Mechanism of Pindolol-Induced Vasoconstriction in Isolated and Perfused Dog Coronary Arteries

Tokio Nakane, Kimiko Kawai and Shigetoshi Chiba*

Department of Pharmacology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390, Japan

Received October 16, 1992 Accepted February 8, 1993

ABSTRACT—The mechanism of pindolol-induced vasoconstriction in isolated and perfused dog coronary arteries was studied. Single injections of pindolol (1–100 μg), propranolol (1–30 μg), and 5-hydroxytryptamine (5-HT, 0.001–1 μg) produced a dose-related vasoconstriction in dog coronary arteries which were dilated by acetylcholine. L-Pindolol constricted coronary arteries, but d-pindolol did not. The responses to pindolol and propranolol were not affected by any of the following compounds (100 μg): bunazosin (a selective α1-adrenergic antagonist), DG 5128 (a selective α2-adrenergic antagonist), atropine (a muscarinic antagonist), chlorpheniramine (a selective H1-antagonist), cimetidine (a selective H2-antagonist), and ketanserin (a selective 5-HT2 antagonist). Methysergide (10 μg, a 5-HT, and 5-HT2 antagonist) significantly reduced pindolol and propranolol-induced vasoconstrictions, although it did not reduce norepinephrine-induced vasoconstriction in the presence of 5 μM propranolol. Methysergide (10 μg) and ketanserin (100 μg) significantly suppressed 5-HT-induced vasoconstriction. Diltiazem (100 μg, a calcium antagonist) and the incubation in Ca2+-free solution containing 1 mM EGTA for 1 hr significantly reduced the vasoconstrictions induced by pindolol and propranolol. The Ca2+-free solution containing 1 mM EGTA abolished the vasoconstriction induced by 5-HT in the presence of 1 μM ketanserine. In a solution containing 20 mM KCl, the vasoconstrictions caused by pindolol and propranolol were enhanced in dog coronary arteries. These results indicate that the direct contractile effects of pindolol on dog coronary arteries are mediated, at least partly, through 5-HT1-like receptors, but not through α-adrenergic receptors. The vasoconstriction induced by pindolol appears to be associated with an increase of Ca2+ influx in the smooth muscle cell membrane.

Keywords: Pindolol, Coronary artery, Vasoconstriction, 5-Hydroxytryptamine, Ketanserin

The administration of β-adrenergic blockers to patients with coronary artery disease is a well-established therapeutic regime (1). β-Adrenergic blockers induce a decrease in myocardial oxygen consumption and a reduction of symptoms of myocardial ischemia in patients with the disease. However, propranolol exacerbated coronary artery spasms in some patients with variant angina (2, 3). It was assumed that the administration of β-adrenergic blockers including propranolol might exacerbate vasospastic angina by inhibiting β-adrenergic receptor-mediated coronary arterial dilation and leaving α-adrenergic receptor mediated vasoconstriction unopposed (3). On the other hand, Vatner and Hintze (4) showed that the vasoconstriction caused by propranolol was not due to the unopposed α-adrenergic vasomotor tone in the conscious dog. Previous reports suggested that propranolol contracted isolated dog coronary arteries by increasing Ca2+ influx across the smooth muscle cell membrane (5, 6). In contrast, it was reported that propranolol dilated isolated pig and dog coronary arteries precontracted by KCl through inhibition of Ca2+ influx across the smooth muscle cell membrane (7). It was also reported that other β-adrenergic blockers, metoprolol and sotalol, did not contract isolated dog coronary arteries (5).

Pindolol, a non-selective β-adrenergic blocker has intrinsic sympathomimetic activities, e.g., marked vasodilator activity in vessels (8, 9). Pindolol dilated human arteries and veins of the forearm, although propranolol constricted them (10). Recently, we showed that pindolol constricted isolated, perfused dog and monkey coronary arteries and that the vasoconstriction of monkey coronary arteries is partially mediated by 5-hydroxytryptamine (5-
HT) receptors (11, 12).

In this study, we analyzed the vascular responses of the isolated and perfused dog coronary arteries to pindolol and compared them with those to propranolol by using subtype-selective antagonists.

