EFFECTS OF DILTIAZEM (CRD-401) ON DEVELOPED CORONARY COLLATERALS IN THE DOG

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Abstract—The effect of diltiazem (CRD-401) on coronary collaterals was studied in the dog. The anterior descending branch of the left coronary artery was occluded for 4 to 6 weeks by an ameroid constrictor. In these dogs, the retrograde flow (RF) from the peripheral coronary artery and peripheral coronary pressure (PCP) were significantly higher than those in acute coronary-ligated dogs, suggesting the development of large supraepicardial intercoronary anastomoses. Diltiazem (100 μ g/kg i.v.) increased circumflex blood flow (CBF) for several min, while nitroglycerin (10 μ g/kg i.v.) increased CBF transiently after which CBF decreased to below control values. Diltiazem (100 μ g/kg) and nitroglycerin (10 μ g/kg) increased RF/MAP (mean aortic pressure) and PCP/MAP and these increases lasted longer than that of CBF. Diltiazem also increased RF in doses of 100 μ g/kg or 20 μ g/kg/min. Therefore, diltiazem possesses the property of dilating coronary collaterals thus causing redistribution of intramyocardial blood flow. In acute preparations, however, both diltiazem and nitroglycerin showed no significant changes in PCP/MAP and RF/MAP.

Diltiazem (CRD-401, d-3-acetoxy-cis-2, 3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride) has been shown to have a potent coronary vasodilating activity in the dog heart lung preparation and in the anesthetized dog (1,2). It reduced myocardial oxygen consumption and caused a hypotension and a bradycardia (1). The compound also possesses a property to oppose cardiac acceleration induced by sympathetic nerve stimulation in the open chest dog (3).

In recent years, as one of the factors concerning the relief of angina pectoris by nitroglycerin, dilation of large coronary arteries and coronary collateral vessels has received more attention than that of arterioles (4-6). Through these actions, nitroglycerin is considered to produce redistribution of myocardial blood flow (7, 8) and to increase blood flow to collateralized ischemic area (9, 10).

In the present experiments, changes in peripheral coronary pressure and retrograde flow were measured together with coronary blood flow in chronic coronary-occluded dogs and the dilator action of diltiazem was examined on the coronary collaterals.

MATERIALS AND METHODS

Male mongrel dogs, 11 to 18 kg in weight, were anesthetized with sodium pentobarbital, 30 mg/kg. An ameroid constrictor (with an initial diameter of 2 or 3 mm) was placed on the proximal anterior descending artery. After operation, animals were treated with penicillin and streptomycin (i.m.).

Experiments were performed in these animals 4 to 6 weeks after placement of the constrictor. Animals were anesthetized with sodium pentobarbital. The trachea was intubated and left thoracotomy was performed. Ventilation was maintained by a positive pressure respirator (Takashima, Model 101). Anticoagulation was produced by injection of 500 $\mu\text{g}/\text{kg}$ of heparin and maintained by succeeding injections of 100 $\mu\text{g}/\text{kg}$ every 1 to 2 hr. The experimental preparation is diagrammed in Fig. 1. Retrograde flow (RF) was determined by the modified method originally described by Anrep and Häusler (11). A cannula,

FIG. 1. Experimental preparation. L.A.D.—the anterior descending branch of the left coronary artery. L.C.—the circumflex branch of the left coronary artery. PT—pressure transducer, MF—flow probe.

inserted into the coronary artery just distal to the constrictor, was connected through a tubing with stopcocks to a pressure transducer (Nihon Kohden, MP-24T) and an electromagnetic flowmeter (Nihon Kohden, MF-26), by which peripheral coronary pressure (PCP) and RF were measured alternately. RF was also measured by collecting for 10 to 15 sec the blood which flows out from the tubing connected to the cannula. The collected blood in the cylinder was transfused into the femoral vein. Coronary blood flow (CBF) was measured with non-cannulating type flow probe placed around the circumflex branch.

In acute experiments, dogs weighing 14 to 20 kg, were anesthetized with sodium pentobarbital and the chest was opened. A cannula was inserted into the proximal of anterior descending coronary artery. RF and PCP were measured as mentioned above.

Aortic pressure was measured by a pressure transducer via a cannula inserted into the aortic root from the left common carotid artery and mean pressure (MAP) was given by electrical integration. Heart rate was monitored by a cardiograph triggered by aortic pulse or ECG. ECG was taken under pentobarbital anesthesia before and after operation. All measurements were recorded on a polygraph (Nihon Kohden, RM-85). Drugs were administered via a polyethylene tube inserted into the saphenous vein of the hind leg. An infusion pump (Natsume, KN-202) was also used for continuous administration of diltiazem. Nitroglycerin (TNG) solution (100 $\mu\text{g}/\text{ml}$ saline) was kindly prepared by Nippon Kayaku Co., Ltd.

