD-BRAIN BARRIER. A ROLE OF THE TRANSPORT CARRIER FOR MONOCARBOXYLIC ACIDS

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The relationship of the transports between acidic drugs and monocarboxylic acids through the blood-brain barrier (BBB) was examined using the carotid artery injection technique in rats. The BBB uptakes of [^3H]acetic acid and [^{14}C]salicylic acid were significantly reduced by the presence of the respective unlabeled compounds, valproic acid, lactic acid, benzoic acid, nicotinic acid or β -lactam antibiotics (benzylpenicillin, propicillin and cefazolin), but was not reduced by choline, phenylalanine and a basic drug, eperisone. A remarkable pH dependency was observed for the BBB uptake of [^{14}C]salicylic acid at the pH region of 4.0 to 7.4. Interestingly, 10 mM of salicylic acid diminished significantly the pH dependent BBB uptake of [^{14}C]salicylic acid. Similar results were obtained in the BBB uptake of [^{14}C]nicotinic acid. No significant difference was observed in the transport of monocarboxylic acids through the BBB between normotensive Wistar KY rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP). From these observations, acidic drugs could be transported by a carrier-mediated system for monocarboxylic acids at the BBB and the transport system was not changed by the disease state.

KEYWORDS — blood-brain barrier; monocarboxylic acid; carrier mediated transport; salicylic acid; nicotinic acid; acetic acid; β -lactam antibiotics; pH dependency; brain uptake index

INTRODUCTION

In a previous study,¹⁾ we have demonstrated that some basic drugs could be transported *via* a carrier-mediated system of choline at the BBB. We have also found that the decreased choline concentration in the brain interstitial fluid is ascribed to the specific dysfunction of the BBB choline transport system in SHRSP.²⁾ The purpose of the present study is to clarify, using the carotid artery injection technique, the mechanism of the BBB transport of acidic drugs in the relation to the transport of monocarboxylic acids because the monocarboxylic acids in the blood are known to be taken up by the brain and incorporated into phospholipids³⁾ and because acidic drugs such as nicotinic acid and salicylic acid which have

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central nervous effects are used as an anti-senile dementia and analgesic, respectively. We also wish to find whether there is a biochemical difference in the transport system for monocarboxylic acids between WKY and SHRSP or not.

MATERIALS AND METHODS

Animals — Normotensive Wistar KY rats (WKY) were purchased from Sankyo Laboratory Co, Toyama, Japan and were used as a control. Stroke-prone spontaneously hypertensive rats (SHRSP) were obtained from breeding colonies maintained at the Institute for the Experimental Animals, Kanazawa University in a manner as described previously.²⁾ Eight-weeks old male rats weighing 230-250 g for WKY and 180-200 g for SHRSP (the brain weight was not significantly different between WKY and SHRSP²⁾) were used throughout the present study. They had free access to the standard food and water.

Chemicals and Radioisotopes — [³H]Acetic acid sodium salt (3.3 Ci/mmol) and N-[1-¹⁴C]butanol (1.1 mCi/mmol) were purchased from New England Nuclear, Boston, U.S.A. [³H]Water (1 mCi/ml) and [carboxyl-¹⁴C]nicotinic acid (53 mCi/mmol) were purchased from Amersham International Ltd., Buckinghamshire, U.K. [7-¹⁴C]Salicylic acid (55 mCi/mmol) was purchased from American Radiolabeled Chemicals Inc., St. Louis, MO. All isotopes were stored at -20°C until use. Eperisone (Hokuriku Seiyaku Co., Katsuyama, Japan), propicillin, phenoxymethylpenicillin (penicillin V) and benzylpenicillin (penicillin G) (Meiji Seika Kaisha, Tokyo, Japan), cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan) and sodium valproate (Kyowa Hakko Co., Tokyo, Japan) were kindly supplied from the cited companies. All other chemicals were of reagent grade and commercially available.

Preparation of Injection Solution — Ringer's-Hepes Buffer (RHB, pH 7.4) containing 141 mM NaCl, 4.0 mM KCl, 2.8 mM CaCl₂, 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) or MES Buffer (pH 4.0, 5.0 and 6.0) containing 141 mM NaCl, 4.0 mM KCl, 2.8 mM CaCl₂, 10 mM 2-(N-morpholino)ethanesulfonic acid, monohydrate (MES), were used for the injection solution. Radioisotope concentration as the test compound was 20 µCi/ml for [³H]acetic acid, 4.0 µCi/ml for [¹⁴C]nicotinic acid, and 4.0 µCi/ml for [¹⁴C]salicylic acid. [¹⁴C]Butanol (0.25 µCi/ml) or [³H]water (20 µCi/ml) were also contained in the injectate as a freely diffusible internal reference.

