Vascular Effects of Betaxolol, a Cardioselective 
\( \beta \)-Adrenoceptor Antagonist, in Isolated Rat Arteries

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ABSTRACT—The effects of betaxolol on isolated rat arteries and the modes of action were investigated. Betaxolol \((10^{-5} - 10^{-3} \, M)\) relaxed the 80 mM K\(^+\)-induced contraction of aortic strips concentration-dependently. The 50% inhibitory concentration of betaxolol in the K\(^+\)-induced contraction was 3 times higher than that of papaverine and about 3 times lower than that of bunitrolol. The relaxations by betaxolol were also demonstrated in renal, mesenteric and femoral arteries. Betaxolol \((3 \times 10^{-6} \, M - 10^{-4} \, M)\) produced rightward parallel shifts of the concentration-response curves for Ca\(^{2+}\) in the K\(^+\)-depolarized aortic strips. On the other hand, betaxolol produced downward shifts as well as rightward shifts of the concentration-response curves for norepinephrine, 5-HT and angiotensin II. In K\(^+\)-depolarized aortic strips, the cytosolic Ca\(^{2+}\) concentration measured with a fluorescent indicator, fura-2, was decreased by betaxolol \((10^{-4} \, M)\) almost concomitantly with the loss of tention. An elevation of external Ca\(^{2+}\) from 2.5 mM to 10 mM restored both the cytosolic Ca\(^{2+}\) concentration and tention. The relaxations of arteries induced by betaxolol were not influenced by glybenclamide, methylene blue, indomethacin or removal of the endothelium. These results suggest that betaxolol possesses a direct vasodilating action, and the action may be due to the inhibition of Ca\(^{2+}\) influx across the cell membrane.

Betaxolol \([(\pm)-1-[4-[2-(cyclopropylmethoxy)-ethyl]phenoxy]-3-(isopropylamino)-2-propanol] hydrochloride is a cardioselective \( \beta \)-adrenoceptor antagonist with no intrinsic sympathomimetic activity and little membrane stabilizing activity (1, 2). In patients with essential hypertension, betaxolol was shown to produce potent and long lasting antihypertensive effects by once daily therapy (2, 3) because of high bioavailability and a long elimination half life (4, 5).

In previous laboratory studies, we (6–8) and other workers (9) have shown that a single oral administration of betaxolol lowers the blood pressure of various hypertensive models, i.e., spontaneously hypertensive rats, renal hypertensive rats, deoxycorticosterone/saline hypertensive rats and renal hypertensive dogs. However, other \( \beta \)-adrenoceptor antagonists such as atenolol are unlikely to produce antihypertensive effects by single administration in these hypertensive models (10–13). These data suggest that betaxolol might have a certain effect other than \( \beta \)-adrenoceptor antagonism. In fact, betaxolol induced an increase in blood flow by intraarterial administration (6) and a decrease in total peripheral resistance by intravenous administration in anesthetized dogs (14).

Although \( \beta \)-adrenoceptor antagonism of betaxolol has been well studied (2), the effects of betaxolol on isolated arteries have not yet
been reported. Therefore, the present study was designed to investigate the effects of betaxolol on vascular smooth muscle using isolated rat arteries and to elucidate the possible mechanism of the vasorelaxing actions. Effects of atenolol, a $\beta_1$-selective adrenoceptor antagonist (15), and bunitrolol, a non selective $\beta$-adrenoceptor antagonist with a vasodilator property (16–18), were also investigated for comparison.

MATERIALS AND METHODS

Preparation of arterial strips for recordings of mechanical activity

Male Wistar rats weighing 250–300 g were killed by blows on the head. The thoracic aorta or distal portion of renal, mesenteric and femoral arteries were rapidly excised, and excess fat and connective tissue were removed. The vessels were cut into rings of 3 to 4 mm in width and mounted in 20-ml tissue baths containing the physiological salt solution bubbled with 95% $O_2$ + 5% $CO_2$. The preparations were allowed to equilibrate under a proper resting tension (aorta, 1 g; other arteries, 0.5 g) at a temperature of 37°C for 60 min. Isometric tension changes were recorded with force transducers (Shinkoh UL-2GR) connected to a polygraph (NEC San-ai 360 system). The physiological salt solution contained 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO$_4$, 2.5 mM CaCl$_2$, 1.2 mM KH$_2$PO$_4$, 25 mM NaHCO$_3$, and 11 mM glucose. The high K$^+$ solution was made by replacing NaCl in the normal solution by equimolar KCl.