After the completion of experimental studies, animals were exsanguinated and the heart was removed. X-ray pictures were taken, after the left circumflex branch had been cannulated and radiopaque mass introduced. The representative sections of the left ventricular myocardium were taken for histologic examination.

RESULTS

Development of intercoronary anastomoses

Control values of several parameters in each experiment in the normal and chronic coronary-occluded dogs are summarized in Table 1. As shown, both PCP and RF were significantly high in the chronic group. Values corrected by systemic blood pressure, i.e. PCP/MAP and RF/MAP $\times 100$, were also significantly higher, whereas no significant differences in blood pressure and heart rate were observed between the two experimental groups.

Fig. 2 illustrates the phasic measurements of PCP and RF as well as CBF. Both PCP and RF increased in systole and decreased in diastole. On the other hand, CBF increased in diastole and decreased in systole. In this representative experiment, PCP was as high as 85% of arterial pressure. When RF was measured under atmospheric pressure, CBF increased. This increment of CBF, however, was smaller than the RF. This indicates that RF comes not only from the normally perfused area by the circumflex branch but also from extra circumflex branch. Intercoronary anastomoses in the

TABLE 1. Peripheral coronary pressure and retrograde flow in the chronic coronary-occluded and acute coronary-ligated dogs.

	N	H.R. beats/min	M.A.P. mmHg	P.C.P. mmHg	R.F. ml/min	P.C.P./M.A.P. %	(R.F./M.A.P.) $\times 100$
Chronic	12	166 $\pm$ 8.8	114 $\pm$ 6.1	86.9 $\pm$ 6.45	26.7 $\pm$ 3.97	74.2 $\pm$ 2.78	22.7 $\pm$ 2.81
Acute	8	157 $\pm$ 3.7	101 $\pm$ 5.5	22.8 $\pm$ 1.60	6.92 $\pm$ 0.82	22.8 $\pm$ 1.78	7.00 $\pm$ 0.91
P		>0.4	>0.5	<0.001	<0.001	<0.001	<0.001

Abbreviations are as follows: H.R.= heart rate, M.A.P.=mean aortic pressure, P.C.P.= peripheral coronary pressure, R.F.=retrograde flow

FIG. 2. Phasic pattern of aortic pressure, peripheral coronary pressure (PCP), retrograde flow (RF) and circumflex blood flow (CBF) in a chronic coronary occluded dog. RF was measured under atmospheric pressure (right of the figure). PCP and RF increase in systole and CBF increases in diastole.

epicardium were observed around the apex, as examined by coronary angiography. Around the blood vessels the developed smooth muscle was observed by histological examination. No change in ECG was observed in chronic occluded dog with higher PCP/MAP, while slight change in ST-T was seen in the preparation of low PCP/MAP.

Effect of nitroglycerin

Fig. 3 shows the effect of TNG ($10 \mu\text{g/kg}$ i.v.) on the coronary collaterals in chronic occluded dogs. TNG caused reductions in both MAP and PCP. CBF increased transiently, and then decreased to below the initial level. PCP and PCP/MAP decreased transiently and then increased by 10% and 15% respectively. Thus, they changed inversely to the change in CBF. RF and RF/MAP did not decrease but increased by 15% and 50%, respectively, after the administration of TNG. As shown in Fig. 3, the increase in RF/MAP was observed even when the increased CBF had recovered to almost the original value. The effect on RF/MAP persisted for several min and thus lasted longer than the increase in CBF.

FIG. 3. Effect of nitroglycerin on coronary collaterals in chronic coronary-occluded dogs. Values of RF were plotted at the start of measurement of RF. C—control measured at 2 min intervals. Broken lines represent PCP/MAP or RF/MAP. Each point and vertical bars indicate the mean value and S.E. * ($p < 0.05$)

FIG. 4. Effect of diltiazem on coronary collaterals in chronic coronary-occluded dogs. Each point and vertical bars indicate the mean value and S.E.

Effect of diltiazem

Fig. 4 represents the effect of diltiazem ($100 \mu\text{g/kg}$ i.v.). CBF increased for approx. 5 min and unlike TNG, it did not decrease to below the initial level. PCP decreased in parallel with the decrease in MAP and increase in CBF. The decrease in PCP/MAP was transient and it immediately turned to a slight but prolonged increase. Increases in

FIG. 5. Effect of diltiazem on coronary collaterals in chronic coronary-occluded dogs. C—control measured at 2.5 min intervals. Each point and vertical bars indicate the mean value and S.E.

RF and RF/MAP were observed soon after the maximum increase in CBF was attained. At the peak increment in CBF no increase in RF was observed, but increase in RF/MAP and decrease in PCP/MAP were evident. The maximum increases in RF (40%) and RF/MAP (50%) occurred from three to seven min after injection of the compound. At that time, the increased CBF had almost recovered to the original value. The increase in RF/MAP continued as long as the increase in PCP/MAP.

