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The Disposition of (*R*)-α-Methylhistamine, a Histamine H_3-Receptor Agonist, in Rats

SEIJI YAMASAKI, EIICHI SAKURAI, NOBORU HIKICHI, NARUHIKO SAKAI*, KAZUTAKA MAEYAMA* AND TAKEHIKO WATANABE*

*Department of Pharmaceutics I, Tohoku College of Pharmacy, 4-1 Komatsushima 4-chome, Aoba-ku, Sendai 981, and *Department of Pharmacology I, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan*

Abstract—Using a modified HPLC method with a fluorescence spectrophotometer and a weak cation exchanger, it was possible to separate (*R*)-α-methylhistamine (α-methylhistamine) from histamine in plasma and various tissues. The assay was used to study the disposition and pharmacokinetic analysis of α-methylhistamine after a bolus intravenous administration to rats. After rapid intravenous administration (12·6 mg kg^{-1}), the plasma concentration declined biexponentially with a half-life of 1·3 min in the elimination phase. The area under the plasma concentration-time curve and total body clearance were 130 μg min mL^{-1} and 97 mL min^{-1} kg^{-1}, respectively. After administration, α-methylhistamine was immediately transferred to various tissues. The concentration was high in the kidney, lung, and liver (kidney > lung > liver), but low in the brain. The tissue-to-plasma concentration ratios in peripheral tissues were greater than 1, suggesting that the transfer of α-methylhistamine to peripheral tissues was due to a specialized transport mechanism or possibly to tissue binding. However, the finding that the tissue/plasma ratio in the brain was lower than unity suggests that the transport system in this tissue depends on a concentration gradient, and that α-methylhistamine crosses the blood-brain barrier in rats with difficulty.

Since the location and distribution of the histaminergic neuron system was elucidated immunohistochemically (Panula et al 1984; Steinbusch & Mulder 1984; Watanabe et al 1984) and the existence and the functions of the presynaptic histamine H_3 receptor were suggested (Arrang et al 1983), the roles of histamine on the central nervous system have been investigated (Wada et al 1985; Schwartz et al 1991). Meanwhile, (*R*)-α-methylhistamine (α-methylhistamine) and thioperamide were developed as highly potent and selective H_3-receptor agonists and antagonists, respectively, which regulate the neuronal release and synthesis of histamine (Arrang et al 1987). These drugs have been used as research tools to clarify the functions of the H_3 receptor (Garbarg et al 1989; Hill 1990; Timmerman 1990; Sakai et al 1991). Moreover, α-methylhistamine is undergoing clinical trials (Arrang et al 1991), but basic pharmacokinetic studies of these drugs have not been carried out. One of the reasons was the lack of a simple and effective method for the measurement of α-methylhistamine, because α-methylhistamine is not separated from histamine in a routine HPLC system.

The aim of this study was to establish a simple and sensitive method for measurement of α-methylhistamine using a direct injection HPLC method, and to investigate its plasma pharmacokinetics and transfer to tissues in rats.

Materials and Methods

Materials

α-Methylhistamine dihydrochloride was kindly supplied by Sumitomo Pharmaceutical Industries, Osaka, Japan. All other chemicals of the highest grade available were purchased from Wako Chemical Co., Osaka, Japan.

Correspondence: N. Hikichi, Department of Pharmaceutics I, Tohoku College of Pharmacy, 4-1 Komatsushima 4-chome, Aoba-ku, Sendai 981, Japan.

Animal experiments

Male Wistar rats, 250–300 g (Japan SLC Inc., Hamamatsu, Japan), were fasted for 18 h before experimentation and anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.). α-Methylhistamine dihydrochloride in 0·9% NaCl (saline) was rapidly injected into the femoral vein at a dose of 20 mg kg^{-1} (12·6 mg kg^{-1} of free base equivalent). Blood samples (0·5 mL) were collected by cardiocentesis into heparinized Vacutainers at appropriate time intervals after drug administration. The plasma was immediately separated by centrifugation at 800 *g* for 10 min. After collection of the blood, the brain, liver, kidneys and lungs were quickly removed and chilled. The liver and kidneys were exsanguinated by infusion of ice-cold saline. The plasma and tissues in 2- and 5-fold vol, respectively, of ice-cold 0·4 M perchloric acid containing 2 mM EDTA Na_2 were sonicated in an ice-bath for 10 s (Sonifier-450, Branson, NJ, USA). The homogenized sample was centrifuged at 9000 *g* for 10 min at 4°C, and the supernatant was stored at −80°C until assayed.

