Effects of Optical Isomers of Ephedrine and Methylephedrine on the Twitch Response in the Isolated Rat Vas Deferens and the Involvement of α_2 -Adrenoceptors

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Abstract

The effects of optical isomers of ephedrine (EPH) and methylephedrine (MEP) on the twitch response to electrical stimulation (1 msec, 1 Hz) in the isolated rat vas deferens were investigated to clarify the action on α_2 -adrenoceptors. *l*-EPH (10^{-7} – 3×10^{-5} M) and *d*-EPH (10^{-6} – 10^{-4} M) markedly inhibited the twitch response in the presence of prazosin (10^{-6} M). *l*-MEP also inhibited the twitch response at high concentrations (3×10^{-5} – 10^{-3} M). The rank order of inhibitory potency was *l*-EPH > *d*-EPH $\gg$ *l*-MEP. Yohimbine (3×10^{-7} M), a selective α_2 -adrenoceptor antagonist, attenuated the twitch-inhibitory effects of EPH isomers and *l*-MEP. Furthermore, the twitch-inhibitory effects of EPH isomers and *l*-MEP were attenuated by reserpine treatment (8 mg/kg, s.c.). On the other hand, *d*-MEP showed the potentiation of twitch response, and competitively antagonized the twitch-inhibitory effect of clonidine (10^{-9} – 10^{-6} M) with the pA_2 value of 4.3 in the presence of prazosin. These results suggest that EPH isomers and *l*-MEP have stimulating activity for presynaptic α_2 -adrenoceptors. In addition, the twitch-inhibitory effect of EPH isomers and *l*-MEP may be at least partly mediated through the release of noradrenaline. It is also suggested that *d*-MEP has competitive α_2 -adrenoceptor antagonist activity.

Key words: Ephedrine; Methylephedrine; Optical isomer; Vas deferens; α_2 -Adrenoceptor

Introduction

Ephedrine and methylephedrine contain two asymmetrical carbon atoms, and then optical isomers exist. Methylephedrine, an antitussive drug, is commonly used as a racemic medicine. Ephedrine is generally accepted to have adrenaline-like activity in peripheral organs (Hoffman and Lefkowitz, 1990). Methylephedrine has been recognized as an ephedrine-like sympathomimetic amine. α -Adrenoceptors are classified into α_1 - and α_2 -adrenoceptors (Langer, 1981). Presynaptic α_2 -adrenoceptor is considered as an adrenoceptor which regulate the neurotrans-

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mission via inhibition of the release of neurotransmitter from nerve ending. Rat vas deferens is preferably used to investigate the effects of adrenergic drugs on α -adrenoceptors. It is generally accepted that α -adrenoceptor agonists produce contractions of rat vas deferens via postsynaptic α_1 -adrenoceptors and/or inhibit the twitch response to electrical stimulation via presynaptic inhibitory α_2 -adrenoceptors. In addition, it is recognized that the twitch response is at least partly mediated through the release of adenosine 5'-triphosphate (ATP) (Dalziel and Sneddon, 1988). Patil *et al.* (1967a; 1967b) reported that *l*- and *d*-ephedrine produce contractions of rat vas deferens and the contractile activities were attenuated by reserpine treatment. These findings indicate that isomers of ephedrine have directly and indirectly stimulating activity for α_1 -adrenoceptors. On the other hand, the actions of optical isomers of ephedrine and methylephedrine on presynaptic α_2 -adrenoceptors have been uncertain (Bauer, 1981; Yamamoto *et al.*, 1989). In the present study, effects of optical isomers of ephedrine and methylephedrine on the twitch response to electrical stimulation in isolated rat vas deferens were investigated to assess the action on α_2 -adrenoceptors.

