Efonidipine, a Long-Acting Dihydropyridine Derivative, Attenuates Coronary Vasoconstriction Induced by Endothelin-1 in Dogs

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ABSTRACT—Effect of efondipine, a long-acting dihydropyridine derivative, on the endothelin-1 (ET-1)-induced coronary vasoconstriction was studied in open-chest anesthetized dogs. Efondipine (0.03 or 0.1 mg/kg) was administered i.v. 10 min before an intracoronary injection of ET-1 (30 pmol/kg). An intracoronary injection of ET-1 decreased coronary blood flow (CBF) that was measured by a flow probe. The ET-1-induced decrease in CBF was sustained for more than 30 min without significant changes in blood pressure and heart rate. Pretreatment with efondipine attenuated the decrease in CBF induced by ET-1 significantly and dose-dependently. ET-1 also reduced coronary diameter for more than 30 min as evaluated by the coronary angiography technique. Pretreatment with efondipine also attenuated the reduction in coronary diameter induced by ET-1 significantly and dose-dependently. These effects of efondipine were sustained for at least 30 min after the ET-1 administration. It is concluded that efondipine attenuates the ET-1-induced vasoconstriction, and therefore the drug would be useful for some patients with variant angina, in which ET-1 is involved in the genesis of coronary vasoconstriction.

Keywords: Coronary blood flow, Angiography, Endothelin-1, Efondipine, Calcium channel blocker

Endothelin-1 (ET-1) is one of the most potent endogenous vasoconstrictors that can be released from the endothelium of blood vessels including the coronary artery (1, 2). Several studies have revealed that ET-1 constricts the coronary artery (3, 4) and decreases coronary blood flow (5, 6). Toyo-oka and co-workers (7) have reported that the levels of ET-1 in venous and coronary sinus blood are high in patients with variant angina. Therefore, it seems that ET-1 is involved in the genesis of coronary vasospasm and hence variant angina pectoris (8).

Calcium channel blockers have been widely used as therapeutic agents for patients with angina pectoris, particularly variant angina, with great success (9). Efondipine hydrochloride ((±)-2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitrophenyl)-3-pyridine-carboxylate hydrochloride ethanol) is a long-acting dihydropyridine derivative synthesized by Nissan Chemical Industries, Ltd. (10) that possesses a calcium channel blocking property (11). The chemical structure of efondipine hydrochloride is shown in Fig. 1. Efondipine decreases blood pressure and increases coronary blood flow in anesthetized dogs, and these effects are slow in onset and long in duration (12). Therefore, it is expected that efondipine may have a long-lasting antivasospastic effect and can be used for patients with variant angina. The present study was undertaken first to examine whether efondipine could attenuate the decrease in coronary blood flow induced by ET-1 and second to examine whether efondipine could suppress constriction of the
coronary artery provoked by ET-1 in anesthetized open-chest dogs.

MATERIALS AND METHODS

Animals
Forty-two healthy mongrel dogs (Animal Laboratory for Medical Research in Asahikawa Medical College, Asahikawa) of either sex weighing 7 to 15 kg were employed.

Study with direct measurement of coronary blood flow
Twelve dogs were anesthetized with sodium pentobarbital (30 mg/kg) given intravenously (i.v.), and their lungs were artificially ventilated with room air. A left thoracotomy was carried out at the forth or fifth intercostal space, and then the left ventricular wall was exposed. After a portion of the left anterior descending coronary artery (LAD) was dissected free from adjacent tissues at the level just proximal to the first diagonal branch, coronary blood flow was measured with an electromagnetic flow probe (FR020T; Nihon Kohden, Tokyo) placed around the LAD. In order to perform intracoronary (i.c.) administration of ET-1, a polyethylene catheter was inserted in a small branch of the LAD in a retrograde manner so that its tip could be positioned near the main trunk of the LAD where the flow probe was placed. Arterial blood pressure was measured by a pressure transducer (MPU-0.5, Nihon Kohden) connected to a polyethylene catheter that was inserted in the left femoral artery. Heart rate was counted by a heart rate meter (model 2140; NEC San-ei, Tokyo).

