Assessment of Antihistaminic and Antimuscarinic Effects of Optical Isomers of Ephedrine and Methylephedrine by Receptor Binding Assay in Guinea Pig Ileal Muscle

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Effects of optical isomers of ephedrine (EPH) and methylephedrine (MEP) on histamine H₁-receptors and muscarinic receptors in guinea pig ileal muscle were investigated by radioligand binding assay and by measuring the mechanical response to histamine and acetylcholine. EPH and MEP inhibited the specific binding of [³H]mepyramine and [³H]quinuclidinyl benzilate (QNB) to microsomal fractions prepared from this tissue. The rank order of inhibitory potency of [³H]mepyramine and [³H]QNB binding was *d*->*l*-isomer and *l*->*d*-isomer, respectively. Furthermore, the rank order of antagonistic potency in the mechanical response study was the same as that in the binding study. *d*-MEP competitively antagonized histamine-induced contraction (pA₂ value; 5.14). These results suggest that each isomer of EPH and MEP has a distinct affinity for histamine H₁-receptors and muscarinic receptors in guinea pig ileal muscle. The antihistaminic and antimuscarinic activity of these compounds may be largely attributed to competition at receptor sites. In addition, it is suggested that *d*-MEP exhibits a competitive antagonist activity for the histamine H₁-receptor.

Key words methylephedrine; ephedrine; optical isomer; receptor binding; [³H]mepyramine; [³H]quinuclidinyl benzilate

Methylephedrine, the *N*-methylated derivative of ephedrine, was developed as an anti-allergic agent about fifty years ago.¹⁾ Ephedrine and methylephedrine contain two asymmetrical carbon atoms and so optical isomers exist. Racemic methylephedrine has been commonly used as an antitussive drug with bronchodilative activity in Japan.²⁻⁷⁾ It is generally recognized that most of the pharmacological actions of ephedrine and methylephedrine in peripheral organs are due to their sympathomimetic activities, which are considered to be in the order ephedrine > methylephedrine.^{8,9)} In addition, it is accepted that the *l*-isomers of sympathomimetic amines exhibit more potent activity than the corresponding *d*-isomers in peripheral tissues.¹⁰⁾ The *l*-isomers of ephedrine and methylephedrine are reported to be more potent than their corresponding *d*-isomers in many experiments.^{9,11-14)} It is also reported that the optical isomers of ephedrine and methylephedrine relax or inhibit contractions induced by histamine and acetylcholine (ACh) in isolated tissues.^{15,16)} However, the rank order of relaxing and inhibitory potency for histamine-induced contraction was *d*->*l*-isomer in previous reports. In addition, the rank order is reported to be methylephedrine > ephedrine in guinea pig small intestine.¹⁶⁾ There is a possibility that these compounds have competitive antagonist activity. In the present study, to clarify the action of ephedrine and methylephedrine on histamine H₁-receptors and muscarinic receptors, a radioligand binding study with [³H]mepyramine and [³H]quinuclidinyl benzilate (QNB) has been carried out. In addition, the ability of optical isomers to inhibit the radioligand binding was compared with the results of mechanical response experiments in guinea pig ileal muscle.

MATERIALS AND METHODS

Preparation of Microsomal Fractions Male guinea pigs,

weighing 300 to 500 g, were killed by a blow on the head. A longitudinal muscle strip was isolated by carefully slipping an ileal segment over a tapered glass rod. Longitudinal muscle strips for radioligand binding assay were washed with ice-cold medium containing 0.25 M sucrose and 10 mM Tris-HCl buffer (pH 7.4 at 4 °C). The isolated tissues were minced with scissors and then homogenized using a Polytron homogenizer (Kinematica, Lucerne, Switzerland) in 20 volumes ice-cold medium. The homogenate was centrifuged at 2500 × *g* for 10 min. Then, the supernatant was centrifuged at 15000 × *g* for 20 min. The supernatant was again centrifuged at 100000 × *g* for 60 min and the pellet suspended in 50 mM Tris-HCl buffer (pH 7.4 at 4 °C). The suspension was used for the binding assay as the microsomal fraction.¹⁷⁾ Protein concentrations were determined by the method of Lowry *et al.*¹⁸⁾ using bovine serum albumin as a standard.

