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Enantioselective Pharmacokinetics of α-Fluoromethylhistidine in Rats and Its Comparison with Histidine

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Abstract—The enantiomer-specific pharmacokinetics of histidine and its analogue, α-fluoromethylhistidine (FMH), were investigated in rats. After bolus intravenous administration of each enantiomer of histidine or FMH at a dose of 40·3 mg kg^{-1} as free base equivalents, the plasma concentrations of L-histidine, D-histidine, (*S*)-FMH and (*R*)-FMH decreased biexponentially with half-lives of 39·2, 20·8, 32·8 and 25·0 min, respectively, in the elimination phase. Although the concentration of L-histidine in the plasma was lower than that of D-histidine, there was no large difference in plasma concentration-time curves of the enantiomers of FMH. The apparent total clearance of L-histidine from rat plasma was about 4 times that of D-histidine or the enantiomers of FMH. L-Histidine was quickly transferred to the peripheral tissues where the concentrations also decrease biphasically. L-Histidine penetrated more rapidly into the brain than either its D-enantiomer or a compound closely related in structure such as FMH. However, the disappearance of L-histidine from the various brain regions was very rapid. In contrast, brain/plasma ratios of D-histidine and (*S*)-FMH increased continuously after injection of these compounds, indicating that D-histidine or (*S*)-FMH partitioned into the brain and was very slowly removed from the brain; (*R*)-FMH was not distributed to the brain. These results suggested stereoselectivity in disposition of histidine and FMH enantiomers in rats.

(*S*)-α-Fluoromethylhistidine ((*S*)-FMH) is a potent and specific inhibitor of L-histidine decarboxylase, a histamine-forming enzyme (Kollonitsch et al 1978). Its in-vivo administration depletes the pool of histamine with rapid turnover (Garbarg et al 1980; Maeyama et al 1982; Mochizuki et al 1991). (*S*)-FMH has, therefore, been widely used in physiological and pharmacological studies of histamine (Bartholeyns & Bouclier 1982; Duggan et al 1984; Tung et al 1985; Watanabe et al 1990). We previously reported the plasma pharmacokinetics and transfer of this drug to various tissues in rats (Sakurai et al 1990). We are now interested in comparing the pharmacokinetic parameters of (*S*)-FMH with those of L-histidine, the parent amino acid of (*S*)-FMH. L-Histidine is present in a variety of mammalian tissues and is a precursor of histamine. Much has been published on the transport, uptake and metabolism of amino acids by animal cells in-vivo (Wolf et al 1956; Baldridge & Tourtellotte 1958; Brown et al 1960; Chirigos et al 1960). There is, however, no report of stereoselective pharmacokinetic studies on L-histidine and its optical isomer, D-histidine, in rats. FMH also has two distinct stereoisomers: (*R*)- and (*S*)-forms. Kollonitsch et al (1978) reported that (*R,S*)-FMH is a potent and rapid inactivator of mammalian histidine decarboxylase, but the time-dependent character of the inactivation caused by α-fluoromethyl amino acids should be attributed to the fluoroalanyl moiety and the selectivity is attributed to the *R* substituents. Kubota et al (1984) reported that L-histidine, a substrate, protected histidine decarboxylase against inactivation by (*S*)-FMH, whereas D-histidine, a non-substrate, did not. There are also quantitative effects of cross-inhibition of transport of related amino acids (Webber et al 1961; Wilson & Scriver 1967; Brenton & Gardiner 1988). Therefore the importance of the enantio-selective pharmacokinetic studies of amino acids and their closely related compounds is recognized.

The present study examines the difference in the disposition of enantiomers of histidine and its analogue, FMH, in rats.

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Materials and Methods

Materials

(*S*)- and (*R*)-FMH were supplied by Dr J. Kollonitsch, Merck Sharp and Dohme Research Laboratories, Rahway, NJ, USA. L- and D-Histidine were obtained from Peptide Institute Inc. (Osaka, Japan). All other reagents were of the highest grade available.