Estimation of mechanical responses

The contractile response to 80 mM K$^+$ was first obtained. Concentration-relaxation curves for the test drugs against the K$^+$-induced contraction were determined cumulatively. At the end of each series of experiments, $10^{-4}$ M papaverine was added to obtain the maximum relaxation. The 50% inhibitory concentration ($IC_{50}$) was calculated by taking the maximum relaxation induced by drugs as 100%. In some experiments, we compared the relaxing responses to betaxolol between endothelium intact and denuded aortic preparations. The removal of endothelium was performed by gently rubbing the intimal surface with the tip of forceps.

In the contraction induced by the addition of Ca$^{2+}$, the preparations were incubated in Ca$^{2+}$-free solution for 20 min and then in Ca$^{2+}$-free 80 mM K$^+$-depolarizing solution for 30 min. A cumulative concentration-response curve for Ca$^{2+}$ was then determined.

In the agonist-induced contractions, nor-epinephrine, 5-hydroxytryptamine (5-HT) or angiotensin II were added in a cumulative manner, and concentration-response curves were obtained. After the wash-out period of 60 min, the test drugs were pretreated for 10 min, and second concentration-response curves for Ca$^{2+}$ or agonists were obtained.

The $pA_2$ values for the test drugs were calculated according to the method described by van Rossum (19).

Measurement of cytosolic Ca$^{2+}$ concentrations

Cytosolic Ca$^{2+}$ concentration was measured with the fluorescent indicator fura-2 according to the method of Ozaki et al. (20). The gently rubbed aorta of the rat was cut helically and made into strips. The muscle strips were treated with acetoxymethyl ester of fura-2 (5 $\mu$M) more than 4 hr at room temperature. Fura-2-Ca$^{2+}$ signals were measured with a fluorimeter (CAF-100, JASCO). The muscle strip was excited alternatively by 340 nm and 380 nm light, and the ratio of the 500 nm fluorescence due to excitation at 340 nm to that at 380 nm (F340/F380) was calculated from successive illumination periods. Since it was difficult to evaluate the dissociation constant of fura-2 for Ca$^{2+}$ in the cytoplasm (21), the ratio of F340 to F380 was used as an indicator of cytosolic Ca$^{2+}$, taking the ratio in the resting state as 0% and that in high K$^+$-stimulated state as 100%. The muscle strip was held horizontally in a temperature controlled, 8-ml tissue bath. One end of the muscle strip was connected to a force transducer to monitor the mechanical activity.
Statistical analysis

Results of the experiments are expressed as the mean value ± S.E. Student’s paired and unpaired t-test were used for statistical analysis of the results, and a P value less than 0.05 was taken as significant.

Drugs and chemicals

The following drugs were used: betaxolol hydrochloride (Synthelabo); bunitrolol hydrochloride (extracted from Betrilol® Nippon Boehringer Ingelheim); atenolol, l-norepinephrine hydrochloride, diltiazem hydrochloride, 5-hydroxytryptamine creatinine sulfate, angiotensin II acetate salt human, cremophor El, glybenclamide, methylene blue, indomethacin (Sigma); acetoxymethyl ester of fura-2 (Dojindo); papaverine hydrochloride (Wako). Glybenclamide and indomethacin were dissolved in dimethylsulfoxide (Sigma) and ethanol, respectively, and diluted with distilled water.

RESULTS

Effects on the K⁺-induced contraction

In aortic strips that were contracted with 80 mM K⁺, the addition of betaxolol at concentrations ranging from 10⁻⁶ M to 10⁻³ M produced a concentration-dependent relaxation (Fig. 1). The relaxation attained at 10⁻⁴ M was 69.5 ± 3.7% of that induced by 10⁻⁴ M papaverine. Bunitrolol also produced a concentration-dependent relaxation. The relaxation at 10⁻⁴ M was 30.5 ± 1.7% of that induced by 10⁻⁴ M papaverine. Atenolol at 10⁻³ M had no effect on the K⁺-induced contraction. Papaverine used as a reference drug showed relaxation of K⁺-induced contraction at concentrations above 10⁻⁶ M. The IC₅₀ of betaxolol, bunitrolol and papaverine were 4.8 ± 0.38 × 10⁻⁵ M, 1.5 ± 0.6 × 10⁻⁴ M and 1.9 ± 0.03 × 10⁻⁵ M, respectively.

