Effects of Dilevalol on Adrenoceptors in Isolated Cat Arteries

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The effects of dilevalol on vascular adrenoceptors were investigated using helical strips of cat arteries. In coronary arteries partially pre-contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), the concentration-relaxant response curves for isoproterenol were shifted to the right by dilevalol with a potency similar to that of propranolol, although dilevalol itself did not relax the arteries. Contractions induced by norepinephrine of mesenteric arteries were attenuated by low concentrations of prazosin but were not influenced by yohimbine of up to 10^{-8} M. In contrast, the norepinephrine-induced contractions of middle cerebral arteries were attenuated by yohimbine but only slightly attenuated by prazosin. With mesenteric arteries, treatment with dilevalol (10^{-7} to 10^{-5} M) attenuated the contractions induced by norepinephrine and phenylephrine in a concentration-dependent manner. On the other hand, contractions induced by norepinephrine and clonidine in middle cerebral arteries were not attenuated by treatment with dilevalol of up to 10^{-6} M. Treatment with low concentrations of dilevalol (10^{-8} to 10^{-7} M) potentiated the contractile response to the electrical stimulation of adrenergic nerves in mesenteric arteries while high concentrations (3×10^{-7} to 10^{-5} M) attenuated it. The potentiation was reversed to attenuation by pretreatment with propranolol. Treatment with isoproterenol (10^{-10} to 10^{-9} M) also potentiated the contractile response to the electrical stimulation in the arteries. Isoproterenol did not cause any relaxation of mesenteric arteries precontracted with $PGF_{2\alpha}$. Attenuations by clonidine of the response to the electrical stimulation in the arteries did not significantly differ in control arteries and those treated with a high concentration (10^{-6} M) of dilevalol. These results suggest that dilevalol has a potent β -receptor blocking activity, weak α_1 -blocking activity and β -agonistic activity but has no effect on the α_2 -adrenoceptor. The potentiating effect of dilevalol on the contractile response to adrenergic nerve stimulation seems to occur only in arteries which do not relax with β -agonist.

Keywords — dilevalol; α -adrenoceptor; β -adrenoceptor; antihypertensive drug; cat isolated artery; adrenergic neurotransmission

Introduction

β -Adrenoceptor blockers have proved to be very useful in the treatment of essential hypertension. Recently, drugs with several action mechanisms have been developed to produce an antihypertensive effect with minimal adverse effects. β - and α -adrenoceptor blockade or direct vasodilation by β_2 -adrenoceptor stimulation is considered to be responsible for the antihypertensive effect.¹⁻³⁾

Dilevalol, the *R,R*-isomer of labetalol, is a potent β -adrenoceptor- but a weak α -adrenoceptor-blocking agent with a vasodilating action.⁴⁻⁷⁾ This vasodilating action of dilevalol was observed using the sympathetically denervated femoral vascular bed of anesthetized dogs.⁴⁾ However, the antagonistic activities of dilevalol were evaluated *in vivo* or were separately evalu-

ated using various isolated preparations such as of atria, trachea or aorta. The purpose of the present study was to clarify the α - and β -antagonistic activities of dilevalol in the adrenoceptors using only isolated arteries and to compare them with those of propranolol, prazosin and yohimbine. Furthermore, the effects of dilevalol on the adrenergic neurotransmission were examined.

Materials and Methods

Preparation — Cats of either sex, weighing 3.5 to 5.5 kg, were anesthetized with intraperitoneal injections of sodium pentobarbital (30 mg/kg) and killed by exsanguination from the common carotid arteries. The brain and heart were quickly removed. Middle cerebral arteries and ventral interventricular and circumflex

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branches of the left coronary arteries were isolated. Distal portions of the superior mesenteric artery were also isolated. The arteries were helically cut into strips, 15–20 mm long. Endothelial cells were expected to remain intact, since acetylcholine (10^{-7} M) produced a relaxation which could be abolished by rubbing the surface of the endothelial cells.⁸⁾ Each strip was fixed vertically between hooks in a muscle bath containing the modified Ringer-Locke solution, which was maintained at 37 ± 0.3 °C and aerated with a mixture of 95% O₂ and 5% CO₂. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 0.3 g for middle cerebral arteries, 1.0 g for coronary arteries and 0.7 g for mesenteric arteries, respectively; these values are the optimal ones for inducing maximum contractions by 30 mM K⁺. Constituents of the solution were as follows (mM): NaCl 120, KCl 5.4, CaCl₂ 2.2, MgCl₂ 1.0, NaHCO₃ 25.0 and dextrose 5.6. The pH of the solution was 7.35 to 7.41. Before the start of the experiments, all strips were allowed to equilibrate for 90 to 120 min in control media, during which time the solution was replaced every 10 to 15 min.