MATERIALS AND METHODS

Arterial preparations
Mongrel dogs (6–20 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After an intravenous injection of sodium heparin (200 units/kg), the animals were killed by rapid bleeding from the common carotid artery. The femoral artery and the circumflex branch of the left coronary artery were isolated and cleaned of loose adipose and connective tissues. The arteries were cut into segments (1.1–2 mm (coronary arteries) and 1.5–3 mm (femoral arteries) in outside diameter and 1.5 cm in length). Side branches were tied with silk threads.

Perfusion system
Segments of the arteries were carefully cannulated with a stainless steel needle (0.95–2.4 mm in outside diameter) with small side holes at a distance of 5 mm from the distal sealed end as described previously (13). The artery was tied to the needle at a position half-way between the holes and the sealed end by a silk thread. Thus, the stream from the holes of the needle passed through the internal surface of the artery. The artery was perfused by Krebs-Henseleit solution with a microtube pump (MP-3, Tokyo Rikakikai, Tokyo). The flow rate was kept constant through the experiments (approximately 2 ml/min). The basal perfusion pressures were 65.5±4.0 mmHg (n=55). The pressure changes were measured with an electric manometer (AP621 G, Nihon Kohden, Tokyo) and recorded by an ink-writing rectigraph (RJG-4028, Nihon Kohden). Therefore, vasoconstriction or vasodilation was recorded as an increase or a decrease in perfusion pressure, respectively. Krebs-Henseleit solution contained: 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 2.5 mM CaCl2, 1.2 mM KH2PO4, 25 mM NaHCO3, and 11 mM glucose. In the Krebs-Henseleit solution containing 20 mM KCl, NaCl was replaced with equimolar KCl. The physiological solution was gassed with 95% O2 and 5% CO2 and was maintained at 37°C with a thermostatic pump (EF2, Haake, Karlsruhe, FRG). The experiments were started when the artery had equilibrated for about 1 hr, until perfusion pressure increased spontaneously and a stable baseline appeared. The responses to KCl and acetylcholine (ACh) were tested at first, and only the coronary arteries dilated by ACh were used in this study.

Drug administration and dose-response curve
The drug solution was administered into the rubber tube connecting the cannula in a volume of 10–30 μl by a microsyringe (Terumo, Tokyo) for approximately 4 sec. After the control dose-response curve was obtained, the preparation was perfused by drug-free Krebs-Henseleit solution for at least 20 min. The second dose-response curve was not significantly different from the first one in our preliminary experiments. Antagonists were administered as a bolus injection. The second dose-response curves were obtained after the perfusion pressure had returned to the baseline except for the cases of methysergide, ketanserin and diltiazem, where a second dose-response curve was obtained shortly after the peak response to the first dose, because the durations of responses to these three antagonists were more than 20 min in our preliminary experiments. The blocking effect of the antagonist usually lasted for 20 min in experiments similar to those reported previously (14–16). Thus, all dose-response curves were made by non-cumulative administration of the agonists.

Drugs
d,l-Pindolol maleate (Sandoz, Basle, Switzerland), l- and d-pindolol (base; Sandoz), d,l-propranolol HCl (propranolol; Sigma, St. Louis, MO, USA), 5-HT creatinine sulfate (Merck, Darmstadt, FRG), ACh Cl (Obisoat Inj.®, Daiichi, Tokyo), isoproterenol HCl (Protanol Inj.®, Nippon Kagaku, Tokyo), norepinephrine HCl (NE, Sanko, Tokyo), bunazosin HCl (Detantol®, Eisai, Tokyo), DG 5128 (2-[2-(4,5-dihydro-1H-imidazol-2-yl)-1-phenylethyl]pyridine dihydrochloride sesquihydrate; Daiichi), methysergide maleate (Sandoz), ketanserin (base, Sigma), diltiazem HCl (Helbesser®, Tanabe, Osaka), chlorpheniramine maleate (Chlortrymeton Inj.®, Shionogi, Osaka), cimetidine (Tagamet Inj.®, Fujisawa, Osaka) and atropine sulfate (Wako, Osaka). The enantiomers of pindolol (100 μg/10 μl) and ketanserin (100 μg/10 μl) were dissolved in an aqueous solution of 0.1 M tartaric acid first and then diluted in physiological solution before the start of the experiments.

Statistical analysis
Results are expressed as the mean ± S.E. Data were evaluated statistically by Student’s t-test for paired observations; a P value of less than 0.05 was considered to indicate significant difference.