Animal experiments

Male Wistar rats, 200–250 g, purchased from Japan SLC, Inc. (Hamamatsu, Japan) were housed at a constant temperature (23 ± 1°C) and a constant humidity (55 ± 5%), and the light cycle was automatically controlled (0700–1900 h). The rats were fasted for 18 h before the experiments and were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.). (*S*)- or (*R*)-FMH monohydrochloride hemihydrate in 0·9% NaCl (saline) was injected rapidly into the femoral vein at a dose of 50·0 mg kg^{-1} (40·3 mg kg^{-1} of free base equivalent), a dosage which is effective pharmacologically. Similarly, L- or D-histidine monohydrochloride monohydrate in saline was intravenously (i.v.) administered at a dose of 54·5 mg kg^{-1} (40·3 mg kg^{-1} as a free base). Blood samples were collected from rats prepared at each defined time by cardiocentesis into heparinized Vacutainers after drug administration. The plasma was promptly separated by centrifugation at 800 *g*

for 10 min. After each collection of the blood, the liver, stomach, proximal small intestine, adrenal glands, kidneys and brain were quickly removed and chilled. The liver and kidneys were freed from blood by infusion of ice-cold saline, and the mucosae were scraped off the stomach and small intestine. The brain was placed on ice and separated into the cerebellum, hippocampus, striatum, amygdala, cerebral cortex, midbrain, thalamus and hypothalamus by the method of Glowinski & Iversen (1966) with slight modifications (Onodera et al 1988). The plasma and tissues were homogenized for 10 s in a sonicator (Sonifier 450, Branson, USA) in an ice bath in 2 and 9 vol, respectively, of ice-cold 0·4 M perchloric acid.

Analytical methods

The sonicated samples were centrifuged at 9000 *g* for 10 min at 4°C, and the supernatant obtained was filtered through an Ultra-free C3GC filter (Millipore, Tokyo, Japan) and stored at −80°C until assayed. The concentrations of (*S*)- or (*R*)-FMH in clear samples were analysed by ion-exchange chromatography as previously described (Sakurai et al 1990). L- or D-Histidine concentrations were determined in a Hitachi Model 835 amino acid analyser equipped with a detector and a stainless steel column (150 × 4·0 mm i.d.) packed with a Hitachi custom ion-exchange resin (#2619, 5 μm particle size). Clear supernatant (50 μL) was injected into the column and material was eluted with 26·3 (containing 13% ethanol), 26·3 (containing 2% ethanol), 50·0 and 90·0 mM citrate buffer (pH 3·3, 3·2, 4·3 and 4·9, respectively) at a flow rate of 0·225 mL min^{-1}. The eluate was mixed at 53°C with ninhydrin solution flowing at a rate of 0·3 mL min^{-1}, and the absorbance of the mixture at 570 nm was measured. The concentrations of L- or D-histidine in the plasma and tissues were obtained by subtracting the concentrations of endogenous L-histidine.

Data analysis

Plasma concentration-time curves were analysed by a two-compartment model according to the least-squares regression analysis program MULTI for biexponential decline (Yamaoka et al 1981). The area under the plasma concentration time curve (AUC) and the mean residence time (MRT) were calculated by standard linear trapezoidal integration with extrapolation to infinite time. The tissue-to-plasma concentration ratios (T/P ratios) were estimated at various times after drug administration. Values are presented as means ± s.e.m. for n experiments and enantiometric differences were analysed for their significance by Student's *t*-test. $P < 0.05$ was considered to be significant.

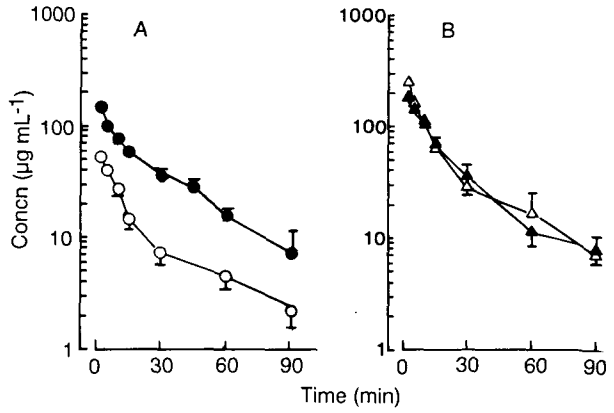

FIG. 1. Plasma concentration of enantiomers of histidine and FMH after intravenous injection into rats. Each point is the mean ± s.e. of 4–5 experiments. ○ L-Histidine, ● D-histidine, △ (*S*)-FMH, ▲ (*R*)-FMH.