Analytical methods

The concentrations of α-methylhistamine in the deproteinized extracts were determined by the HPLC method described by Fukuda et al (1991) with slight modifications as follows. A solution of 37·5 mM citric acid and 0·18 M imidazole adjusted to pH 6·52 was mixed with methanol (82·5:17·5 v/v%) and used as a mobile phase. α-Methylhistamine was separated with an ion exchange resin, CM-2SW (Tosoh, Tokyo, Japan, 250 × 4·6 mm i.d.; particle size 5 μm) eluted with the mobile phase at a flow rate of 0·6 mL min^{-1} using an HPLC pump L-6000 (Hitachi, Tokyo, Japan). α-Methylhistamine in the eluate was derivatized using an on-line automated *o*-phthalaldehyde method (Shore et al 1959), and the fluorescence intensity was determined in a fluoromonitor (FS-8000, Tosoh, Tokyo, Japan) using excitation and emission wavelengths at 360 and 450 nm, respectively.

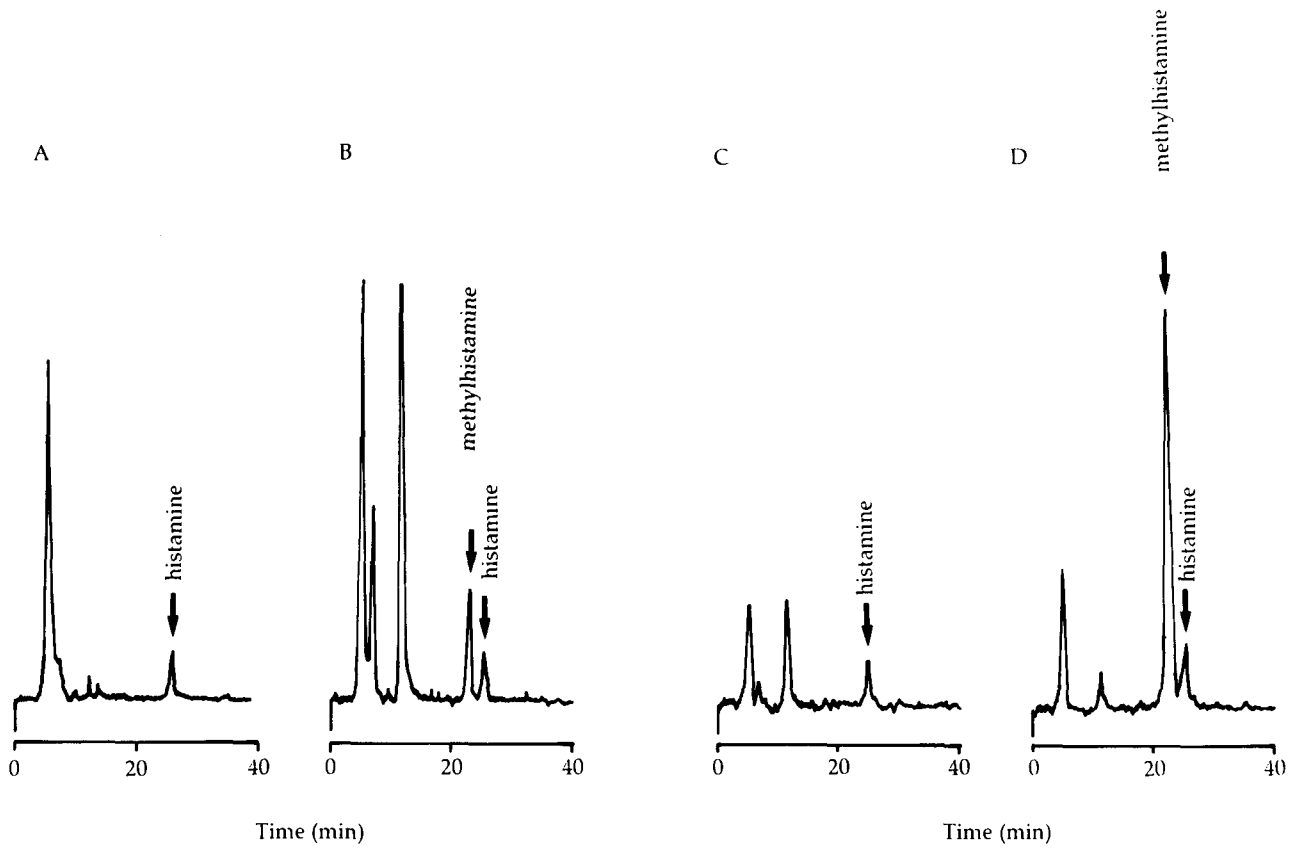

FIG. 1. Chromatograms of deproteinized samples of the plasma (A, B) and lung (C, D) of rats 30 min after intravenous injection of 12·6 mg kg^{-1} α-methylhistamine. Control rats were treated with saline only. A, C, Control samples; B, D, α-methylhistamine-treated samples.