Methods

Measurement of twitch response to electrical stimulation: Male Wistar strain rats, weighting 220–310 g, were killed by a blow on the head and exsanguinated. Vas deferens was isolated and immersed in oxygenated McEwen's solution. The composition of the solution was: 130 mM NaCl, 5.9 mM KCl, 2.2 mM CaCl_2 , 0.9 mM NaH_2PO_4 , 25.0 mM NaHCO_3 , 13.1 mM sucrose and 11.1 mM glucose. Because different portions of rat vas deferens are reported to show different responses to drugs (Brown *et al.*, 1979), only the prostatic portion was used in this study. The preparation (about 1.5 cm) was suspended under a resting tension of about 0.2 g in a 30-ml organ bath filled with McEwen's solution, kept at 34°C and gassed with a mixture of 95% O_2 and 5% CO_2 . The preparation was stimulated by rectangular pulses of 0.1 Hz in frequency, 1 msec in duration and 65 V, delivered by a pair of platinum ring electrodes connected to an electrostimulator (Biomedical science, Japan). The ring electrode was enveloped by polyethylene plastic except for the inside of ring. Longitudinal contractions of the preparation were recorded isometrically with an isometric transducer (TB-652T, Nihon Kohden, Japan). The preparation was allowed to equilibrate for more than 60 min in electrically stimulated condition. For the first 20 min, electrical stimulation was performed at lower frequency (0.0125 Hz). The twitch experiments were performed in the presence of 10^{-6} M prazosin to block α_1 -adrenoceptors. Prazosin was added to the bath about 30 min before the cumulative application of test drug. It is reported that prazosin causes the reduction of twitch response (Doxey *et al.*, 1977). However, in the present study, the twitch response was maintained for long periods of time sufficient for cumulative application of test drugs in the presence of prazosin. This experimental advantage is considered to be at least partly due to the lack of Magnesium in McEwen's solution. The time interval between two successive concentrations of test drug was allowed to be 5 to 20 min. The cumulative application of test drug was not repeated in a same preparation to avoid possible change in sensitivity. The ability to inhibit the twitch response caused by electrical stimulation was expressed as the pIC₃₀ value which was a negative

logarithm of molar concentration of test drug necessary to inhibit 30% of amplitude of twitch response. When yohimbine was used as an α_2 -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 hr before the isolation of vas deferens.

To clarify whether antagonism is competitive or not, Schild plot analysis was performed. The pA_2 value, which is the negative logarithm of dissociation constant of a competitive antagonist, was calculated according to the method of Tallarida *et al.* (1979), which was originally reported by Arunlakshana and Schild (1959). The concentration ratio was obtained from the ratio of IC_{50} values (50% inhibitory concentrations) of clonidine in the presence and absence of test drug. In this study, the basal IC_{50} value in the absence of test drug was obtained from the mean curve of 5 experiments. The log (concentration ratio-1) was plotted against logarithm of molar concentration of test drug, and the regression line and its slope were calculated.

Statistical analysis: The results were expressed as the mean value $\pm$ S.E. Statistical significance was analysed by Student's *t*-test or Welch's *t*-test after F-test. A *P* value less than 0.05 was considered to be significant.

Drugs: *l*-Ephedrine, *d*-ephedrine, *l*-methylephedrine and *d*-methylephedrine were kindly donated by Fuji Chemical Industries. Test drugs were used as a solution of hydrochloride salt in distilled water. Other drugs mainly used were as follows; prazosin hydrochloride, yohimbine hydrochloride and reserpine (all from Wako Pure Chemical Industries, Japan), clonidine hydrochloride (Tokyo Kasei Kogyo, Japan). These agents were used as a solution in distilled water except for prazosin and reserpine. Prazosin was suspended in ethanol, and final concentration of ethanol was less than 0.05% in the organ bath. Reserpine was used as a suspension

Fig. 1. Typical recording of the twitch response to electrical stimulation in the isolated rat vas deferens. Drugs were cumulatively added to the bath in the presence of 10^{-6} M prazosin at the points indicated. For further details, see the Methods.

in saline containing 0.5% Tween 80 (Kanto Chemical Co., Japan).

Results

In electrically stimulated rat vas deferens, *l*-ephedrine (10^{-7} – 3×10^{-5} M) and *d*-ephedrine (10^{-6} – 10^{-4} M) markedly inhibited the twitch response in the presence of 10^{-6} M prazosin (Figs. 1 and 2). *l*-Methylephedrine (3×10^{-5} – 10^{-3} M) also inhibited the twitch response (Figs. 1 and 3). On the other hand, *d*-methylephedrine showed potentiation of twitch response. The pIC₃₀

Fig. 2. Effects of *l*-ephedrine (A) and *d*-ephedrine (B) on the twitch response and the influence of yohimbine against them in the isolated rat vas deferens. Yohimbine (3×10^{-7} M) was added 15 min before drug application. Each point is presented as a mean $\pm$ S.E. of 5 experiments. Symbols represent: $\bullet$, *l*-ephedrine; $\circ$, yohimbine + *l*-ephedrine; $\blacktriangle$, *d*-ephedrine; $\triangle$, yohimbine + *d*-ephedrine. For further details, see the Methods. Significantly different from the value of untreated group: **p < 0.01 and ***p < 0.001.