Transmural Stimulation of Adrenergic Nerves — Mesenteric arterial strips were placed between a pair of stimulating electrodes made of platinum plate, 5 × 15 mm in size and approximately 2 mm apart from each other.⁹⁾ The gap between the strip and the electrodes was wide enough to allow undisturbed contractions, and yet sufficiently narrow to permit stimulation of intramural nerve terminals. The strips were transmurally stimulated by a train of 0.3 ms square pulses of supramaximum intensity (approximately 10 V), at frequencies of 5 Hz for periods of 40 s. Stimulations at 5 Hz were applied repeatedly at 10-min intervals until steady state responses were obtained.

Recording — Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Nihonkohden Kogyo Co.). The contractile response to 30 mM K⁺ was first obtained, then the preparations were repeatedly washed with fresh solution and equilibrated for

30 to 40 min. Concentration–response curves for isoproterenol, norepinephrine, phenylephrine and clonidine were obtained by adding the drugs directly to the bathing media in cumulative concentrations. To test the relaxant effect of isoproterenol or acetylcholine, arterial strips were partially contracted with prostaglandin F_{2α} (PGF_{2α}); the contraction was in a range between 15% and 30% of the contraction induced by 30 mM K⁺. At the end of each series of experiments, papaverine at 10^{-4} M was added to obtain maximal relaxation.¹⁰⁾ The preparations were treated for 20 min with blocking agents before the concentration–response curve for agonist and the contractile response to transmural stimulation were obtained.

Statistics and Drugs — The results shown in the text and figures are expressed as mean values ± S.E.M. The slope of the Schild plot and the pA₂ value were calculated for each drug by computerized linear regression analysis using the method of least squares. The difference between groups was evaluated by Tukey's method after one-way analysis of variance with $p < 0.05$ being taken as significant. Drugs used were dilevalol hydrochloride (Schering-Plough), PGF_{2α} (Ono Pharmaceutical Co.), propranolol hydrochloride (Sumitomo Pharmaceutical Co.), tetrodotoxin (Sankyo Co.), clonidine hydrochloride, (–)-isoproterenol hydrochloride, (–)-norepinephrine bitartrate and (–)-phenylephrine hydrochloride (Sigma Chemical Co.), yohimbine hydrochloride (Nakarai Chemicals, Ltd.), prazosin hydrochloride (Tokyo Kasei Co.), acetylcholine chloride (Daiichi Pharmaceutical Co.), phentolamine mesylate (Ciba-Geigy Co.) and papaverine hydrochloride (Dainippon Pharmaceutical Co.).

Results

Effects of Propranolol and Dilevalol on the Relaxant Responses to Isoproterenol in Coronary Arteries Partially Contracted with PGF_{2α}

In coronary arteries partially precontracted with PGF_{2α}, the addition of isoproterenol (10^{-9} to 10^{-7} M) produced a concentration-related relaxation (Fig. 1). The concentration–relaxant response curve for isoproterenol was shifted to

Fig. 1. Effects of Propranolol (A) and Dilevalol (B) on the Relaxant Response to Isoproterenol of Coronary Arteries Partially Contracted with $\text{PGF}_{2\alpha}$

(A) ●, control (6); ○, propranolol 10^{-9} M (6); ×, propranolol 10^{-8} M (6); △, propranolol 10^{-7} M (6). (B) ●, control (7); ○, dilevalol 10^{-9} M (7); ×, dilevalol 10^{-8} M (7); △, dilevalol 10^{-7} M (7).

Relaxation induced by 10^{-4} M papaverine was taken as 100%; mean absolute values in the upper and the lower figures were 544 ± 57 mg ($n = 6$) and 544 ± 72 mg ($n = 7$), respectively. Numbers in parentheses indicate the number of preparations used. Vertical bars represent S.E.M.

the right by treatment with propranolol (10^{-9} to 10^{-7} M) in a concentration-dependent manner (Fig. 1A). The pA_2 value for propranolol in the coronary arteries was 8.87 and the slope of the Schild plot was 1.04. The addition of dilevalol (10^{-9} to 10^{-5} M) did not relax or contract the arteries precontracted with $\text{PGF}_{2\alpha}$. Treatment with dilevalol (10^{-9} to 10^{-7} M) shifted the concentration-response curves for isoproterenol to the right (Fig. 1B). The pA_2 value for dilevalol in the arteries was 8.85 and the slope of the Schild plot was 1.00.