The relaxations of K⁺-induced contraction by betaxolol were also observed in renal, mesenteric and femoral arteries (Fig. 2): the IC₅₀'s of betaxolol were 2.2 ± 0.17 × 10⁻⁵ M, 3.1 ± 0.26 × 10⁻⁵ M and 3.9 ± 0.33 × 10⁻⁵ M, respectively.

Effects on the concentration-response curves for Ca²⁺, norepinephrine, 5-HT or angiotensin II

Betaxolol (3 × 10⁻⁶–10⁻⁴ M) produced a
rightward parallel shift of the concentration-response curve for Ca\(^{2+}\) in a concentration-dependent manner (Fig. 3). Diltiazem (10\(^{-7}\) – 3 \times 10\(^{-7}\) M) also produced a rightward parallel shift of the curve. The calculated pA\(_2\) values were 5.4 ± 0.06 for betaxolol and 7.6 ± 0.07 for diltiazem.

On the other hand, betaxolol showed a downward shift as well as rightward shift of the concentration-response curves for noradrenaline, 5-HT and angiotensin II. The effects of betaxolol (10\(^{-5}\) M) on noradrenaline, 5-HT and angiotensin II-induced contractions were less prominent than that on Ca\(^{2+}\)-induced contraction (Fig. 4).

Effects of betaxolol on cytosolic Ca\(^{2+}\) concentrations

Figure 5 shows the effects of betaxolol on fura-2 fluorescence and tension of the rat aorta. When the aorta was exposed to 80 mM K\(^+\), F340/F380 promptly increased and tension gradually increased, as reported by Ozaki et al. (20). In the presence of 80 mM K\(^+\), an application of 10\(^{-4}\) M betaxolol lowered the F340/F380 almost concomitantly with the loss of tension. Twenty minutes after the application, betaxolol decreased F340/F380 and tension to 34.8 ± 6.3% (n = 5) and 29.9 ± 5.5% (n = 5) of the pre-drug values, respectively. During the falling phase of F340/F380 and
tension, an elevation of external Ca\(^{2+}\) concentration from 2.5 mM to 10 mM restored F340/F380 and tension to 96.0 ± 6.8% (n = 4) and 118.3 ± 8.6% (n = 4) of the pre-drug values, respectively. These results suggest that the inhibitory action of betaxolol on the F340/F380 and tension in the presence of 80 mM K\(^+\) is antagonized by increased external Ca\(^{2+}\) concentrations.

**Involvement of K\(^+\) channel, cGMP, prostaglandins or endothelium derived relaxing factors**

The relaxant effects of betaxolol on the K\(^+\)-induced contraction in the aorta were not influenced by treatment with 10\(^{-7}\) M glybenclamide, 10\(^{-6}\) M methylene blue or 3 × 10\(^{-6}\) M indomethacin. The relaxant responses were also unaffected by removal of endothelium. The IC\(_{50}\) of betaxolol in the aortic strips pre-treated with glybenclamide, methylene blue, indomethacin or removal of endothelium were 6.2 ± 0.56 × 10\(^{-5}\) M, 5.7 ± 0.44 × 10\(^{-5}\) M, 5.7 ± 0.64 × 10\(^{-5}\) M and 5.2 ± 1.2 × 10\(^{-5}\) M, respectively (n = 7).