Fig. 3. Effects of optical isomers of methylephedrine on the twitch response and the influence of yohimbine against them in the isolated rat vas deferens. Yohimbine (3×10^{-7} M) was added 15 min before drug application. Each point is presented as a mean $\pm$ S.E. of 5–6 experiments. Symbols represent: $\blacksquare$, *l*-methylephedrine; $\square$, yohimbine + *l*-methylephedrine; $\blacktriangledown$, *d*-methylephedrine; $\triangledown$, yohimbine + *d*-methylephedrine. For further details, see the Methods. Significantly different from the value of untreated group: **p < 0.01 and ***p < 0.001.

Fig. 4. Influence of reserpinization against the effects of *l*-ephedrine (A) and *d*-ephedrine (B) on the twitch response in the isolated rat vas deferens. Reserpine (8 mg/kg, s.c.) was administered 24 hr before the isolation of vas deferens. Each point is presented as a mean $\pm$ S.E. of 5-6 experiments. Symbols represent: ●, *l*-ephedrine; ○, *l*-ephedrine (reserpinized rats); ▲, *d*-ephedrine; △, *d*-ephedrine (reserpinized rats). For further details, see the Methods. Significantly different from the value of untreated group: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Fig. 5. Influence of reserpinization against the effects of optical isomers of methylephedrine on the twitch response in the isolated rat vas deferens. Reserpine (8 mg/kg, s.c.) was administered 24 hr before the isolation of vas deferens. Each point is presented as a mean $\pm$ S.E. of 5-7 experiments. Symbols represent: ■, *l*-methylephedrine; □, *l*-methylephedrine (reserpinized rats); ▼, *d*-methylephedrine; ▽, *d*-methylephedrine (reserpinized rats). For further details, see the Methods. Significantly different from the value of untreated group: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

values of *l*-ephedrine, *d*-ephedrine and *l*-methylephedrine were 6.20 ± 0.06 , 5.44 ± 0.01 and 3.82 ± 0.04 , respectively. By pretreatment with yohimbine (3×10^{-7} M) or by reserpinization, twitch-inhibitory effect of these drugs were attenuated or abolished (Figs. 2, 3, 4 and 5). Reserpine-induced attenuation of maximal inhibition of *d*-ephedrine was larger than that of *l*-ephedrine.

Antagonism by d-methylephedrine against the twitch-inhibitory effect of clonidine

Schild plot analysis was performed for *d*-methylephedrine which did not show the twitch-

Fig. 6. Antagonism by *d*-methylephedrine against the twitch-inhibitory effect of clonidine in the isolated rat vas deferens. A: Antagonism of clonidine-induced twitch-inhibition by *d*-methylephedrine (*d*-MEP). Clonidine was added cumulatively 15 min after drug application. Each point is presented as a mean $\pm$ S.E. of 5 experiments. Symbols represent: ●, control; ▲, *d*-MEP 10^{-4} M; ■, *d*-MEP 3×10^{-4} M; ▼, *d*-MEP 10^{-3} M. Significantly different from the value of untreated group: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. B: Schild plot for antagonism of clonidine by *d*-MEP. For further details, see the Methods.

inhibitory effect. As shown in Fig. 6, the concentration-response curves of clonidine were shifted to the right in a parallel manner by *d*-methylephedrine. The slope of the regression line was not being significantly different from unity (1.10 ± 0.13). The pA_2 value was 4.27 ± 0.05 .

Discussion

l-Ephedrine, *d*-ephedrine and *l*-methylephedrine inhibited the twitch response in the presence of prazosin. The twitch-inhibitory effects of these drugs were attenuated by the pretreatment with yohimbine (a selective α_2 -adrenoceptor antagonist). Therefore, it is suggested that ephedrine isomers and *l*-methylephedrine have stimulating activity for presynaptic α_2 -adrenoceptors. Based on the pIC_{30} values, *l*-ephedrine was about 5 times and about 240 times more potent than *d*-ephedrine and *l*-methylephedrine, respectively.