Fig. 5 illustrates the effect of diltiazem which was administered continuously to the chronic occluded dog at a constant rate of $20 \mu\text{g/kg/min}$ i.v. Under this condition only slight decreases in MAP and PCP were observed. The increase in PCP/MAP was slight but longer lasting. On the other hand, RF and RF/MAP increased markedly. RF/MAP increased approx. by 50%, measured at ten min infusion. As demonstrated in the present experiments, both PCP/MAP and RF/MAP increased after either single or continuous injection of diltiazem, indicating that diltiazem dilates not only coronary arterioles but also coronary collateral vessels. The dilator action of diltiazem on collaterals was longer-lasting than that on arterioles, as in the case of TNG.

FIG. 6. Effect of nitroglycerin and diltiazem on coronary collaterals in acute coronary-ligated dogs. Each point and vertical bars indicate the mean value and S.E.

Effects of nitroglycerin and diltiazem on PCP and RF in acute preparations

In acute preparation (Fig. 6), TNG

decreased PCP/MAP in all experiments and tended to decrease RF/MAP, while diltiazem tended to increase RF/MAP. Both TNG and diltiazem caused no persisting increases in PCP/MAP and RF/MAP, as observed in chronic preparations.

DISCUSSION

It is well known that PCP and RF increase after sustained partial or total coronary artery occlusion (12, 13). Elliot et al. observed that the increase in PCP paralleled the increase in collateral flow development (14). In our chronic coronary-occluded dogs, prepared by the implantation of an ameroid constrictor during 4 to 6 weeks, PCP and RF levels were found to be much higher than those in acute preparations. Thus the development of collaterals was suggested. The phasic flow pattern shows that RF increased in systole, whereas circumflex flow increased in diastole. This may indicate that the measured RF was the flow via the area where extravascular compression was little affected, i.e. via large anastomoses located in the supraepicardium, as suggested by others (14, 15). In case of well developed collateral vessels, RF which was measured under atmospheric pressure, may represent mainly the changes in large intercoronary anastomoses. The development of large anastomoses was also confirmed by postmortem radiopaque mass injection. On the other hand, PCP may be considered to reflect the reactivity of small anastomotic arteries as well as large intercoronary arteries and the peripheral coronary bed. Therefore, PCP is the pressure readily affected by a reduction in coronary peripheral resistance. Since PCP and RF depend on systemic pressure (11, 16), the changes in PCP/MAP and RF/MAP may be considered an index of the extent of redistribution of intramyocardial blood flow.

Fam and McGregor (5) found that both RF/MAP and PCP/MAP were increased by TNG which was administered sublingually. Cohen et al. (10) demonstrated the increase in PCP by TNG associated with the increase in contractility in the anesthetized dog. In patients with coronary occlusive disease, the increase in PCP (as a fraction of aortic pressure) by TNG was also reported in intraoperative experiments (17). We also observed that TNG increased RF/MAP and PCP/MAP and that these increases lasted longer than that of the total CBF. These results suggest that TNG dilates the developed collateral artery as well as the relatively large coronary artery (6, 18). The increase in CBF indicates the dilation of arterioles.

The increase in RF was induced by diltiazem with both a single administration and continuous infusion in which decrease in blood pressure was slight. This suggests that diltiazem has a dilating action on the large intercoronary anastomoses. Diltiazem increased RF/MAP and PCP/MAP. These increases occurred a little later and lasted longer than the increase in CBF. At the peak time of the increment of CBF, PCP/MAP decreased as observed with the case of TNG, while the RF did not change. This may be attributable to a redistribution of blood to the lower resistant area caused by a marked dilation of coronary arterioles and would mask the dilation of collateral arteries at this time.

Both TNG and diltiazem dilate the collaterals longer than the coronary arterioles.

As suggested by Winbury et al. (7) and as demonstrated by Cohen and Kirk (19), this may be explained as follows. If a drug acts on both arterioles and the collateral vessels, the total blood flow is apt to return to the control level as the result of autoregulation and the increase in flow appears to be short lasting. However, since autoregulation does not appear to include collaterals and large arteries, the effect of drugs on collaterals may be rather longer lasting.

Although in our acute experiments, TNG caused a slight decrease in PCP/MAP, both TNG and diltiazem showed no significant effects on collaterals, as estimated by RF/MAP and PCP/MAP. In the acute preparation, RF and PCP may reflect the responses of small collaterals originally present and of the peripheral coronary resistance. Therefore, responses of collaterals may be masked by the change in total coronary flow.

Our results show that diltiazem possesses the property of dilating well developed collaterals, such as seen in the case with TNG. The vasodilator action of diltiazem was reported to be direct on blood vessels (2). In a potassium depolarizing solution, diltiazem antagonized competitively against calcium ions in perfused rabbit ear artery (20). These results suggest that diltiazem exerts its direct action on the smooth muscle of coronary collaterals.

The dilator action of diltiazem observed in well developed intercoronary anastomoses probably contributes to its therapeutic effect on angina pectoris (21) by resultant redistribution of blood flow in myocardium as suggested in the case of TNG.

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