**DISCUSSION**

In the present study, betaxolol was found to exert a concentration-dependent relaxing effect on the isolated aortic strips, which was 3 times less potent than that of papaverine. In the previous in vivo observations with the hindlimb perfusion model in dogs, the vasodilating action of betaxolol was also 3 times less potent than that of papaverine (6). Bunitrolol is known to lower the blood pressure of renal hypertensive dogs, like betaxolol, by single oral administration (13), and this antihypertensive action may be partly due to its direct vasodilating action (16). Compared with bunitrolol, betaxolol relaxed the K\(^+\)-induced contraction at lower concentrations. Thus, it is suggested that the vasorelaxing action of betaxolol also plays some role in its antihypertensive action by decreasing the total peripheral resistance. On the other hand, in the present experiments, atenolol showed no vasorelaxing effect, as previously reported (22), suggesting that a vasorelaxation is not involved in mechanisms of antihypertensive action of atenolol. Atenolol's lack of vasorelaxing action may be related to the fact that this drug has no antihypertensive action in renal hypertensive dogs (13). The involvement of vasorelaxation in the antihypertensive mechanism of betaxolol has also been suggested by Satoh et al. (14), who demonstrated that betaxolol decreased the total peripheral resistance but atenolol increased it when both drugs decreased the blood pressure.
for the beneficial renal hemodynamic changes in patients shown by Pathe and Schwartz.

It has been shown that $K^+$-induced contraction may be associated mainly with increased influx of $Ca^{2+}$ across the depolarized cell membrane (24). Therefore, it can be suggested that betaxolol produced the relaxation of the vascular smooth muscles by inhibiting the $Ca^{2+}$ influx across the membrane depolarized by $K^+$. In support of this suggestion, we found that betaxolol produced a concentration-dependent rightward parallel shift of the concentration-response curve for $Ca^{2+}$ in the $K^+$-depolarized aortic strips, suggesting a competitive antagonism. Such an inhibitory manner was also seen in the case of diltiazem. Against the concentration-response curve for norepinephrine, 5-HT and angiotensin II, betaxolol exhibited a downward shift as well as a rightward shift. Such inhibition was also observed with nifedipine and other $Ca$ antagonists (25, 26). No interaction of betaxolol with either $\alpha_1$, $\alpha_2$ or 5-HT$_2$-receptors has been observed in receptor binding studies (27).

Cytosolic $Ca^{2+}$ concentrations measured with fura-2 fluorescence and tension were reduced by betaxolol in the $K^+$-depolarized aorta. The extent of reductions in both parameters was almost similar. These effects were antagonized by increased external $Ca^{2+}$ concentration. These results further suggest that betaxolol possesses a Ca antagonist action.

The possibility that betaxolol relaxed the 80 mM $K^+$-induced contraction of arteries by a mechanism other than inhibition of $Ca^{2+}$ influx was also investigated. It has been reported that cromakalim, a $K^+$ channel opener, exerts no inhibition on the aorta contracted with 80 mM $K^+$ (28). Moreover, glybenclamide, a $K^+$ channel blocker, did not affect the relaxation response to betaxolol. Thus, the $K^+$ channel opening action can be ruled out in the vasorelaxing mechanism of betaxolol. The betaxolol induced relaxation was also unaffected by methylene blue (an inhibitor of guanylate cyclase), indomethacin (an inhibitor of cyclooxygenase) or removal of endothelium. These data indicate that cyclic GMP, prostaglandins and endothelium derived relaxing factors are not also involved in the betaxolol-induced relaxation of $K^+$-contracted arteries.

In the present experiments with isolated rat arteries, the concentration of betaxolol showing vasorelaxation in the presence of 80 mM $K^+$ was above $3 \times 10^{-7}$M. The plasma concentration of oral betaxolol in healthy volunteers at a dose of 20 mg is reported to be about 50 $\mu$g/l ($1.5 \times 10^{-7}$M) at peak (2). This plasma concentration is not so remote from the effective concentration observed in the present experiments. Felodipine, a $Ca$ antagonist, has been reported to cause relaxation in the presence of high $K^+$ at much lower concentrations in mesenteric resistance vessels than in aorta (29, 30). Thus betaxolol may also induce vasorelaxation in resistance vessels at lower concentration than in the aorta.

Considering these observations, it seemed that in clinical use, the direct vasorelaxing action of betaxolol may be partly involved in the antihypertensive mechanism as suggested in animal experiments, although the main mechanism is $\beta$-adrenoceptor antagonism.

In conclusion, betaxolol has a direct vasorelaxing action in isolated rat arteries due to the inhibition of $Ca^{2+}$ influx across the cell membrane, and this action may partly contribute to its potent antihypertensive action in addition to the $\beta_1$-selective adrenoceptor antagonist action.

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