Radioligand Binding Assay Binding studies for histamine H₁-receptors and muscarinic receptors were performed with [³H]mepyramine and [³H]QNB, respectively. Microsomal fractions were incubated with 1 nM [³H]mepyramine or 0.2 nM [³H]QNB and various concentrations of the test drugs in a total volume of 300 μl 50 mM Tris-HCl buffer (pH 7.4 at 32 °C). After an incubation period of 60 min, the incubation mixture was rapidly filtered through a Whatman GF/C glass-fiber filter using a Cell Harvester (Brandel, Gaithersburg, MD, U.S.A.). The filters were then dried and the radioactivity determined in a toluene-based scintillator using a liquid scintillation spectrometer (Aloka LSC-3100, Tokyo). The specific binding was determined as the difference between total and nonspecific binding. Nonspecific binding was determined in the presence of 10⁻⁴ M mepyramine for [³H]mepyramine binding and 10⁻⁵ M atropine for [³H]QNB binding. Inhibition of specific binding by unlabeled test drug was determined. The ability to inhibit specific binding was expressed as the pIC₅₀ value which

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was the negative logarithm of the molar concentration of unlabeled drug necessary to displace 50% of the specific binding. The Hill coefficient for the inhibition by test drug was obtained by pseudo Hill plot analysis.

Mechanical Response Experiment Male guinea pigs were fasted overnight and then killed. Longitudinal muscle preparations from guinea pig ileum were suspended in a 30 ml-organ bath filled with Tyrode solution, kept at 32°C and gassed with a mixture of 95%O₂ and 5%CO₂. The composition of the solution was; 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 0.4 mM NaH₂PO₄, 11.9 mM NaHCO₃ and 5.6 mM glucose. The mechanical response of the muscle preparation was recorded under a tension of 0.5 g using an isotonic transducer (TD-112S, Nihon Kohden, Tokyo). The preparations were allowed to equilibrate for more than 30 min before the start of the experiments. Cumulative administration of histamine or ACh was performed repeatedly. After constant concentration-response curves were obtained, test drug was pretreated for 5 min before the addition of agonist. To clarify the rank order of inhibitory potency, optical isomers including the *dl*-form of the drug (equimolar mixture) were applied. The competitive antagonist potency of the drug was expressed as the pA₂ value which is the negative logarithm of the dissociation constant. The pA₂ value was calculated according to the method of Tallarida *et al.*,¹⁹⁾ which was originally reported by Arunlakshana and Schild.²⁰⁾

Drugs *l*-Ephedrine, *d*-ephedrine, *l*-methylephedrine and *d*-methylephedrine were kindly donated by Fuji Chemical Industries Ltd. (Toyama). Test drugs were used as solutions of the hydrochloride salts in distilled water. Radioactive ligands were purchased from New England Nuclear (Boston, MA, U.S.A.); [³H]mepyramine (specific activity: 24.7 Ci/mmol) and [³H]QNB (specific activity: 52.3 Ci/mmol). Other chemicals used were of analytical grade. The drugs were dissolved in distilled water.

Statistical Analysis The results were expressed as mean value ± S.E. Statistical significance of means was analyzed by Duncan's new multiple range test. A *p* value less than 0.05 was considered to be significant.

RESULTS

Receptor Binding Assay The specific binding of [³H]-mepyramine and [³H]QNB to microsomal fractions from guinea pig ileum were displaced in a concentration-dependent manner by the simultaneous addition of non-radioactive *l*- and *d*-isomers of ephedrine and methylephedrine. Inhibition of [³H]mepyramine binding by ephedrine and methylephedrine is shown in Figs. 1A and 1B, respectively. Similarly, displacement curves of [³H]QNB binding are shown in Figs. 2A and 2B. The pIC₅₀ values as the negative logarithm of the 50% inhibitory concentrations estimated from the displacement curves and Hill coefficients are summarized in Table 1. The pIC₅₀ values of the *d*-isomers for [³H]mepyramine binding were significantly greater than those of the *l*-isomers (*p* < 0.05). On the other hand, the pIC₅₀ values of the *l*-isomers for [³H]QNB binding were significantly greater than those of the *d*-isomers (*p* < 0.05). The Hill

Fig. 1. Effects of Optical Isomers of Ephedrine and Methylephedrine on the Specific [³H]Mepyramine Binding to the Microsomal Fractions from Guinea Pig Ileum

A: *l*-ephedrine (●), *d*-ephedrine (○). B: *l*-methylephedrine (▲), *d*-methylephedrine (△). Abscissa: log concentration (M) of ephedrine or methylephedrine. Each point is presented as a mean ± S.E. of 3 separate experiments.

coefficients calculated from the displacement curve of [³H]mepyramine binding by *l*- and *d*-methylephedrine were not significantly different from unity, while those for *l*- and *d*-ephedrine were. In addition, the Hill coefficients calculated from the displacement curve of [³H]QNB binding by *l*- and *d*-methylephedrine were not significantly different from unity, while those for *l*- and *d*-ephedrine were.