In 4 dogs, ET-1 (30 pmol/kg) was administered i.c. Changes in coronary blood flow and other hemodynamic parameters were monitored until 30 min after the i.c. administration of ET-1. In 4 other dogs, ET-1 (30 pmol/kg) was administered i.c. after the i.v. injection of 0.03 mg/kg of efonidipine. Coronary blood flow and other hemodynamic parameters were monitored until 30 min after the i.v. administration of efonidipine.

Study with measurement of coronary artery diameter by the coronary angiography technique
Thirty dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and their lungs were artificially ventilated with room air. A Judkins catheter was inserted into the left coronary artery through the right femoral artery. Coronary angiography was performed before and after an i.c. administration of ET-1 (30 pmol/kg). We were able to recognize a significant reduction of the coronary diameter on a monitor without any interference from cardiac movement at a bolus injection of 30 pmol/kg of ET-1. Left coronary angiograms were obtained in a left anterior oblique projection by manually injecting 2 to 3 ml of contrast medium (Urografin 60; Nihon Schering K.K., Osaka) through the catheter. Angiograms were recorded on a videocassette by a radiographic imaging system (Hitachi DR-125HM; Hitachi Medical Corporation, Tokyo). The posture of the dog and the distance between the dog and the image intensifier were kept constant during the experiment in order to avoid postural effects on magnification of the angiograms.

In the first group consisting of 6 dogs, ET-1 alone was administered i.c. after taking control coronary angiograms, which were recorded 1, 2, 3, 4, 5, 10, 20 and 30 min after the i.c. administration of ET-1. In the second group consisting of 12 dogs, the effect of efonidipine on the coronary diameter was examined. Six out of 12 dogs were injected with 0.03 mg/kg of efonidipine and the remaining 6 dogs were injected with 0.1 mg/kg of the drug via the left femoral vein. Coronary angiograms were taken 1, 2, 3, 4, 5, 10, 20, 30 and 40 min after the i.v. administration of efonidipine. In the third group consisting of 12 dogs, the effect of efonidipine on the ET-1-induced coronary vasoconstriction was examined. In 6 dogs, 0.03 mg/kg of efonidipine was injected i.v., and in the remaining 6 dogs, 0.1 mg/kg of the drug was injected 10 min before the i.c. administration of ET-1. Coronary angiograms were taken again as described earlier.

Angiograms on a videomonitor were selected, matching end-diastolic views, and photographs were taken for measurements of the coronary artery. The luminal diameter of the coronary arteries was obtained by using the diameter of the Judkins catheter (1.2 mm as reference). The site of measurement of the coronary artery diameter was the portion just proximal to the first diagonal branch of the left anterior descending artery or the first posterolateral branch of the left circumflex coronary artery. The measuring point was not changed during the whole course of study in each experimental animal.

Statistical analyses
All the values are expressed as means±S.E.M. Time course data were evaluated by the paired t-test. When the data were compared between groups, Dunnett’s multiple comparison test was used. Differences were considered statistically significant at the 5% level.

Drugs
Efonidipine hydrochloride (the former name is NZ-105) was kindly supplied by the Central Research Laboratories of Nissan Chemical Industries, Ltd. (Funabashi, Chiba). ET-1 (human) was purchased from
Funakoshi (Tokyo). Efonidipine hydrochloride was dissolved in a polyethylene glycol 400 : ethanol : purified water (2 : 3 : 5) mixture, and the dose of efonidipine hydrochloride was expressed as that of efonidipine in the text.

RESULTS

Study with direct measurement of coronary blood flow

Figure 2 shows the effect of an i.c. administration of ET-1 (30 pmol/kg) on coronary blood flow (CBF), heart rate (HR), and systolic and diastolic blood pressure (BP) in anesthetized open-chest dogs (n=4). *P<0.05, significantly different from the value immediately before intravenous administration of ET-1 (paired t-test).

Fig. 2. Effect of an i.c. administration of 30 pmol/kg of endothelin-1 (ET-1) on coronary blood flow (CBF), heart rate (HR), and systolic and diastolic blood pressure (BP) in anesthetized open-chest dogs (n=4). *P<0.05, significantly different from the value immediately before intravenous administration of ET-1 (paired t-test).

