Chronotropic Effects of Optical Isomers of Ephedrine and Methylephedrine in the Isolated Rat Right Atria and in Vitro Assessment of Direct and Indirect Actions on $\beta_1$-Adrenoceptors

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Effects of optical isomers of ephedrine (EPH) and methylephedrine (MEP) on the spontaneous beating rate of isolated right atrium of normal and reserpinized rat were investigated to assess direct and indirect actions on $\beta_1$-adrenoceptors. L-EPH ($3 \times 10^{-7}$--$3 \times 10^{-5} \text{M}$) and d-EPH ($10^{-6}$--$10^{-5} \text{M}$) markedly increased beating rate of rat right atrium. L-MEP ($10^{-5}$--$3 \times 10^{-4} \text{M}$) showed slight increase in heart rate. The potency of positive chronotropic effect is L-EPH > d-EPH > L-MEP. L-EPH was about 3 times as potent as d-EPH. In addition, d-MEP ($3 \times 10^{-5}$--$3 \times 10^{-4} \text{M}$) caused a decrease in heart rate. Positive chronotropic effects of EPH isomers and L-MEP were attenuated by pretreatment with atenolol (a selective $\beta_1$-adrenoceptor antagonist) or reserpine treatment (8 mg/kg, s.c.). In reserpinized atria, the maximal increase by d-EPH was quite small, and L-MEP decreased heart rate. On the other hand, d-MEP, at $3 \times 10^{-4} \text{M}$, did not show antagonist activity against the positive chronotropic effect of isoprenaline ($10^{-10}$--$10^{-9} \text{M}$). These results suggest that L-EPH, d-EPH and L-MEP have $\beta_1$-adrenoceptor agonist activity, while d-MEP is suggested to have only low or no affinity for $\beta_1$-adrenoceptors. The relatively weak activity of L-MEP is believed to be mainly mediated by released noradrenaline. It is also suggested that d-EPH has very potent noradrenaline-releasing activity.

Key words ephedrine; methylephedrine; optical isomer; $\beta_1$-adrenoceptor; chronotropic effect; atrium

Ephedrine and methylephedrine contain two asymmetrical carbon atoms, so that four stereoisomers exist.19 dl-Methylephedrine, a mixture of two optical isomers, has been used in Japan as an antitussive drug with bronchodilator activity.2–4 It is generally recognized that methylephedrine is an ephedrine-like sympathomimetic amine.6 Naturally occurring l-ephedrine is well known as a mixed-acting sympathomimetic amine possessing both direct and indirect actions.7 The indirect action is said to be mediated through the release of noradrenaline from storage sites in adrenergic nerve endings. There are a few reports in which sympathomimetic actions of optical isomers of methylephedrine were comparatively studied.8,9,9 but direct and indirect actions were not investigated. It was observed that isomers of methylephedrine produce tachyphylaxis to some extent, which is recognized to be related to indirect action.10,11 The results concerning tachyphylaxis were qualitative or semi-quantitative, however.

A catecholamine-depleted preparation such as one reserpin-pretreated is a pharmacological tool used for assessing direct and indirect actions of sympathomimetic amines.12 l-Ephedrine and d-ephedrine reportedly produce contractions of isolated rat vas deferens and these contractile activities are reduced by reserpine pretreatment.13,14 After reserpine pretreatment, l-ephedrine was observed to retain quite potent activity. These results are concerned with the actions on $\alpha_2$-adrenoceptors. The proportion of direct and indirect actions is thought to vary between different tissues in which several $\alpha$- and $\beta$-adrenoceptors are functionally distributed.

There are several reports using isolated cardiac tissues of frog, guinea pig and rabbit to compare the actions of optical isomers of ephedrine and methylephedrine on heart.8,9,15 Cardiac excitatory activity of l-ephedrine is 2 to 10 times more potent than that of d-ephedrine. The difference in potency between optical isomers seems to vary considerably between species. Catecholamine-depleted cardiac preparation was not used in these reports, and the overall (direct + indirect) action was comparatively studied in the isolated tissues. Reserpine-pretreated rat atrium was previously used as a tool for testing $\beta_1$-adrenoceptor-mediated chronotropic effects of noradrenaline isomers, and marked difference in direct activity between isomers was reported.16

In the present study, chronotropic effects of l- and d-isomers of ephedrine and methylephedrine were investigated in normal and reserpinized rat right atria, to assess direct and indirect actions on cardiac $\beta_1$-adrenoceptors. Lack of $\beta_1$-adrenoceptor antagonist activity of d-methylephedrine possessing no positive chronotropic effect was also tested.