Modification by Yohimbine and Prazosin of the Contractile Responses of Mesenteric Arteries and Middle Cerebral Arteries to Norepinephrine

The addition of norepinephrine (10^{-9} to 10^{-5} M) produced a concentration-dependent contraction of mesenteric arteries and middle cerebral arteries treated with 10^{-6} M propranolol (Figs. 2 and 3). Further increase in the concentration of norepinephrine to 5×10^{-5} M produced no or only a slight additional contractions. In mesenteric arteries, the concentration-response curve for norepinephrine was shifted to the right by low concentrations of prazosin (10^{-9} M) but was not influenced by 10^{-8} M yohimbine (Fig. 2). The pA_2 values for prazosin and yohimbine were 9.08 and 7.45, respectively. In contrast, the concentration-response curves for norepinephrine in middle cerebral arteries were shifted to the right and downward by treatment with yohimbine (10^{-8} and 10^{-7} M) and only slightly shifted by treatment with prazosin at 10^{-8} M (Fig. 3).

Modification by Dilevalol of the Contractile Response of Mesenteric Arteries and Middle Cerebral Arteries to Norepinephrine, Phenylephrine or Clonidine

In mesenteric arteries pretreated with 10^{-6} M propranolol, treatment with dilevalol (10^{-6} and 10^{-5} M) significantly shifted the concentration-response curves for norepinephrine and phenylephrine to the right (Fig. 4). The pA_2 values for dilevalol to norepinephrine and phenylephrine in the arteries were 6.32 and 6.46, respectively. The slopes of the Schild plots were 0.78 and 0.88, respectively. In middle cerebral arteries treated with 10^{-6} M propranolol, the contractile responses to norepinephrine and clonidine were attenuated by treatment with dilevalol at 10^{-5} M (Fig. 5).

Modification by Dilevalol of the Contractile Response of Mesenteric Arteries to Transmural Stimulation

Transmural stimulation at frequencies of 5 Hz for periods of 40 s produced contraction, which was abolished by 3×10^{-7} M tetrodotoxin or 10^{-6} M phentolamine. The addition of dilevalol (10^{-8} to 10^{-5} M) did not alter the arterial resting tension. Treatment with dilevalol at 10^{-8} to 10^{-7} M potentiated the contractile response to

Fig. 2. Modification by Yohimbine (A) and Prazosin (B) of the Contractile Response to Norepinephrine of Mesenteric Arteries Treated with 10^{-6} M Propranolol

(A) ●, control (7); ○, yohimbine 10^{-8} M (7); ×, yohimbine 10^{-7} M (7); △, yohimbine 3×10^{-7} M (5). (B) ●, control (6); ○, prazosin 10^{-9} M (6); ×, prazosin 10^{-8} M (6).

Contractions induced by 10^{-5} M norepinephrine in control media were taken as 100%; mean absolute values in experiments with yohimbine and prazosin were 2004 ± 275 mg ($n = 7$) and 1931 ± 154 mg ($n = 6$), respectively. Data for yohimbine at 3×10^{-8} M and prazosin at 3×10^{-10} and 3×10^{-9} M are not shown.

Fig. 3. Modification by Yohimbine (A) and Prazosin (B) of the Contractile Response to Norepinephrine of Middle Cerebral Arteries Treated with 10^{-6} M Propranolol

(A) ●, control (8); ○, yohimbine 10^{-8} M (7); ×, yohimbine 10^{-7} M (8). (B) ●, control (7); ○, prazosin 10^{-9} M (7); ×, prazosin 10^{-8} M (7).

Contractions induced by 10^{-5} M norepinephrine in control media were taken as 100%; mean absolute values in experiments with yohimbine and prazosin were 254 ± 31 mg ($n = 8$) and 266 ± 42 mg ($n = 7$), respectively. Data for yohimbine at 3×10^{-8} M and prazosin at 3×10^{-9} and 3×10^{-8} M are not shown.

Fig. 4. Modification by Dilevalol of the Contractile Responses to Norepinephrine (A) and Phenylephrine (B) of Mesenteric Arteries Treated with 10^{-6} M Propranolol

(A) ●, control (10); △, dilevalol 10^{-7} M (10); ○, dilevalol 10^{-6} M (10); ×, dilevalol 10^{-5} M (10). (B) ●, control (8); △, dilevalol 10^{-7} M (8); ○, dilevalol 10^{-6} M (8); ×, dilevalol 10^{-5} M (8).