Data analysis

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

Results

Determination of α-methylhistamine in plasma and tissues

Fig. 1 shows chromatograms of deproteinized samples of rat plasma (A,B) and lung (C,D) obtained 30 min after rapid intravenous injection of saline (A,C) or α-methylhistamine (B,D). There were no interfering peaks in the region with a retention time corresponding to that of α-methylhistamine (22·3 min) in the chromatogram: the α-methylhistamine peak was symmetrical and well separated from histamine (eluted at 25·8 min) and other compounds. Similar chromatograms were also obtained from other tissue samples (data not shown). The calibration curve was linear over the range of 10 ng mL^{-1}–300 μg mL^{-1} with a correlation coefficient of more than 0·98 (n = 10). The lower limit of determination was 5 ng mL^{-1} and the coefficient of variance was 5·3%.

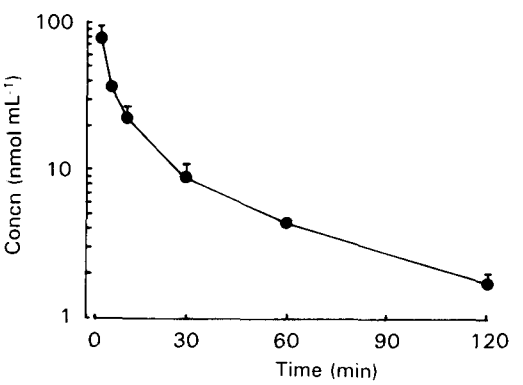

FIG. 2. Plasma concentrations of α-methylhistamine after intravenous injection of 12·6 mg kg^{-1} α-methylhistamine into rats. Each point is the mean ± s.e. of 4–7 rats.

Time-course of decrease in plasma concentration of α-methylhistamine

Fig. 2 shows the time-course of changes in plasma concentrations of α-methylhistamine after its rapid intravenous administration to rats. The pharmacokinetic parameters are listed in Table 1.

Distribution to tissues

The time-course of changes in the concentrations of α-methylhistamine in the brain, liver, kidney and lung after its rapid intravenous injection is shown in Fig. 3. The

Table 1. Pharmacokinetic parameters of α-methylhistamine after rapid intravenous administration to rats.

Parameter	Meaning	Value	Unit
A	Defined in the equation below	17·6	$\mu g\ mL^{-1}$
α	Defined in the equation below	0·54	min^{-1}
$t_{\frac{1}{2}\alpha}$	Half-life time of distribution phase	1·3	min
B	Defined in the equation below	3·98	$\mu g\ mL^{-1}$
β	Defined in the equation below	0·04	min^{-1}
$t_{\frac{1}{2}\beta}$	Half-life of elimination phase	17·3	min
Vd_1	Distribution volume of C*	586	$mL\ kg^{-1}$
Vd_2	Distribution volume of P*	1240	$mL\ kg^{-1}$
Vd_{ss}	Steady-state distribution volume (Vd_1+Vd_2)	1826	$mL\ kg^{-1}$
K_{12}	Transfer rate constant from C* to P*	0·28	min^{-1}
K_{21}	Transfer rate constant from P* to C*	0·13	min^{-1}
K_{el}	Elimination rate constant from C*	0·17	min^{-1}
CL	Total body clearance	97·0	$mL\ min^{-1}\ kg^{-1}$
AUC	Area under the plasma concentration-time curve	130	$\mu g\ min\ mL^{-1}$

α-Methylhistamine dihydrochloride (20 mg kg^{-1}) was administrated intravenously. The data were fitted to the equation $C_t = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ for the plasma concentrations. C* and P* represent the central and peripheral compartments, respectively. Values are means of n = 7–12.

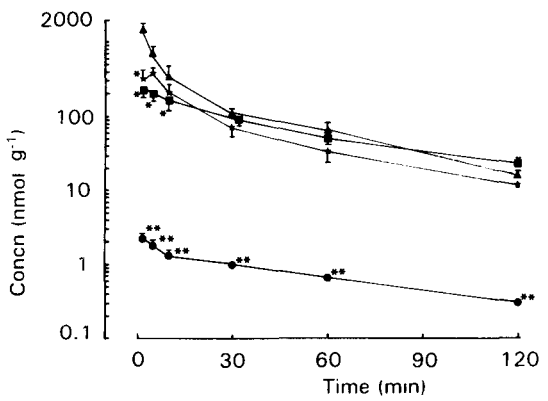

FIG. 3. Time-course of transfer of α-methylhistamine to tissues. Injection of α-methylhistamine and measurement of its concentrations in tissues were as described in Materials and Methods. Each point is the mean ± s.e. of 4–7 rats. ▲ Kidney, ■ liver, ★ lung, ● brain. $*P<0·05$, $**P<0·001$ compared with kidney concentrations at the same time point.