RESULTS

Responses of dog coronary and femoral arteries to ACh, isoproterenol, pindolol and propranolol
In the dog coronary artery, ACh (0.01–1 μg) and isoproterenol (0.0001–1 μg) induced a dose-related vasodila-
tion (data not shown) as reported previously (17). The removal of endothelium by a bolus injection of 1 mg saponin (18) did not affect the vasoconstriction induced by d,l-pindolol (data not shown).

d,l-Pindolol (1–100 µg (2.74–274 nmol)) and propranolol (1–30 µg (3.38–101 nmol)) constricted the dog coronary artery in a dose-related manner, whereas the same doses of d,l-pindolol produced no responses, and only large doses (more than 100 µg) constricted the dog femoral artery (Fig. 1). Large doses of d,l-pindolol (more than 10 µg (27.4 nmol)) sometimes induced a slight vasodilation in the dog coronary artery as shown previously (11).

Responses of dog coronary arteries to pindolol enantiomers

The vehicle (tartaric acid), in which pindolol enantiomers were dissolved, produced a dose-related vasoconstriction, when pindolol enantiomers were injected at doses of more than 10 µg. The responses to the vehicle were subtracted from those to pindolol enantiomers. l-Pindolol (1–100 µg (4.03–403 nmol)) constricted the dog coronary artery in a dose-related manner, but d-pindolol (1–100 µg (4.03–403 nmol)) caused no response (Fig. 2). The response to 100 µg (274 nmol) of d,l-pindolol (maleate) was 79.5 ± 11.2% (n = 8) of that to 100 µg (403 nmol) of l-pindolol (base), and the ED$_{50}$ values of d,l-pin-

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**Fig. 1.** Dose-response curves for the contractile effects of propranolol and pindolol on isolated and perfused dog coronary and femoral arteries. Dog coronary artery: pindolol (○), propranolol (●). Dog femoral artery: pindolol (□), propranolol (■). Each point represents the mean values of 6 to 10 determinations; vertical bars show S.E.M.

**Fig. 2.** Effects of pindolol enantiomers on isolated and perfused dog coronary arteries. The maximal response is that to l-pindolol. l-Pindolol (○), d-pindolol (●), d,l-pindolol (△). Each point represents the mean values of 8 determinations; vertical bars show S.E.M.

**Fig. 3.** Representative recordings of the inhibitory effects of methysergide (10 µg) and diltiazem (100 µg) on pindolol-induced vasoconstrictions in isolated and perfused dog coronary arteries.
dolol and l-pindolol were 46.0±9.9 µg (126±27.1 nmol, n = 8) and 12.6±2.6 µg (50.8±10.5 nmol, n = 8), respectively.

**Effects of α-adrenergic, muscarinic, and histamine antagonists on pindolol- and propranolol-induced vasoconstrictions**

Bunazosin (100 µg) and DG 5128 (100 µg), which are selective α1- and α2-adrenergic antagonists, respectively, did not alter the pindolol- and propranolol-induced vasoconstrictions in the dog coronary artery (data not shown). These doses of bunazosin and DG 5128 produced vasodilations of 10 to 20 mmHg and about 10 mmHg in the dog coronary artery, respectively.

The muscarinic antagonist, atropine at 100 µg and the histamine antagonists, chlorpheniramine (a selective H1-antagonist) at 100 µg and cimetidine (a selective H2-antagonist) at 100 µg, did not affect d, l-pindolol- and propranolol-induced vasoconstrictions (data not shown).

**Effects of 5-HT antagonists on pindolol-, propranolol-, NE-, and 5-HT-induced vasoconstrictions**

The 5-HT1 and 5-HT2 antagonist methysergide (10 µg) significantly reduced pindolol-, propranolol-, and 5-HT-induced vasoconstrictions in the dog coronary artery (Figs. 3 and 4), whereas it did not attenuate the norepinephrine-induced vasoconstriction in the presence of 5 µM propranolol (data not shown). Ketanserin (100 µg), a selective 5-HT2 antagonist, did not affect pindolol- (Fig. 5) and propranolol-induced vasoconstrictions (data not shown), although it significantly reduced the 5-HT-induced vasoconstriction (Fig. 5). These doses of methysergide and ketanserin produced vasoconstrictions of 10 to 50 mmHg but the response to ketanserin was not different from that to the vehicle in the dog coronary artery.