Efonidipine increased CBF and decreased HR and also decreased systolic and diastolic BP dose-dependently. In the dog pretreated with 0.03 mg/kg efonidipine, ET-1 decreased CBF rapidly, but the CBF returned to the basal level within 5 min after the ET-1 administration (Fig. 3). In the presence of 0.03 mg/kg of efonidipine, ET-1 did not affect HR, while it decreased systolic and diastolic BP slightly 1 min after the administration. Efonidipine (0.1 mg/kg) increased CBF, and decreased HR, and also decreased systolic and diastolic BP. The systolic and diastolic BP that had been decreased by efonidipine was not affected by ET-1 (Fig. 4). Figure 5 shows percent change in CBF after the ET-1 administration in dogs pretreated or not pretreated with efonidipine. The value of CBF measured immediately before the ET-1 administration was taken as 100%. Pretreatment with efonidipine significantly and dose-dependently attenuated the reduction in CBF induced by ET-1. The attenuation was significant...
in both early (within 5 min after the administration of ET-1) and late phases (from 10 min to 30 min after the ET-1 administration).

**Study with measurement of the coronary artery diameter by the coronary angiography technique**

An i.c. administration of ET-1 at the dose of 30 pmol/kg decreased the diameter of the coronary artery (Fig. 6, upper panel). The decrease in coronary diameter induced by ET-1 reached maximum during a period from 1 min (19.2% reduction) to 10 min (20.8% reduction) after the ET-1 administration. The decrease in coronary diameter was sustained until 30 min (12.4% reduction) after the ET-1 administration. An i.v. injection of efonidipine increased the coronary diameter dose-dependently (Fig. 6, middle panel). The increase in diameter induced by efonidipine was sustained until 40 min after the injection. Pretreatment with efonidipine dose-dependently attenuated the decrease in coronary diameter induced by ET-1 (Fig. 6, lower panel). The efonidipine-induced increase in coronary diameter was temporarily counteracted by ET-1 in the early phase of ET-1 injection, but all the values of diameter measured after the injection of ET-1 were higher than the value of coronary diameter before the injection of efonidipine.

Figure 7 shows the percent change in coronary diameter after the i.c. administration of ET-1 in the presence or absence of efonidipine. The value measured immediately before ET-1 was taken as 100%. Pretreatment with efonidipine significantly and dose-dependently attenuated the ET-1-induced reduction of coronary diameter. The effect of efonidipine to attenuate the ET-1-induced reduction in coronary diameter was marked in both the early and late phases and was sustained at least until 30 min after the ET-1 administration.

**DISCUSSION**

Studies have demonstrated that ET-1 contracts the coronary artery (3, 13, 14). In fact, an i.c. administration of ET-1 decreases coronary blood flow (4–6, 15, 16). In the present study, the i.c. administration of ET-1 (30 pmol/kg) decreased both blood flow and diameter of the coronary artery. The degree of coronary diameter reduction induced by ET-1 was small when compared with that of coronary blood flow. Nevertheless, if the change in
The diameter of the coronary artery is converted to that of cross-sectional area, it is consistent with the change in coronary flow; values of percent change of decrease in coronary blood flow 3, 10 and 30 min after the i.c. administration of ET-1 were 34%, 31% and 23%, respectively, while those in cross-sectional area were 32%, 37% and 23%, respectively.

The response of the coronary artery to ET-1 has two phases: early and late phases. It is suggested that the early phase of the ET-1-induced vasoconstrictor response is due to the increase in the intracellular Ca\(^{2+}\) level caused by Ca\(^{2+}\) release from the intracellular store sites and that the late phase of the response is due to the increase in the intracellular Ca\(^{2+}\) level caused by the increase in Ca\(^{2+}\) influx probably through voltage-dependent Ca\(^{2+}\) channels (14). In fact, some studies have demonstrated that the ET-1-induced decrease in coronary blood flow is inhibited by calcium channel blockers (13, 17, 18). However, the effects of calcium channel blockers have been investigated in only the early phase of the response to ET-1. Because there is no study on the effect of calcium channel blockers on the late phase of coronary vasoconstriction response to ET-1 in vivo and because the duration of action of efonidipine is long (10), we performed the present study with the expectation that efonidipine would inhibit the late phase as well as the early phase of the response to ET-1. Our results demonstrated that the i.v. pretreatment with efonidipine attenuates the decrease in both the early and late phases of the response of coronary blood flow and coronary diameter to the i.c. administration of ET-1, suggesting that efonidipine attenuates the ET-1-induced vasoconstriction, at least in part, through a mechanism involving inhibition of the voltage-dependent Ca\(^{2+}\) channel (14, 19, 20).