Results

Time course of decrease in plasma concentrations of enantiomers of histidine and FMH

Fig. 1 shows the time course of changes in plasma concentrations of enantiomers of histidine and FMH after rapid intravenous injection of each form of histidine or FMH. The pharmacokinetic parameters of the enantiomers are listed in Table 1.

Distribution of enantiomers of histidine and FMH to tissues

Figs 2A and 3A show the time courses of changes in concentration of each enantiomer of histidine and FMH in the liver, kidney, small intestinal mucosa and brain after their rapid intravenous injections. The tissue-to-plasma concentration ratios (T/P ratios) of enantiomers of histidine and FMH

Table 1. Pharmacokinetic parameters of enantiomers of histidine and FMH after rapid intravenous administration of their hydrochloride salts to rats.

Parameter	meaning	Value				Units
		L-Histidine	D-Histidine	(*S*)-FMH	(*R*)-FMH	
A	Defined in the equation below	57·3 ± 0·8	117·7 ± 13·9[b]	258·7 ± 13·5	150·9 ± 8·5[a]	$\mu g\ mL^{-1}$
B	Defined in the equation below	10·0 ± 1·6	95·1 ± 11·8[b]	58·6 ± 1·1	75·8 ± 7·2[a]	$\mu g\ mL^{-1}$
α	Defined in the equation below	0·129 ± 0·001	0·451 ± 0·094[b]	0·161 ± 0·012	0·133 ± 0·009	min^{-1}
β	Defined in the equation below	0·016 ± 0·001	0·033 ± 0·001[b]	0·022 ± 0·003	0·029 ± 0·005	min^{-1}
k_{12}	Transfer rate constant from C* to P*	0·045 ± 0·004	0·182 ± 0·059[b]	0·062 ± 0·002	0·038 ± 0·001	min^{-1}
k_{21}	Transfer rate constant from P* to C*	0·034 ± 0·002	0·242 ± 0·030[b]	0·048 ± 0·006	0·064 ± 0·011	min^{-1}
k_{e1}	Elimination rate constant from C*	0·067 ± 0·007	0·061 ± 0·004	0·073 ± 0·007	0·061 ± 0·005	min^{-1}
Vd_1	Volume of distribution of C*	605·4 ± 7·4	196·4 ± 21·6[b]	127·4 ± 5·6	177·8 ± 1·2	$mL\ kg^{-1}$
Vd_2	Volume of distribution of P*	786·4 ± 28·6	134·2 ± 10·5[b]	166·3 ± 6·1	109·6 ± 19·2	$mL\ kg^{-1}$
Vd_{ss}	Steady-state volume of distribution ($Vd_1 + Vd_2$)	1391·8 ± 23·2	262·1 ± 66·4[b]	293·7 ± 3·1	287·5 ± 18·1	$mL\ kg^{-1}$
$t_{\frac{1}{2}\alpha}$	Half-time of distribution phase	5·4 ± 0·02	1·7 ± 0·3[b]	4·4 ± 0·3	5·2 ± 0·3	min
$t_{\frac{1}{2}\beta}$	Half-time of elimination phase	39·2 ± 1·9	20·8 ± 0·3[b]	32·8 ± 4·3	25·0 ± 4·3	min
AUC	Area under the plasma concentration time curve	964·3 ± 96·5	3345·8 ± 154·8[b]	4036·9 ± 430·6	3798·7 ± 376·1	$\mu g\ min\ mL^{-1}\ kg^{-1}$
CL	Total body clearance ($k_{e1} \cdot Vd_1$)	40·9 ± 4·6	11·8 ± 0·6[b]	9·4 ± 1·3	10·8 ± 1·0	$mL\ min^{-1}\ kg^{-1}$
MRT	Mean residence time	30·7 ± 3·0	29·6 ± 0·7	27·3 ± 0·2	28·3 ± 4·9	min