**Effects of KCl, diltiazem and Ca2+-free solution on pindolol-, propranolol-, and 5-HT-induced vasoconstrictions**

When dog coronary arteries were incubated in a solution containing 20 mM KCl for 1 hr, the perfusion pressure increased by 50–100 mmHg. d,l-Pindolol and propranolol induced no vasodilation, and the vasoconstrictions...
Diltiazem (100 µg) decreased the perfusion pressure by 10–50 mmHg and significantly inhibited both pindolol- and propranolol-induced vasoconstrictions in the dog coronary artery (Figs. 3 and 6). In a Ca^{2+}-free solution containing 1 mM EGTA, the perfusion pressure was decreased by 10 to 20 mmHg, and pindolol and propranolol induced no response after 1-hr incubation in the dog coronary artery (Fig. 6). The incubation in Ca^{2+}-free solution containing 1 mM EGTA for 1 hr also abolished the vasoconstriction induced by 5-HT in the presence of 1 µM ketanserine (Fig. 7).

**DISCUSSION**

The results in the present study indicate that pindolol and propranolol constricted isolated and perfused dog coronary arteries dose-dependently. Those vasoconstrictions are mediated, at least partly, by 5-HT_{1}-like receptors, not by α-adrenoceptors, because α-adrenoceptor antagonists and ketanserin (a selective 5-HT_{2} receptor antagonist) did not affect the vasoconstrictions, but methysergide (a 5-HT_{1} and 5-HT_{2} antagonist) significantly inhibited them. Furthermore, the vasoconstrictions are induced by increasing Ca^{2+} influx in the vascular smooth muscle cell membrane, because diltiazem (a calcium antagonist) or free calcium ion perfusate with 1 mM EGTA significantly suppressed them.

Relaxations caused by several β-adrenergic blockers were endothelium-dependent in the rat aorta (20). Cyanopindolol (1 µM), a derivative of pindolol, contracted rings of dog coronary arteries without endothelium (21). Pindolol induced vasoconstrictions in isolated and perfused monkey and dog coronary arteries that had been vasodilated by ACh (ref. 12 and this study). In this study, the removal of the endothelium by a bolus injection of saponin did not affect the pindolol-induced vasoconstriction (data not shown).

The antagonists changed the perfusion pressures by 10 to 50 mmHg. In Fig. 3, methysergide (10 µg) and diltiazem (100 µg) increased and decreased the perfusion pressures, respectively. However, both antagonists suppressed the responses to pindolol. Although ketanserin (100 µg) increased the perfusion pressures by 10 to 50 mmHg (actually the vehicle constricted the coronary arteries), it depressed the responses to 5-HT significantly and did not affect those to pindolol, as shown in Fig. 5. Therefore, it seems that the changes in perfusion pressure do not contribute to the reductions of the responses to pindolol.

Pindolol, a β-adrenergic blocker, has intrinsic sympathomimetic activities (8). Namely, pindolol dilated human arteries and veins (10, 19) and dog mesenteric and femoral arteries (8, 9). However, pindolol constricted monkey and dog coronary arteries either non-preconstricted or preconstricted by 20 mM KCl (refs. 11 and 12, and this study). Clinically, it was assumed that β-adrenergic...
blockers induced coronary vasospasm by inhibiting β-adrenergic receptor-mediated vasodilation and leaving α-adrenergic receptor-mediated vasoconstriction unopposed (3). Neither bunazosin nor DG 5128, which are selective α₁ and α₂-adrenergic antagonists, respectively (22, 23), altered pindolol- and propranolol-induced vasoconstrictions in this study. One hundred micrograms of bunazosin is the dose that significantly reduced phenylephrine (a selective α₂-adrenergic agonist)-induced vasoconstrictions (24). There are no functional α₂-adrenergic receptors in large dog coronary arteries (24, 25). Therefore, α-adrenergic receptors do not mediate pindolol- and propranolol-induced vasoconstrictions in somewhat large dog coronary arteries.