It should be pointed out that the inhibitory effect of 0.03 mg/kg of efonidipine on the ET-1-induced coronary vasoconstriction was more pronounced when the coronary artery diameter was used as an index of vasoconstriction (Fig. 7) than when coronary blood flow was used (Fig. 5). The results with diameter of the coronary artery reflect the response of the large coronary artery, while those with coronary blood flow reflect the response of all the coronary vessels including large and small arteries. Therefore, the discrepancy between the results in Fig. 7 and those in Fig. 5 suggests that efonidipine inhibits the ET-1-induced coronary vasoconstriction of the large coronary artery.

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**Fig. 6.** Effect of efonidipine on the ET-1-induced coronary vasoconstriction. Efonidipine was injected i.v. 10 min before the i.c. administration of ET-1 (30 pmol/kg). Upper panel: open circle, ET-1 alone (n=6). Middle panel: closed circle, efonidipine (0.03 mg/kg, i.v.) alone (n=6); closed triangle, efonidipine (0.1 mg/kg, i.v.) alone (n=6). Lower panel: closed circle, ET-1 in the presence of 0.03 mg/kg of efonidipine (n=6); closed triangle, ET-1 in the presence of 0.1 mg/kg of efonidipine (n=6). *P<0.05, significantly different from the value immediately before intravenous administration of ET-1 (upper panel) (paired t-test) or efonidipine (middle and lower panels) (paired t-test).

**Fig. 7.** Comparison of the percent decrease in coronary artery diameter induced by ET-1 among three groups: no efonidipine, 0.03 mg/kg efonidipine, and 0.1 mg/kg efonidipine groups. Data are those in Fig. 6. Efonidipine was injected i.v. 10 min before the i.c. administration of ET-1 (30 pmol/kg). Open circle, no efonidipine group (ET-1 alone); closed circle, 0.03 mg/kg efonidipine group (ET-1 in the presence of 0.03 mg/kg of efonidipine); closed triangle, 0.1 mg/kg efonidipine group (ET-1 in the presence of 0.1 mg/kg of efonidipine). *P<0.05, significantly different from the value in dogs in the “no efonidipine” group (Dunnett’s multiple comparison test).
coronary artery preferentially under the conditions in the present study.

ET-1 also stimulates phosphatidyl inositol turnover and releases inositol triphosphate and diacylglycerol; the former releases Ca^{2+} from the sarcoplasmic reticulum and the latter activates protein kinase C leading to vasoconstriction (21, 22). In addition, ET-1 increases Ca^{2+}-sensitivity during the late (or sustained) phase of constriction (20). Accordingly, ET-1 has many sites of action to produce vasoconstriction. Efonidipine may not counteract all of the effects induced by ET-1, but it attenuated the coronary vasoconstriction induced by ET-1, when efonidipine was given i.v. 10 min before the i.c. administration of ET-1. In addition, the effect of efonidipine is long-lasting in terms of increase in coronary blood flow and decrease in blood pressure (12, 23), and therefore efonidipine is expected to produce an antivasospastic effect for a long period of time compared with the effect of other calcium channel blockers such as nicardipine (10). Efonidipine is superior to other calcium channel blockers in that it inhibits both early and late phases of the vasoconstrictor response to ET-1, producing a long-lasting inhibitory effect on the ET-1-induced vasoconstriction in vivo. In fact, efonidipine counteracted the vasoconstriction induced by ET-1 for more than 30 min.

It is concluded that efonidipine attenuates the ET-1-induced vasoconstriction, suggesting that the drug would be useful for patients with variant angina, in which ET-1 is involved in the genesis of coronary vasoconstriction.

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