Brain Uptake Study — According to the carotid artery injection technique as described previously,^{1,2)} rats were decapitated at 5 s after the injection of about 200 µl of injectate and the uptake of radiolabeled acidic drugs into the brain was measured. The percentages of the brain extraction of [³H]acetic acid, [¹⁴C]nicotinic acid and [¹⁴C]salicylic acid were estimated in a manner described previously.^{1,2)}

RESULTS AND DISCUSSION

The percentages of the brain extraction determined for [^3H]acetic acid, [^{14}C]nicotinic acid and [^{14}C]salicylic acid both in WKY and SHRSP are listed in Table I. The value of [^3H]acetic acid in WKY was similar to that reported previously in an another strain of rats.⁴⁾ As shown in Table I, no significant difference was observed for the extraction value of [^3H]acetic acid, [^{14}C]nicotinic acid and [^{14}C]salicylic acid at pH 7.4 ($p > 0.05$) between WKY and SHRSP. At pH 6.0 of the injectate, the values of [^3H]acetic acid, [^{14}C]nicotinic acid and [^{14}C]salicylic acid increased significantly in WKY ($p < 0.05$). Similarly for [^3H]acetic acid in SHRSP, the significant increase of the extraction at pH 6.0 than that at pH 7.4 was observed ($p < 0.05$). These results showing pH dependent uptake of acetic acid are very similar to the result of the BBB L-lactate transport.⁵⁾

Table I. Comparison of [^3H]Acetic Acid, [^{14}C]Salicylic Acid and [^{14}C]Nicotinic Acid Transport through the Blood-Brain Barrier between Normotensive Wistar KY Rats (WKY) and Stroke-Prone Spontaneously Hypertensive Rats (SHRSP)

Substrate	Concn.	pH	Extraction (%)	
			WKY	SHRSP
[^3H]Acetic acid	6 μM	7.4	6.83 $\pm$ 0.08 (3)	6.28 $\pm$ 0.72 (3) ^{a)}
		6.0	10.5 $\pm$ 0.4 (3) ^{b)}	11.2 $\pm$ 0.4 (3) ^{c)}
[^{14}C]Salicylic acid	72 μM	7.4	5.01 $\pm$ 0.51 (4)	4.35 $\pm$ 0.13 (3) ^{a)}
		6.0	9.88 $\pm$ 1.20 (5) ^{c)}	n.d ^{d)}
[^{14}C]Nicotinic acid	76 μM	7.4	2.14 $\pm$ 0.12 (4)	2.10 $\pm$ 0.12 (4) ^{a)}
		6.0	3.91 $\pm$ 0.51 (5) ^{c)}	n.d ^{d)}
	20 mM	7.4	1.27 $\pm$ 0.12 (4) ^{e)}	n.d ^{d)}
		6.0	1.64 $\pm$ 0.17 (5) ^{e)}	n.d ^{d)}

Data are presented as the mean $\pm$ S.E.M. with the number of rats in parentheses. a) not significant between WKY and SHRSP, with a significance limit of $p=0.05$. b) pH 6.0 $>$ pH 7.4 with a significance limit of $p=0.001$. c) pH 6.0 $>$ pH 7.4 with a significance limit of $p=0.05$. d) n.d: not determined e) Tracer $>$ + cold 20 mM in the respective pH with a significance limit $p=0.05$.

Table II shows the inhibitory effects of various compounds on the percentage of brain extraction of [^3H]acetic acid. The acidic compounds such as acetic acid itself, valproic acid, salicylic acid, benzoic acid, nicotinic acid, and β -lactam antibiotics (penicillin G, penicillin V, propicillin and cefazolin) diminished significantly [^3H]acetic acid uptake through the BBB ($p < 0.001$), whereas choline, phenylalanine and a basic drug (eperisone) did not reduce significantly the uptake of [^3H]acetic acid. The inhibitory effect of various compounds on the uptake of [^{14}C]salicylic acid was also examined and is summarized in Table III. With the similarity to the

inhibitory effects obtained in Table II, the uptake of [^{14}C]salicylic acid was decreased by the presence of monocarboxylic acids (acetic acid and *L*-lactic acid) and acidic drugs such as valproic acid, salicylic acid itself and β -lactam antibiotics (penicillin G, propicillin and cefazolin), but not affected by dicarboxylic acid (succinic acid), tricarboxylic acid (citric acid), a basic drug (eperisone), choline and phenylalanine. From these results, acidic drugs appear to be transported *via* the monocarboxylic acid carrier system. This is very similar to the transport characteristics reported by Spector and Lorenzo⁶⁾ in the rabbit choroid plexus that penicillin and salicylate were transported by the same carrier system of monocarboxylic acids. The difference of the inhibitory effect between *L*-lactic acid and its *D*-isomer showed a clear stereo-specificity on the BBB monocarboxylic acid transport. The result is in accordance with a report by Oldendorf⁷⁾ and Nemoto and Severinghaus⁸⁾ showing the stereospecific BBB transport of lactate. Choline and basic drug used in the present study did

Table II. Effect of Acidic and Basic Compounds on [^3H]Acetic Acid Uptake into Blood-Brain Barrier at pH 6.0