MATERIALS AND METHODS

Measurement of Heart Rate Response Male Wistar strain rats weighing 220—310 g were killed by a blow on the head and exsanguinated. The heart was isolated and immersed in oxygenated McEwen’s solution.17,18 The composition of the solution was: 130 mM NaCl, 5.9 mM KCl, 2.2 mM CaCl₂, 0.9 mM NaH₂PO₄, 25.0 mM NaHCO₃, 13.1 mM sucrose and 11.1 mM glucose. The right atrium was dissected from the heart. The atrial preparation was suspended under a resting tension of about 0.15 g in a 30-ml organ bath filled with McEwen’s solution (pH 7.8), kept at 34 °C and gassed with a mixture of 95% O₂ and 5% CO₂. MgCl₂ (1.0 mM) was added to the bath after the suspension of preparation until the finish of experiment. It was observed that the addition of MgCl₂ tended to stabilize the spontaneous beating of atria in many cases.

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The final concentration of Mg$^{2+}$ is believed not too high, as compared with other physiological solutions. The tension developed in the preparation was recorded isometrically with an isometric transducer (TB-652T, Nihon Kohden, Tokyo). Heart rate was measured by cardiographometer (AT-601G, Nihon Kohden), triggered by the atrial tension developed. The preparation was allowed to equilibrate for more than 60 min. The cumulative application of test drug was not repeated in the same preparation to avoid possible change in sensitivity. The ability to increase the heart rate was expressed as the pD$_2$ value which was the negative logarithm of molar concentration of test drug necessary to produce half maximal effect.$^{19}$ When atenolol was used as an $\beta_1$-adrenoceptor antagonist, it was added to the bath 15 min before drug application. To assess the involvement of endogenous noradrenaline, reserpine (8 mg/kg) was administered subcutaneously 24 h before the isolation of heart. Ptosis was observed in all rats pretreated with reserpine. To test the antagonism by test drug against the positive chronotropic effect of isoprorenaline, the drug was added to the bath 15 min before cumulative application of isoprorenaline.

**Drugs**  
$\beta$-Ephedrine, $d$-ephedrine, $l$-methylephedrine and $d$-methylephedrine were generously donated by Fuji Chemical Industries, Ltd. (Toyama). Test drugs were used as a solution of hydrochloride salt in distilled water. Other drugs used were: (±)-atenolol (Research Biochemicals International, Natick, MA, U.S.A.), reserpine (Wako Pure Chemical Industries, Osaka), tyramine hydrochloride (Wako), $l$-isoprorenaline hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.). Atenolol was dissolved in distilled water. Reserpine was used as a suspension in a saline containing 0.5% Tween 80 (Kanto Chemical Co., Tokyo). Isoprorenaline was used as a solution in distilled water containing 0.01% ascorbic acid to prevent oxidation.

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

**RESULTS**

**Effect on the Spontaneous Beating Rate in Atria**  
The basal heart rate in isolated rat right atria was 258.2 ± 4.0 beats/min ($n = 22$). $l$-Ephedrine ($3 \times 10^{-7} - 3 \times 10^{-5}$ M) and $d$-ephedrine ($10^{-5} - 10^{-4}$ M) increased the spontaneous beating rate concentration-dependently (Fig. 3), while $l$-methylephedrine ($10^{-5} - 3 \times 10^{-4}$ M) only slightly increased the rate (Figs. 2 and 4). The pD$_2$ value of $l$-ephedrine ($5.57 ± 0.09$) was significantly ($p < 0.05$) larger than those of $d$-ephedrine ($5.07 ± 0.04$) and $l$-methylephedrine ($4.04 ± 0.08$). Based on the pD$_2$ values, $l$-ephedrine was about 3 times and about 30 times more potent than $d$-ephedrine and $l$-methylephedrine, respectively. When the mean value of maximal increase by isoprorenaline ($143.5$ beats/min, $n = 4$) was taken as 100%, the maximal effects of $l$-ephedrine, $d$-ephedrine and $l$-methylephedrine were $58.9 ± 4.5$, $68.6 ± 8.3$ and $18.5 ± 2.9$%, respectively. The maximal increase by $d$-ephedrine ($98.4 ± 12.0$ beats/min) was not significantly different from that of $l$-ephedrine ($84.5 ± 6.5$ beats/min). $d$-Methylephedrine ($3 \times 10^{-5} - 3 \times 10^{-4}$ M) caused a concentration-dependent decrease in heart rate, and atenolol, a selective

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**Fig. 1.** Chemical Structures of $l$ and $d$-Isomers of Ephedrine and Methylephedrine

**Fig. 2.** Typical Recording of the Heart Rate Response to Ephedrine (EPH) and Methylephedrine (MEP) in the Isolated Rat right Atria  
Drugs were cumulatively added to the bath at the points indicated. Logarithm of molar concentration is indicated by the value at the heart rate tracing.
$\beta_1$-adrenoceptor antagonist, did not change the rate at $10^{-5}$ M. The positive chronotropic effects of l-ephedrine, d-ephedrine and l-methylphedrine were attenuated by pretreatment with atenolol, as shown in Figs. 3 and 4. In the atenolol-treated atria, l-methylphedrine decreased heart rate.