The equation fitted to the data was, $C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ for the plasma concentration, C_t, at time t, and pharmacokinetic parameters (average of 4–5 experiments) were calculated. C* and P* represent the central and peripheral compartments, respectively. Enantiomeric differences in histidine or FMH. [a] $P < 0.05$, [b] $P < 0.01$.

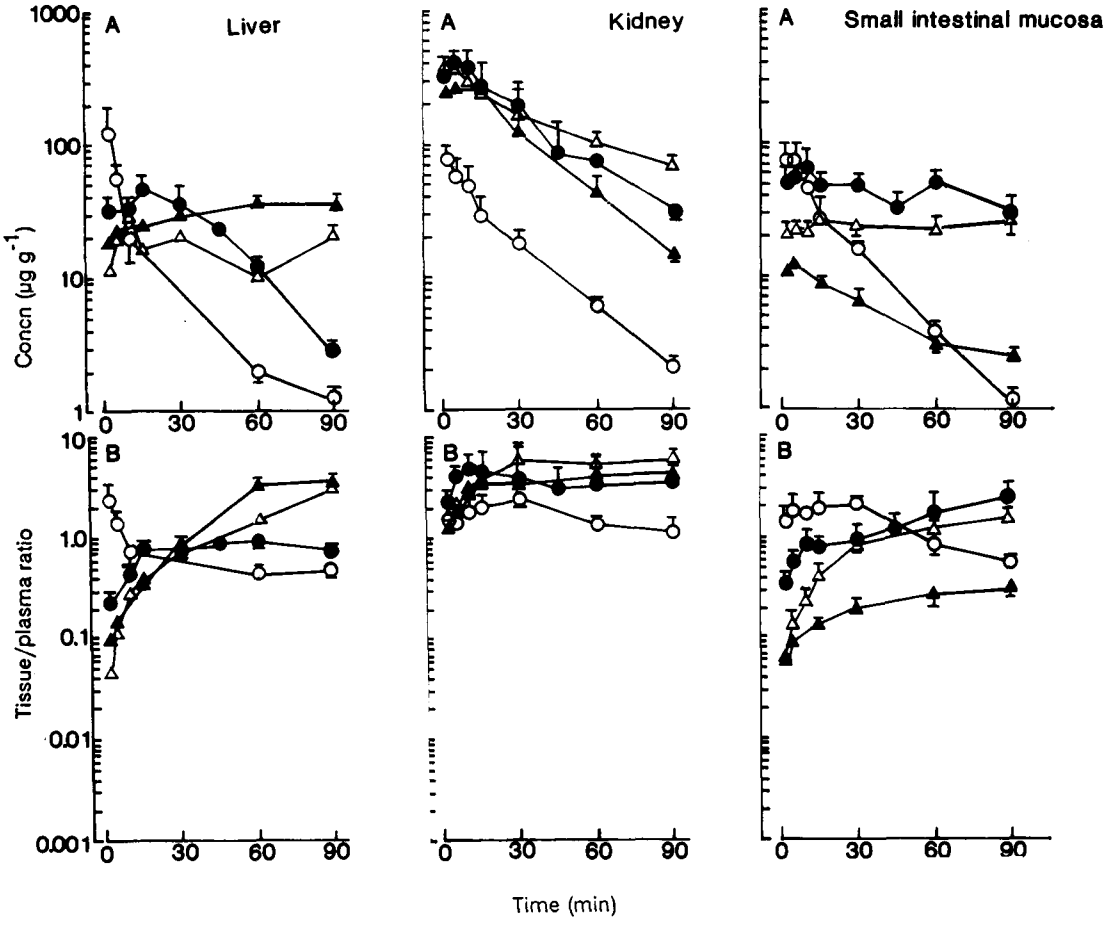

FIG. 2. Time courses of transfer of enantiomers of histidine and FMH to the liver, kidney and small intestinal mucosa. Each point represents the mean ± s.e. of 4–5 experiments. ○ L-Histidine, ● D-histidine, △ (*S*)-FMH, ▲ (*R*)-FMH.

determined at various times after administration of each histidine or FMH enantiomer are shown in Figs 2B and 3B.