Addition	% of control
None	100 (9)
Acetic acid	55.3 $\pm$ 0.5 ^{a)} (3)
Valproic acid	29.8 $\pm$ 1.7 ^{a)} (4)
Salicylic acid	45.9 $\pm$ 2.3 ^{a)} (4)
Benzoic acid	38.3 $\pm$ 5.1 ^{a)} (4)
Nicotinic acid	43.0 $\pm$ 3.4 ^{a)} (4)
Penicillin G	48.5 $\pm$ 1.8 ^{a)} (4)
Penicillin V	47.6 $\pm$ 3.5 ^{a)} (4)
Propicillin	34.2 $\pm$ 3.6 ^{a)} (4)
Cefazolin	52.9 $\pm$ 6.4 ^{a)} (4)
Eperisone	91.3 $\pm$ 5.7 (3)
Choline	93.9 $\pm$ 3.7 (4)
Phenylalanine	91.6 $\pm$ 7.9 (4)

Data are presented as the mean $\pm$ S.E.M. with the number of rats in parentheses. The concentration of [^3H]acetic acid used for the uptake was 6 μM . The concentration of the compounds added was 20 mM except 10 mM for salicylic acid.

a) Level of significance was set at $p < 0.001$.

Table III. Effect of Acidic and Basic Compounds on [^{14}C]Salicylic Acid Uptake into Blood-Brain Barrier at pH 4.0

Addition	% of control
None	100
Acetic acid	26.2 $\pm$ 1.5 ^{a)}
Valproic acid	29.8 $\pm$ 11.2 ^{b)}
Salicylic acid	35.1 $\pm$ 3.6 ^{a)}
<i>L</i> -Lactic acid	47.3 $\pm$ 2.9 ^{c)}
<i>D</i> -Lactic acid	78.9 $\pm$ 16.3
Penicillin G	69.6 $\pm$ 6.7 ^{c)}
Propicillin	47.4 $\pm$ 1.5 ^{c)}
Cefazolin	39.3 $\pm$ 6.5 ^{c)}
Succinic acid	105.1 $\pm$ 19.4
Citric acid	121.6 $\pm$ 20.5
Eperisone	90.3 $\pm$ 10.7
Choline	107.0 $\pm$ 6.6
Phenylalanine	107.0 $\pm$ 2.7

Data presented as the mean $\pm$ S.E.M. ($n=3$). The concentration of [^{14}C]salicylic acid used for the uptake was 72 μM . The concentration of the compounds added was 20 mM except 5 mM for propicillin and 10 mM for salicylic acid.

a) $p < 0.001$, b) $p < 0.01$, c) $p < 0.05$.

not inhibit the BBB extraction of [^3H]acetic acid and [^{14}C]salicylic acid, supporting our previous suggestion that some basic drugs are transported *via* choline carrier system at the BBB.

Figure 1 illustrates the pH dependency of the [^{14}C]salicylic acid uptake into the BBB. The percentage of the brain extraction of [^{14}C]salicylic acid was the highest at pH 4.0 and lowered significantly at pH 5.0, 6.0 and 7.4. As matched to the results in Table III, the value was significantly diminished by the presence of 10 mM of salicylic acid at pH 4.0, 5.0 and 6.0. Being similar to the result with salicylic acid, the brain extraction of [^3H]acetic acid and [^{14}C]nicotinic acid was also inhibited at pH 6.0 by 20 mM acetic acid and nicotinic acid (Table I, II). The pH dependent BBB transport of these monocarboxylic acids is suggested to be ascribed not to a pH-partition hypothesis but to a proton-gradient dependent and carrier-mediated mechanism. Recently, we have demonstrated similar transport characteristics in that the intestinal transport of [^{14}C]nicotinic acid was pH dependent⁹⁾ and occurred *via* a proton-coupled and active transport system, although Elbert *et al.*¹⁰⁾ reported that nicotinic acid is transported into the small intestine by a passive diffusion following a pH partition hypothesis. However, the physiological meaning for the enhanced BBB uptakes of these monocarboxylic acids in the acidic pH region at the BBB remains to be elucidated.

Fig. 1. pH and Concentration Dependency of [^{14}C]Salicylic Acid Uptake into the Blood-Brain Barrier.

Open and closed circles represent the percentage of brain extraction of [^{14}C]salicylic acid in the absence and presence of the unlabeled salicylic acid at 10 mM, respectively. Each point represents the mean $\pm$ S.E.M. of 3-5 rats. When the S.E.M. was small, it was included in the symbol.

In conclusion, our present study indicates that acidic drugs such as salicylic acid, benzoic acid, nicotinic acid, valproic acid and some of β -lactam antibiotics are taken up in a carrier-mediated manner through the BBB most probably *via* a monocarboxylic acid transport system and that this carrier system acts more effectively in the acidic pH. We also found that there is no difference of the BBB transport of monocarboxylic acids between SHRSP and WKY.

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