Contractions induced by 10^{-5} M norepinephrine or 10^{-5} M phenylephrine were taken as 100%; mean absolute values in experiments with norepinephrine and phenylephrine were 2129 ± 153 mg ($n = 10$) and 2048 ± 191 mg ($n = 8$), respectively.

Fig. 5. Modification by Dilevalol of the Contractile Responses to Norepinephrine (A) and Clonidine (B) of Middle Cerebral Arteries Treated with 10^{-6} M Propranolol

(A) ●, control (10); ○, dilevalol 10^{-6} M (10); ×, dilevalol 10^{-5} M (10). (B) ●, control (9); ○, dilevalol 10^{-6} M (9); ×, dilevalol 10^{-5} M (9).

Contractions induced by 10^{-5} M norepinephrine or 10^{-6} M clonidine were taken as 100%; mean absolute values in experiments with norepinephrine and clonidine were 318 ± 61 mg ($n = 10$) and 146 ± 30 mg ($n = 9$), respectively.

Fig. 6. Response to Transmural Stimulation at Frequency of 5 Hz of Mesenteric Arteries, before and after Treatment with Dilevalol

transmural stimulation. Percent increases caused by 10^{-8} , 3×10^{-8} and 10^{-7} M dilevalol in the response were 12.3 ± 3.8 ($n = 8$), 15.8 ± 4.8 ($n = 8$) and 12.7 ± 6.9 ($n = 8$), respectively. However, further increase of the concentration of dilevalol (3×10^{-7} to 10^{-5} M) attenuated the contraction induced by transmural stimulation. Typical recordings of the response to transmural stimulation before and after treatment with dilevalol are shown in Fig. 6. In mesenteric arteries pretreated with 10^{-6} M propranolol, the potentiation by dilevalol (10^{-8} to 10^{-7} M) of the response to transmural stimulation was reversed to attenuation, and attenuation by dilevalol (up to 10^{-6} M) was enhanced (Fig. 7). Treatment with isoproterenol (10^{-10} to 10^{-9} M) also potentiated the contraction induced by transmural stimulation of mesenteric arteries in a concentration-dependent manner (Fig. 8A); potentiations caused by 10^{-10} , 3×10^{-10} and 10^{-9} M in the response were $28.5 \pm 2.5\%$ ($n = 4$), $44.6 \pm 7.6\%$ ($n = 5$) and $77.1 \pm 13.5\%$ ($n = 5$), respectively. Dilevalol and isoproterenol did not cause relaxation in the mesenteric arteries precontracted with $\text{PGF}_{2\alpha}$ (data not shown). On the other hand, the contraction induced by transmural stimulation was significantly attenuated by clonidine; attenuations caused by 10^{-8} and 10^{-7} M clonidine were $22.8 \pm 8.4\%$ ($n = 4$) and $44.0 \pm 7.6\%$ ($n = 4$), respectively. Attenuations by clonidine (10^{-8} and 10^{-7} M) of the response to transmural stimulation did not

significantly differ in control arteries and those treated with a high concentration (10^{-6} M) of dilevalol (Fig. 8B). In mesenteric arteries treated with dilevalol (10^{-6} M), attenuations caused by 10^{-8} and 10^{-7} M clonidine in the response were $26.6 \pm 3.6\%$ ($n = 4$) and $48.1 \pm 3.6\%$ ($n = 4$), respectively.

Fig. 7. Modification by Dilevalol of the Contractile Responses of Mesenteric Arteries to Transmural Stimulation in the Absence and Presence of 10^{-6} M Propranolol ●, control (8); ○, propranolol 10^{-6} M (7).

Contractions induced by the stimulation at 5 Hz in the absence and presence of propranolol were 200 ± 39 mg ($n = 8$) and 232 ± 72 mg ($n = 7$), respectively.

Fig. 8. (A) Modification by Isoproterenol of the Response to Transmural Stimulation in a Mesenteric Arterial Strip
(B) Modification by Clonidine of the Response to Transmural Stimulation in a Mesenteric Arterial Strip Treated with 10^{-6} M Dilevalol

Discussion

In cat coronary arteries partially precontracted with $\text{PGF}_{2\alpha}$, the addition of isoproterenol produced a concentration-related relaxation. Dilevalol antagonized the relaxant responses for isoproterenol in the arteries showing similar potency to propranolol. The pA_2 values for dilevalol and propranolol were 8.85 and 8.87, respectively. The value for dilevalol agreed well with those reported by other investigators; 8.26 for guinea pig left atrium and 8.52 for guinea pig trachea.⁷⁾ Thus, our results offer further evidence that dilevalol has a potent β -blocking activity similar to that of propranolol.