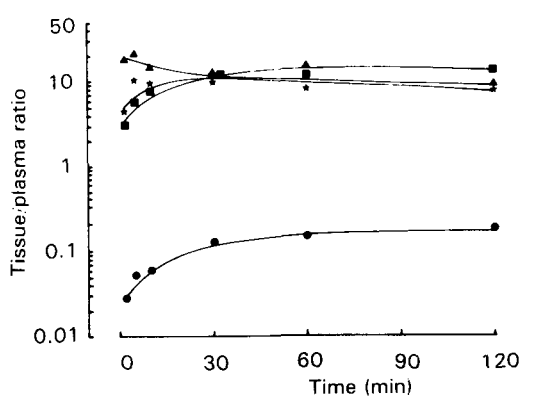

FIG. 4. Tissue-to-plasma concentration (T/P) ratios of α-methylhistamine in various tissues after intravenous administration to rats. Each point is the mean ± s.e. of 4–7 rats. ▲ Kidney, ■ liver, ★ lung, ● brain.

concentration of α-methylhistamine in the kidney was significantly higher than that in the other tissues, but each curve decreased biphasically. The T/P ratios of α-methylhistamine estimated at various times are shown in Fig. 4. The values in the peripheral tissues (liver, kidney and lung) were much larger than 1, but the T/P ratio in the brain was less than 1. The values in most tissues reached a steady state at 60 min after administration.

Discussion

In the present study, we clarified the disposition of α-methylhistamine. The histamine H_3 receptor which regulates histamine release and synthesis exists in the brain and peripheral tissues (Arrang et al 1987; Barnes 1991; Kilpatrick & Michel 1991). The histaminergic neurons are concentrated in the tuberomammillary nucleus of the posterior hypothalamus (Panula et al 1984; Steinbusch & Mulder 1984; Watanabe et al 1984), and project their fibres to almost all regions in the mammalian brain from the olfactory bulb to the spinal cord (Inagaki et al 1990). These observations suggest that the histaminergic neurons participate widely in various functions of the central nervous system. Studies on the role of brain histamine, such as the sleep-wakefulness cycle, feeding, drinking, thermoregulation, catalepsy, locomotion, convulsion and other functions have already been reported (Watanabe & Wada 1991; Yokoyama et al 1992). Thus, a histamine H_3-receptor agonist such as α-methylhistamine that inhibits histamine release could be expected to have potential clinical applications for disorders induced by histamine. This drug is under initial clinical trials (Arrang et al 1991). In addition, pentagastrin-stimulated gastric acid secretion in cats is inhibited by α-methylhistamine (Coruzzi et al 1991), and its clinical application for gastric ulceration may also be expected. Thus, it is very important to clarify the disposition of the drug for clinical application.

The HPLC method developed is specific, sensitive and stable enough to study the disposition of α-methylhistamine and was applicable to the investigation of the effect of α-methylhistamine on tissue histamine concentrations. Recently, Itoh et al (1992) reported a method for assay of histamine using ion-pair HPLC, which also allows measurement of α-methylhistamine.

Although the disappearance of α-methylhistamine from rat plasma was very rapid, it was taken up readily by rat peripheral tissues. However, the concentration of α-methylhistamine in the brain was much lower than those in peripheral tissues, suggesting that its transfer to the brain was not so efficient.

The T/P ratio is one of the most useful methods to characterize the transport mechanism of a drug. If the T/P ratio is equal to or smaller than 1, its transport is through the passive diffusion mechanism, and if the ratio is greater than 1, there is a special transport mechanism, such as a facilitated, or active, or phagocytic, or receptor-mediated transport. In this study, the T/P ratio of α-methylhistamine in peripheral tissues (kidneys, liver and lung) was much higher than 1 whereas that in brain was smaller than 1. These results suggest the presence of a specific transport mechanism in peripheral tissues for uptake of α-methylhistamine. An alternative explanation is that α-methylhistamine binds to the tissue proteins more strongly than to plasma proteins, resulting in higher concentrations in the tissues after the drug has been substantially cleared from the blood. The distribution mechanism to the brain depended on a gradient of drug concentration, and it was evident that α-methylhistamine crossed the rat blood-brain barrier with difficulty. This information is helpful in pharmacological studies on H_3 ligands.

Since concentrations of α-methylhistamine and histamine in the plasma and tissues can be measured simultaneously using our HPLC method, it is now possible to investigate the relation between the pharmacokinetic and pharmacological effects of α-methylhistamine.

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