It is generally accepted that ephedrine has direct and indirect stimulating action on adrenoceptors. Indirect action of sympathomimetic amine is defined as the action mediated through the release of noradrenaline from nerve endings. In the present study, the twitch-inhibitory effects of ephedrine isomers and *l*-methylephedrine were attenuated or abolished by reserpinization. These results suggest that the released noradrenaline plays an important role in the twitch-inhibition by ephedrine isomers and *l*-methylephedrine.

Phenylethylamine derivatives are reported to show weak antinociceptive action, and the mechanism of the action is suggested to be due to the α_2 -adrenoceptor stimulating action at least partly mediated through the release of endogenous noradrenaline in central nervous system (Matsuoka *et al.*, 1993). Ephedrine contains the structure of phenylethylamine. From the data in the present experiments, ephedrine is considered to have α_2 -adrenoceptor stimulating action partly mediated through noradrenaline release. Therefore, ephedrine may produce antinociceptive action.

By addition of yohimbine, potentiation of basal twitch response was observed (data not shown). The twitch response observed in electrically stimulated vas deferens was considered to be already inhibited by α_2 -adrenoceptor-mediated feedback mechanism. Then, yohimbine-induced potentiation of the twitch response may be resulted from the cancellation of feedback regulation. *d*-Methylephedrine also showed the potentiation of twitch response as well as yohimbine. Then, it is thought to be possible that *d*-methylephedrine has yohimbine-like action. *d*-Methylephedrine shifted concentration-response curve of clonidine to the right in a parallel manner. When Schild plot analysis was performed, the slope of regression line was not being significantly different from unity, and then the pA_2 value was 4.3. From these results, it is suggested that *d*-methylephedrine has competitive and weak antagonist activity for α_2 -adrenoceptors. In the vas deferens of reserpinized rat, *l*-methylephedrine showed the potentiation of twitch response as well as *d*-methylephedrine. It is considered that *l*-methylephedrine has stimulating and antagonistic activity for α_2 -adrenoceptors.

In the present study, *d*-methylephedrine potentiated the twitch response in reserpinized and normal vas deferens. However, the percentage of potentiation in reserpinized preparations was much larger than that in normal preparations. Reserpinization tended to lower the control twitch response which was used as 100% in calculating percent potentiation. It is suggested that the difference in percent value of potentiating effect of *d*-methylephedrine between in normal and reserpinized preparations is mainly resulted from the difference in the magnitude of control twitch response. There are several reports indicating that reserpine treatment reduce the twitch response of vas deferens (Urabe *et al.*, 1987; Marshall *et al.*, 1978). It is considered that reserpine-induced depletion of tissue noradrenaline reduce noradrenaline-mediated component of twitch response. In addition, it is presumed that the twitch response of reserpinized preparation may be still inhibited by the release of residual noradrenaline. These are thought to be the reason why the control twitch response tended to be low in reserpinized preparations.

In the rat vas deferens, adenosine is known to inhibit the twitch response to electrical stimulation as well as ATP (Clanachan, 1977). Adenosine is a common metabolite of ATP which is released by nerve stimulation in addition with noradrenaline. It is generally accepted that indirectly acting sympathomimetic amines release catecholamines from nerve endings without exocytotic release of granular contents such as ATP (Lefkowitz *et al.*, 1990). Therefore, it is considered that presynaptic inhibitory purinoceptors are not involved with the twitch-inhibitory effect of ephedrine isomers and *l*-methylephedrine.

In additional study, *dl*-methylephedrine (equimolar mixture of isomers) showed potentiation. The concentration-response curve was located between two curves of methylephedrine

isomers (data not shown).

In conclusion, *l*-ephedrine, *d*-ephedrine and *l*-methylephedrine are considered to have stimulating activity for presynaptic α_2 -adrenoceptors in rat vas deferens. The α_2 -adrenoceptor agonist activity may be at least partly mediated through the release of noradrenaline. In addition, *d*-methylephedrine is suggested to have competitive antagonist activity for α_2 -adrenoceptors.

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