Discussion

Although (*S*)-FMH, a new specific inhibitor of histidine decarboxylase, strongly inhibits histamine formation in-vivo after a single administration, the decrease of histamine is not prolonged; the histamine level begins to increase again after 8–12 h and returns to the original level after 24 h (Garbarg et al 1980; Maeyama et al 1982). We previously suggested that the reason for this short duration of histamine depletion by (*S*)-FMH was due to its rapid clearance or its low transfer to tissues because it is an amino acid analogue and is transported through an amino acid transporting system (Sakurai et al 1990). However, the present study shows that the disposition of an α-fluoromethyl derivative, a synthetic amino acid, was quite different from that of the parent amino acid, L-histidine. Although the disappearance of L-histidine from rat plasma was very rapid, L-histidine was indeed taken up readily by animal tissue (Figs 2, 3). It seems, therefore, that L-histidine penetrates into various peripheral tissues by the same mechanisms as do the natural amino acids such as a tyrosine, confirming the previous findings (Chirigos et al 1960). However, D-histidine and each enantiomer of FMH remained for longer periods in the various peripheral tissues (except kidney) than did L-histidine. Also, the findings that the T/P ratio in the kidney reached equilibrium within 30 min after administration of each enantiomer of FMH or D-histidine and was greater than unity (Figs 2, 3) suggested that rat kidney has a specific transport mechanism for the uptake of these compounds.

The transport of amino acids in the mammalian central nervous system has been extensively investigated (Yudilevich et al 1972), and the existence of separate transport systems at the blood-brain barrier and in brain cells is well established (Brenton & Gardiner 1988). The plasma membrane of the brain capillary endothelial cell is now recognized as the site of several facilitated diffusion systems (Bradbury 1984) including those for glucose, monocarboxylic acid and amino acids. Brenton & Gardiner (1988) reported that fractional extraction of phenylalanine and alanine was stereospecific with preference for the L-enantiomer, using a single-pass indicator dilution technique. In the lamb, the fractional extraction value was about 3 times larger for L-phenylalanine than for the D-enantiomer. It was also shown that L-tyrosine penetrated more rapidly into rat brain than either its D-isomer or a number of other compounds of closely related structure (Chirigos et al 1960). In this work, we also found that in rats there was a large difference in transfer to the brain between the enantiomers of histidine. The rapid transfer of L-histidine to the various regions was observed after its administration, and the regional distribution of L-histidine 2 min after its bolus intravenous injection was positively correlated with the