Mechanical Response Experiment In the longitudinal muscle of guinea pig ileum, ephedrine and methylephedrine inhibited histamine- and ACh-induced contractions. The rightward shift of the histamine concentration-response curve by *l*-, *dl*- and *d*-ephedrine and methylephedrine, at 10⁻³ M, is shown in Table 2. The shift by the *l*-isomers was significantly different from that by the *d*-isomers (*p* < 0.05). Then, the rank order of inhibitory potency for the response to histamine and ACh was *d*->*l*-isomer and *l*->*d*-isomer, respectively. The antihistaminic effect of methylephedrine was more potent than that of ephedrine, when each isomer is compared with the other compound possessing the same optical activity (*p* < 0.05). To obtain the pA₂ value of *d*-methylephedrine which showed relatively potent antihistaminic activity, a Schild plot analysis was performed at low concentrations ranging from 3 × 10⁻⁵ to 3 × 10⁻⁴ M. As shown in Fig. 3A, the concentration-response

Fig. 2. Effects of Optical Isomers of Ephedrine and Methylephedrine on the Specific $[^3\text{H}]\text{QNB}$ Binding to Microsomal Fractions from Guinea Pig Ileum

A: *l*-ephedrine (●), *d*-ephedrine (○). B: *l*-methylephedrine (▲), *d*-methylephedrine (△). Abscissa: log concentration (M) of ephedrine or methylephedrine. Each point is presented as a mean $\pm$ S.E. of 3 separate experiments.

curves of histamine were shifted to the right in a parallel manner by *d*-methylephedrine. Each pD_2 value, the negative logarithm of the molar concentration producing half maximum response, in the presence of *d*-methylephedrine was significantly different from the control value ($p < 0.05$). The slope of the regression line was not significantly different from unity (0.93 ± 0.06), and the pA_2 value was 5.14 ± 0.03 (Fig. 3B).

DISCUSSION

Ephedrine and methylephedrine inhibited the specific binding of $[^3\text{H}]\text{mepyramine}$ (histamine H_1 -receptor) and $[^3\text{H}]\text{QNB}$ (muscarinic receptor) to microsomal fractions from the guinea pig ileum. It is suggested that these drugs have affinity for histamine H_1 -receptors and muscarinic receptors. In addition, ephedrine and methylephedrine inhibited the contractile response to histamine and ACh in isolated longitudinal muscle of guinea pig ileum. The rank order of inhibitory potency of optical isomers in the binding study was the same as that in the mechanical response study. These results suggest that ephedrine and methylephedrine inhibit the contractile response to histamine and ACh by blocking histamine H_1 -receptors and muscarinic receptors in the guinea pig ileal muscle.

Table 1. The pIC_{50} Values and Hill Coefficients for the *l*- and *d*-Isomers of Ephedrine and Methylephedrine

	$[^3\text{H}]\text{Mepyramine}$ (Histaminergic)		$[^3\text{H}]\text{QNB}$ (Muscarinic)	
	pIC_{50}	n_H	pIC_{50}	n_H
<i>l</i> -EPH	2.79 ± 0.06	0.70 ± 0.04	2.46 ± 0.13	0.74 ± 0.08
<i>d</i> -EPH	$3.69 \pm 0.05^*$	0.78 ± 0.03	$2.00 \pm 0.11^*$	0.84 ± 0.04
<i>l</i> -MEP	3.68 ± 0.09	0.91 ± 0.07	3.22 ± 0.14	0.88 ± 0.11
<i>d</i> -MEP	$4.96 \pm 0.08^*$	0.94 ± 0.06	$2.86 \pm 0.01^*$	1.32 ± 0.15

$[^3\text{H}]\text{Mepyramine}$, 1 nM; $[^3\text{H}]\text{QNB}$, 0.2 nM; EPH, ephedrine; MEP, methylephedrine; n_H , the Hill coefficient. Each value is presented as a mean $\pm$ S.E. of 3 separate experiments. * Significantly different from the pIC_{50} value of the corresponding *l*-isomer at $p < 0.05$ (Duncan's new multiple range test).

Table 2. The Rightward Shift of Histamine and Acetylcholine Concentration-Response Curve by Optical Isomers of Ephedrine and Methylephedrine (10^{-3} M)

	Rightward shift (log units)	
	Histamine	Acetylcholine
<i>l</i> -EPH	0.79 ± 0.03	1.17 ± 0.05
<i>dl</i> -EPH	$1.04 \pm 0.07^*$	1.07 ± 0.06
<i>d</i> -EPH	$1.17 \pm 0.10^*$	$0.57 \pm 0.07^*$
<i>l</i> -MEP	1.21 ± 0.01	1.27 ± 0.04
<i>dl</i> -MEP	$1.77 \pm 0.05^*$	$1.06 \pm 0.06^*$
<i>d</i> -MEP	$1.93 \pm 0.04^*$	$0.85 \pm 0.05^*$

EPH, ephedrine; MEP, methylephedrine. Each value is presented as a mean $\pm$ S.E. ($n = 4-6$). * Significantly different from the corresponding *l*-isomer at $p < 0.05$ (Duncan's new multiple range test).