**Influence of Reserpination on the Chronotropic Response** The basal heart rate in reserpinized (8 mg/kg, s.c.) atria was 253.1 ± 3.8 beats/min ($n = 22$), which was not significantly different from that in normal atria. Tyramine ($3 \times 10^{-7} - 3 \times 10^{-5}$ M), a representative indirectly-acting sympathomimetic amine, caused concentration-dependent increase in heart rate (Fig. 5), although its maximal effect (156.4 ± 9.1 beats/min) was not significantly different from that of isoprenaline. The positive chronotropic effect of tyramine was markedly attenuated by reserpination, and the $pD_2$ value in reserpinized atria (4.38 ± 0.07) was significantly smaller than that (5.70 ± 0.10) in normal atria ($p < 0.05$). The positive chronotropic effects of l-ephedrine, d-ephedrine and l-methylphedrine were attenuated by reserpination, as shown in Figs. 6 and 7. In reserpinized atria, the maximal increase (20.8 ± 6.3 beats/min) by d-ephedrine was significantly smaller than that (61.2 ± 2.5 beats/min) by l-ephedrine ($p < 0.05$). L-Methylphedrine showed a negative chronotropic effect as did d-methylphedrine in reserpinized atria.

**Effect on Positive Chronotropic Action of Isoprenaline** Isoprenaline ($10^{-10} - 10^{-5}$ M) caused a concentration-dependent increase in heart rate (Fig. 8). d-Methylphedrine ($3 \times 10^{-8}$ M), which decreases heart rate, showed slight or no inhibition on the chronotropic effect of isoprenaline. The $pD_2$ value (8.80 ± 0.13) and maximal effect (165.5 ± 3.8 beats/min) in the presence of d-methylphedrine were not significantly different from those values (8.99 ± 0.04; 143.5 ± 14.2 beats/min) in normal atria.

**DISCUSSION**

l-Ephedrine, d-ephedrine and l-methylphedrine increased spontaneous beating rate in isolated rat right atria. The positive chronotropic effect of these drugs was blocked by pretreatment with atenolol (a selective $\beta_1$-adrenoceptor antagonist). It is suggested that l-ephedrine and d-ephedrine have stimulating activity for $\beta_1$-adrenoceptors. In addition, the very weak activity of l-methylphedrine is considered to be $\beta_1$-adrenoceptor-mediated. On the other hand, d-methylphedrine did not cause a
positive chronotropic effect, nor did it show antagonism against the positive chronotropic effect of isoprenaline. It is suggested that d-methylephedrine has low or no affinity for β₁-adrenoceptors.

Tachyphylaxis of sympathomimetic amines is generally recognized to be produced by depletion of a small available pool of noradrenaline with or without other mechanisms such as inhibition of the transport of noradrenaline out of the nerve terminal. It was previously reported that the rate of development of tachyphylaxis to pressor effect was l-ephedrine < d-ephedrine, when equipressor doses were intravenously administered to anesthetized dogs. The in vivo data suggest that d-ephedrine acts...
much more indirectly than l-ephrine. In that report, the relative pressor potency of l-ephrine to d-ephrine was calculated as 100:33, and the effects of ephrine isomers on heart rate were said to parallel the pressor effects. Chen et al. reported that the pressor action of l-ephrine is 2.95 times as strong as that of d-ephrine in pithed cats. In the present study using isolated rat right atria, the positive chronotropic potency of l-ephrine was about 3 (3.2) times more potent than that of d-ephrine. The relative potency of d-ephrine for the in vivo pressor action is believed to be in good agreement with that for the in vitro positive chronotropic activity in rat right atria. L-Ephrine, in contrast, was reported to be 10 times more potent than d-ephrine in experiments using guinea pig atria. The potency of d-ephrine was thought to be relatively small. Thus the chronotropic response of rat right atrium may be a preferable indicator to detect indirect actions of sympathomimetic amines.