In the present study, atropine (100 µg), chlorpheniramine (100 µg) and cimetidine (100 µg) had no effect on pindolol- and propranolol-induced vasoconstrictions. These doses of antagonists were the same or higher than those that blocked the vascular responses to ACh and histamine in isolated and perfused dog coronary arteries, respectively (17, 26). On the other hand, the 5-HT antagonist methysergide (10 µg) significantly inhibits pindolol-, propranolol-, and 5-HT-induced vasoconstrictions (Fig. 4). Although methysergide has a weak α-blocking activity (27), it did not affect the NE-induced vasoconstriction in the presence of 5 µM propranolol in this study. Furthermore, methysergide did not suppress the KCl-induced vasoconstriction (data not shown). Single injection of methysergide (10 and 100 µg) inhibited the pindolol-induced vasoconstriction of monkey coronary arteries in a dose-related manner (12). In the rat central nervous system, Hjorth and Carlsson (28) reported that l-pindolol is a mixed 5-HT receptor agonist-antagonist. A radioligand binding study showed that pindolol enantiomers interact with 5-HT receptor sites in rat brain (29). Therefore, pindolol constricts isolated and perfused dog coronary arteries through activation of 5-HT receptors as previously shown in isolated and perfused monkey coronary arteries.

Ketanserin (100 µg), which significantly suppressed the 5-HT-induced vasoconstriction, did not affect the pindolol-induced vasoconstriction (Fig. 5), whereas methysergide (10 µg) reduced it in this study. Bradley et al. (30) proposed a framework for the classification and nomenclature of functional 5-HT receptors. In short, three main types of 5-HT receptors, 5-HT₁, 5-HT₂ and 5-HT₃, are distinguished. Recently, 5-HT₄ receptors have been characterized (31). Methysergide is a weak 5-HT₁-like and 5-HT₃ receptor antagonist (30). Ketanserin shows negligible affinity for 5-HT₁ binding sites (32). Pindolol shows stereoselectivity for binding to 5-HT₁, but not 5-HT₂ recognition sites in rat brain membranes (30, 33). Furthermore, it was reported that ketanserin only weakly inhibited the contraction of rings elicited by 5-HT and non-5-HT₂ receptors predominates in dog coronary arteries (21, 34, 35). Therefore, pindolol may cause a vasoconstriction, at least partly, through stimulation of 5-HT₁-like receptors in isolated and perfused dog coronary arteries.

d-Pindolol had no effect, whereas l-pindolol constricted dog coronary arteries in this study (Fig. 2). l- and d-pindolol have fundamentally different properties. d-Pindolol had only weak β-adrenergic blocking effects, although its β-adrenergic receptor stimulating effects were equal to those of l-pindolol in isolated and perfused dog mesenteric arteries (9). The affinity of l-pindolol for the 5-HT₁ binding sites in rat cerebral membrane was approximately 100 times higher than that of d-pindolol (33). Therefore, only l-pindolol may induce a vasoconstriction mediated through 5-HT₁-like receptors in isolated and perfused dog coronary arteries.

Pindolol also constricted dog coronary arteries preconstricted by KCl (20 mM) in this study. Turlapaty and Altura (6) reported that 15 mM KCl potentiated the propranolol-induced contraction in isolated dog coronary arteries. In contrast, propranolol relaxed isolated and pig coronary arteries precontracted by 30 or 127.7 mM KCl through inhibition of Ca²⁺ influx across the smooth muscle cell membrane (7). Pindolol also relaxed isolated human arteries (except coronary artery) and dog mesenteric arteries contracted by 40 or 127 mM KCl through stimulation of β-adrenergic receptors (9, 19). These discrepancies may be due to the differences of experimental conditions, e.g., the different concentrations of KCl. Further studies are needed, however, to confirm the mechanism of the potentiation.

The calcium antagonist diltiazem (100 µg) significantly attenuated pindolol- and propranolol-induced vasoconstrictions (Fig. 6), and these drugs and 5-HT in the presence of 1 µM ketanserin produced no response in a Ca²⁺-free solution containing 1 mM EGTA (Fig. 7). Diltiazem (100 µg) blocked the KCl-induced vasoconstriction in isolated and perfused dog coronary arteries (25). Our results on propranolol agree with the previous reports (5, 6). Therefore, the vasoconstrictor responses of isolated and perfused dog coronary arteries to pindolol and propranolol are predominantly associated with increasing Ca²⁺ influx across the vascular smooth muscle membrane.

In conclusion, pindolol and propranolol produce a vasoconstriction by increasing Ca²⁺ influx through the vascular smooth muscle membrane, and these effects are mediated by 5-HT₁-like receptors in isolated and perfused dog coronary arteries.
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