Fig. 3. Determination of the pA_2 Value for *d*-Methylephedrine (*d*-MEP)

A: Antagonism of histamine-induced contraction by *d*-MEP. Control (○); *d*-MEP at 3×10^{-5} M (●), 10^{-4} M (▲) or 3×10^{-4} M (■). Histamine was added cumulatively 5 min after drug application. Ordinate: contraction (%), expressed as a percentage of the maximal contraction induced by histamine. Each point is presented as a mean $\pm$ S.E. ($n = 6$). * Significantly different from control group at $p < 0.05$ (Duncan's new multiple range test). B: Schild plot for antagonism of histamine by *d*-MEP.

In the present study, the antihistaminic effect of the *d*-isomer was more marked than that of the *l*-isomer. In addition, the *d*-isomer showed a higher affinity for the histamine H₁-receptor than the *l*-isomer. *d*-Methylephedrine was the most potent among the optical isomers of ephedrine and methylephedrine. The results of the Schild plot analysis suggest that *d*-methylephedrine has a competitive antagonist action on histamine H₁-receptors.

In our latest study using isolated guinea pig trachea,²¹⁾ it was also demonstrated that *d*-methylephedrine competitively antagonizes the contractile response to histamine. There are some reports in the literature that *d*-isomers showed more potent inhibition of the response to histamine than *l*-isomers in smooth muscle tissues.^{15,16)} The mechanism behind these curious observations is clarified by the present study using a radioligand binding assay and Schild plot analysis.

The *d*-methylephedrine-induced rightward shift of the histamine concentration-response curve was much greater than the shift of the ACh curve at the same concentration (10⁻³M). In addition, the pA₂ value of 5.1 against histamine means that the rightward shift of the histamine curve is about 0.3 (log units) at the low concentration of 10⁻⁵M. Furthermore, *d*-methylephedrine exhibits only slight or no sympathomimetic activity.^{9,21)} From these findings, it seems that *d*-methylephedrine is markedly selective for the histamine H₁-receptor. It is thought that *d*-methylephedrine should be recognized as a histamine H₁-receptor antagonist rather than a sympathomimetic amine. Antihistaminic activity is useful for the treatment of allergic disease and inflammation which is mediated through histamine release. *d*-Methylephedrine may play a role as an antihistaminic drug, when racemic methylephedrine is administered to patients with allergic diseases such as asthma.

In general, histamine H₁-receptor antagonists have antimuscarinic actions,^{22,23)} and they have affinity for the two kinds of receptors, the histamine H₁-receptor and the muscarinic receptor.²⁴⁾ Ephedrine and methylephedrine are considered to have a receptor-blocking action on these receptors as well as many antihistamines. The structure of the tertiary ethylamine of methylephedrine is similar to the common structure of antihistamines.^{22,25,26)} This structural similarity is thought to support the finding that methylephedrine has a higher affinity for histamine H₁-receptors than ephedrine.

In the present study using optical isomers of ephedrine and methylephedrine, the antihistaminic effect of the *d*-isomer was more potent than that of the *l*-isomer. It is well known that the *d*-isomer of chlorpheniramine has a more potent antihistaminic effect than the *l*-isomer.²⁶⁾ Rekker *et al.* reported that the antihistaminic and antimuscarinic effects of optical isomers of diphenhydramine derivatives, which possess an alkyl substituent in phenyl ring, are stereospecific.²³⁾ In this report, it is proposed that the two phenyl groups of the diphenhydramine structure play quite dissimilar roles, and only one phenyl group is involved in binding. In addition, it has been observed that the antihistaminic activity of 2-*tert*-butyldiphenhydramine is in the order *d*-isomer > *l*-isomer, and the antimuscarinic activity is *l*-isomer > *d*-

isomer. It is thought possible that the phenyl group of ephedrine and methylephedrine is involved in binding to both histamine H₁-receptors and muscarinic receptors, and the configuration for binding is different for the two receptors as well as the antihistamines.

In conclusion, it is suggested that the *d*-isomers of ephedrine and methylephedrine have a higher affinity for histamine H₁-receptors than the corresponding *l*-isomers, while the *l*-isomers have a higher affinity for muscarinic receptors than the corresponding *d*-isomers. In addition, *d*-methylephedrine is suggested to have a competitive antagonist action on histamine H₁-receptors.

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