The positive chronotropic effects of l-ephrine, d-ephrine and l-methyllephrine were attenuated by reserpine pretreatment. These findings suggest that indirect action is at least partly involved in the positive chronotropic effects of these isomers. Noradrenaline-releasing activity of d-ephrine is believed to be more potent than that of l-methyllephrine. In normal atria, the maximal increase in heart rate by d-ephrine is almost the same as that by l-ephrine. In reserpinated atria, however, d-ephrine showed a slight increase, while the l-ephrine-induced increase remained largely the same. In regard to ephrine isomers, similar results were reported by Patil and co-workers who carried out experiments using normal and reserpine-pretreated rat vas deferens. They observed that d-ephrine showed slight contraction in reserpine-pretreated vas deferens, although its contractile activity in normal rat vas deferens was quite potent. Therefore, it is suggested that the direct actions of d-ephrine on ß1- and ß2-adrenoceptors are weaker than those of l-ephrine. L-Ephrine is reported to cause greater release of noradrenaline than d-ephrine from perfused rabbit heart, and it is thought possible that the indirect action of l-ephrine may also be more potent than that of d-ephrine in rat right atria.

Plasma concentration of ephrine reportedly reached about 200 ng/ml (10^-6 M) 1 h after a single 50 mg oral dose of dl-ephrine hydrochloride to healthy male volunteers. In the present study, positive chronotropic effects of l- and d-ephrine were observed at 3 x 10^-7 M and 10^-6 M, respectively, and the results from this study using rat right atria may agree with the appearance of tachycardia in clinical treatment. It was also reported that a single 200 mg oral dose of methyllephrine did not influence heart rate in patients with obstructive pulmonary diseases. In the present study, a positive chronotropic effect of l-methyllephrine was observed at 10^-5 M and higher concentrations; in addition, d-methyllephrine did not have a positive chronotropic effect. These findings in rat right atria support that dl-methyllephrine rarely causes tachycardia in clinical treatment.

d-Methyllephrine, at 3 x 10^-7 M and higher concentrations, decreased heart rate in this study. L-Methyllephrine also showed a concentration-dependent decrease in heart rate in atenolol-treated atria or reserpine-treated atria. These results suggest that l- and d-methyllephrine have negative chronotropic effect. In normal atria, l-methyllephrine showed an initial decrease following by an increase in heart rate. The initial decrease is thought to be related to the negative chronotropic component of l-methyllephrine. There is a possibility that methyllephrine has non-specific depressant action on cardiac tissues. In atenolol (10^-5 M)-treated atria, ephrine caused a slight decrease in heart rate at 10^-7 M. Toxicity in rabbit by intravenous injection of ephrine and related compounds was reported earlier by Chen et al.; they proposed that death was due to cardiac collapse. Ephrine and methyllephrine do not contain hydrophilic catechol moiety. A tertiary amine structure of methyllephrine is believed to enhance the lipid solubility. It is presumed that the accumulation of methyllephrine in biological membranes may disturb the physiological functions of various cells such as pacemaker cells.

Our present study began with the hypothesis that methyllephrine might be a considerably selective ß2-adrenoceptor agonist, because it has been generally recognized as an ephrine-like bronchodilator possessing only slight or no ephrine-like adverse action such as heart acceleration. It is reported that l-methyllephrine causes slight pressor activity in pithed cats. However, our recent study using guinea pig trachea indicated that the bronchodilator action of methyllephrine is very unlike that of ephrine. The bronchodilator activity of d-methyllephrine may be mediated by antihistaminic action of d-methyllephrine rather than by the minimal or complete lack of stimulating action of l-methyllephrine on ß-adrenoceptors, while l-methyllephrine reportedly inhibits a centrally-induced cough response. In the report, the antitussive action of l-methyllephrine is presumed to be due to depression of the cough center. The antitussive action of methyllephrine thus may not be related to ß-adrenoceptor-mediated actions.

In conclusion, l-ephrine, d-ephrine and l-methyllephrine are suggested to have stimulating activity for ß1-adrenoceptors, and d-methyllephrine to have low or no affinity for these adrenoceptors. The relatively weak activity of l-methyllephrine is thought to be largely mediated by released noradrenaline. It is also suggested that d-ephrine has a very potent noradrenaline-releasing activity.

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REFERENCES

1) Racemic ephrine and racemic methyllephrine are mixtures of ehtyro pairs of diastereomers, while the three pairs of diastereomers are called pseudoephrine and pseudomethyllephrine.
4) Kase Y., J. Practical Pharmacy, 12, 151—331 (1961).