With mesenteric arteries, the contractions induced by norepinephrine were significantly attenuated by low concentrations of prazosin but were resistant to yohimbine. With middle arteries, the contractions induced by norepinephrine were attenuated by yohimbine but were slightly attenuated by prazosin. Highly selective blockade by prazosin of α_1 -adrenoceptors and by yohimbine of postsynaptic α_2 -adrenoceptors has been reported.¹¹⁾ Thus, our results suggest that norepinephrine-induced contractions in mesen-

teric arteries and middle cerebral arteries are mediated predominantly *via* α_1 - and α_2 -adrenoceptors, respectively. Similar findings have also been documented with isolated cat cerebral arteries.^{12,13)} Using these arteries, the α_1 - and α_2 -adrenoceptor blocking activities of dilevalol were evaluated. Dilevalol antagonized the contractile responses to norepinephrine and phenylephrine in mesenteric arteries with the pA_2 values of 6.32 and 6.46, respectively. The pA_2 values for dilevalol to the contractile responses to norepinephrine have been reported to be 6.40 in rat,⁵⁾ 5.90 in guinea pig⁷⁾ and 5.87 in rabbit aorta.³⁾ In contrast, inhibitory effects on the contractile responses by norepinephrine and clonidine in middle cerebral arteries were observed at a concentration above 10^{-5} M. Thus, it appears that dilevalol has weak α_1 -adrenoceptor blocking activity and the involvement of α_2 -adrenoceptor blocking activity is, if any, minimal.

The response to transmural stimulation was abolished by tetrodotoxin and phentolamine, suggesting the involvement of α -adrenoceptors which are stimulated by norepinephrine released from adrenergic nerve terminals. Treatment with

low concentrations of dilevalol (10^{-8} to 10^{-7} M) potentiated the contractile response to electrical stimulation in mesenteric arteries but high concentrations (3×10^{-7} to 10^{-5} M) attenuated it. Percent increases caused by 10^{-8} , 3×10^{-8} and 10^{-7} M dilevalol in the response were 12.3 ± 3.8 ($n = 8$), 15.8 ± 4.8 ($n = 8$) and 12.7 ± 6.9 ($n = 8$), respectively. The potentiation was reversed to attenuation by treatment with propranolol. In contrast, the concentration-response curves for norepinephrine (10^{-9} to 10^{-5} M) in mesenteric arteries were not potentiated by treatment with 10^{-8} and 10^{-7} M dilevalol (data not shown). Treatment with isoproterenol also potentiated the contractile response of the mesenteric arteries to transmural stimulation in a concentration-dependent manner. Cat mesenteric arteries have a particular nature because β -agonist such as isoproterenol does not cause any relaxation mediating postsynaptic β -adrenoceptors. It appears, therefore, that the potentiating effect of dilevalol results from an interaction with the presynaptic β -adrenoceptor, and that its weak α_1 -adrenoceptor blocking activity at low concentrations (up to 10^{-7} M) of dilevalol is masked by its β -adrenoceptor agonistic activity in the present experiment. Furthermore, the potentiating effect of dilevalol may not interact with the presynaptic α_2 -adrenoceptor, since the attenuations caused by clonidine of the response to transmural stimulation did not differ in control arteries and those treated with a high concentration of dilevalol. Presynaptic β -adrenoceptors have been thought to mediate a positive feedback mechanism for the release of norepinephrine.¹⁴⁾ Recently, this mechanism in some vascular tissues has been suggested at least in part, to be associated with local angiotensin II formation.^{15–18)} However, contrary evidence has also been reported with some preparations.^{19–21)} Thus, further studies are needed to determine whether or not the potentiating effects of dilevalol in mesenteric arteries are mediated by the presynaptic β -adrenoceptor.

Dilevalol did not cause relaxation in the coronary arteries precontracted with $\text{PGF}_{2\alpha}$, although isoproterenol did. However, Baum and Sybertz⁴⁾ reported that intra-arterial administra-

tion of dilevalol into the sympathetically denervated femoral vascular bed resulted in marked vasodilation, which is mediated by vascular β_2 -adrenoceptors. Matsunaga *et al.*⁶⁾ also reported that dilevalol caused relaxation in guinea pig trachea precontracted with histamine, which was attenuated by the pretreatment with propranolol. The nature of β -adrenoceptors or their signal transduction in cat coronary arteries may differ from those in dog femoral arteries and guinea pig trachea.

In the present study, we used isolated cat arteries to demonstrate that dilevalol has a potent β -adrenoceptor blocking activity, weak α_1 -adrenoceptor blocking activity, and β -adrenoceptor agonistic activity.

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