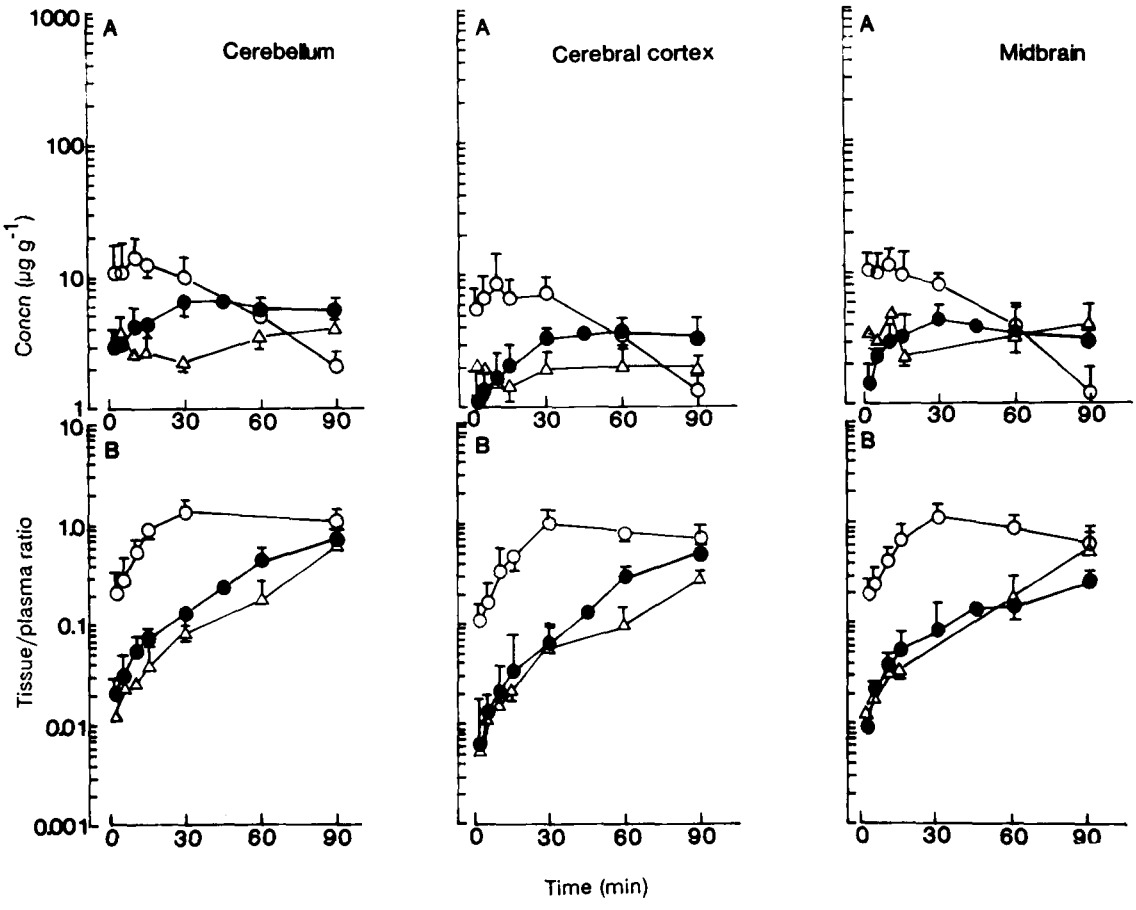

FIG. 3. Time courses of transfer of enantiomers of histidine and FMH to the various regions of rat brain. Each point represents the mean ± s.e. of 4–5 experiments. ○ L-Histidine, ● D-histidine, △ (*S*)-FMH. (*R*)-FMH was not detected in the brain.

histamine concentration in the various regions of rat brain (data not shown). However, the concentration of L-histidine in the brain very quickly decreased. Conversely, although the uptake rate of D-histidine by rat brain in-vivo was slow, the concentration increased continuously until 30 min after D-histidine injection and reached an equilibrium in various parts thereafter. Our results, therefore, suggested the existence of respective stereoselective transport and elimination systems of histidine enantiomers at the blood-brain barrier or in brain cells. In contrast with the transfer of histidine to brain, the (*S*)-FMH concentrations in brain regions were lower, but its tissue/plasma ratios in various tissues increased continuously after injection as for D-histidine, indicating that (*S*)-FMH partitioned into these regions and was lost from them very slowly, whereas no transfer of (*R*)-FMH to brain was observed. Thus, the transfer system was changed by the α-fluoromethylation of histidine, suggesting that a stereoselective transport mechanism of FMH at the blood-brain barrier may also exist.

August et al (1985) indicated little biotransformation of (*S*)-FMH in man. Our experiments also showed that (*S*)- or (*R*)-FMH may be unmetabolized in rats because of its rapid clearance and its low transfer to tissues. The mammalian metabolism of L-histidine has been studied extensively (Tabor 1954; Cowgill & Freeburg 1957; Baldridge & Tourtellotte 1958; Brown et al 1960; Aures et al 1968; Schayer & Reilly 1973). D-Histidine may be unmetabolized in rat tissues because most of the enzymes responsible for metabolism of histidine are specific to the L-enantiomer.

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