SIMULTANEOUS ASSESSMENT OF EFFECTS OF CORONARY VASODILATORS ON THE CORONARY BLOOD FLOW AND THE MYOCARDIAL CONTRACTILITY BY USING THE BLOOD-PERFUSED CANINE PAPILLARY MUSCLE

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Abstract—Effects of 6 coronary vasodilators on the coronary blood flow and the contractile force of the ventricular muscle were examined simultaneously by injecting these drugs to the arterially blood-perfused canine papillary muscle preparation. All compounds produced a dose-dependent increase in blood flow rate, and relative potencies determined on the basis of doses producing a 100% increase in blood flow rate, ED100, were in the descending order: nifedipine > verapamil > diltiazem > dilazep > dipyridamole > carbochromen, and approximately 1 : 1/12 : 1/26 : 1/100 : 1/300 : 1/500. All drugs except for dipyridamole caused a dose-dependent decrease in the developed tension of the papillary muscle, although nifedipine and diltiazem in low doses produced a slight increase. Relative potencies determined on the basis of doses producing a 50% decrease in developed tension, ID50, were as follows: nifedipine (1), verapamil (1/13), diltiazem (1/40), dilazep (1/100), and carbochromen (1/270). Ratios of the ID50 to ED100 were as follows: diltiazem (5.2), nifedipine (3.5), verapamil (3.5), dilazep (2.5), and carbochromen (1.8). The higher the value the more predominant on the coronary vascular bed or the less depressant on the myocardial contractility were their actions.

A coronary vasodilator, dipyridamole has no cardiodepressant action, but does increase the venous return and the cardiac output in the dog (1). This suggests that dipyridamole may have rather a cardiotonico-stimulant action. In contrast to dipyridamole, a coronary vasodilator, verapamil reduces the contractile force of the mammalian myocardium (2), and the mechanism of the action underlying the negative inotropic and vasodilator actions has been generally attributed to a calcium-antagonistic inhibition of the excitation-contraction coupling in the cardiac muscle and vascular smooth muscle fibers (3, 4). A recently-developed coronary vasodilator, nifedipine also exerts a negative inotropic effect on the mammalian myocardium (5, 6, 7) and its mechanism of action has also been ascribed to the calcium antagonism (4, 7). The negative inotropic action of these calcium-antagonistic vasodilators is considered to be favorable for the treatment of ischemic heart disease as the reduction in the myocardial contractile force leads to the decreased myocardial oxygen consumption (4). However, an excess of the negative inotropic action is one of the untoward effects as it produces the congestive heart failure. Thus, it is of value to obtain precise information about the potency producing coronary vasodilation and that affecting the myocardial contractility in each coronary vasodilator.

Investigators studying the effects of coronary vasodilators on the coronary circulation
and on the myocardial contractility have utilized different preparations for each purpose. Which of the two effects is predominant in each coronary vasodilator remains as a matter of speculation. The present experiments were an attempt to obtain more direct information about this, by utilizing the blood-perfused canine papillary muscle preparation (8) which permits the simultaneous observation of the contractile force and the blood flow as a part of the coronary circulation. The preparation is free from extracardiac influences and its blood flow is free from intraventricular pressure.

The effects of carbochromen, dilazep and diltiazem (9) were also investigated in addition to those of dipyridamole, nifedipine and verapamil. Diltiazem has a negative inotropic action on the mammalian ventricular muscle and is thought to be a calcium antagonist (10, 11). The mechanism of the vasodilator action of dilazep has been ascribed to potentiation of the action of endogenous adenosine (12) as that of dipyridamole (13). A similar mechanism of action has been suggested for carbochromen (14, 15).

MATERIALS AND METHODS

Experiments were performed on 28 preparations of the anterior papillary muscle of the right ventricle of the heart obtained from mongrel dogs, weighing from 8.5 to 14.5 kg. The preparation was essentially the same as described by Endoh and Hashimoto (8). After cannulation of the anterior septal artery, the preparation was fixed at the base of the papillary muscle to a plastic plate with a hole and placed in a water jacket maintained at 38-39°C (see inset in Fig. 1). The preparation was perfused through the cannulated anterior septal artery with blood conducted from the right carotid artery of a supporting dog by a peristaltic pump (Harvard, Model 1215). Constant pressure perfusion at about 100 mm Hg was accomplished by shunting a fraction of the blood to a reservoir through a Starling pneumatic resistance set in parallel with the preparation. The blood from the preparation was also collected in the blood reservoir and returned to the supporting dog through the right external jugular vein. Perfusion circuit is shown diagrammatically in Fig. 1. Supporting dogs, weighing from 14 to 26.5 kg, were anesthetized with sodium pentobarbital, 30 mg/kg i.v., and given sodium heparin, 500 units/kg i.v., at the start of the experiment. Sodium pen-

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Fig. 1. Diagram of circuit for perfusion of the papillary muscle preparation with blood from a supporting dog. The papillary muscle preparation in detail is illustrated in inset.
tofarbital, 4–5 mg/kg, and sodium heparin, 150 units/kg, were added at hourly intervals to the blood reservoir. The preparation was driven through bipolar electrodes placed at the base of the papillary muscle with rectangular pulses of 0.6–1.5 V (about twice threshold) and 5-msec duration at a rate of 120 beats/min delivered by an electronic stimulator (Nihon Kohden, MSE-3). Isometric tension of the papillary muscle stretched with a weight of 1.5 g was picked up with a strain-gauge transducer (Grass, FT 03 B). The rate of blood flow through the anterior septal artery was measured with an electromagnetic flowmeter (Nihon Kohden, MF-46). The two parameters were recorded on an ink-writing rectigraph (San-ei Instrument, Rectiholiz 8S).

Drugs used were carbochromen hydrochloride (Cassella AG), dilazep dihydrochloride monohydrate (Asta AG), diltiazem hydrochloride (Tanabe Pharmaceutical Co.), dipyridamole (C.H. Boehringer Sohn Ingelheim, solution in ampule), nifedipine (Bayer AG, solution in ampule) and verapamil hydrochloride (Knoll AG). These drugs except for dipyridamole and nifedipine were dissolved in 0.9% saline. All drug solutions were diluted with 0.9% saline to desired concentrations and were injected in a volume of 30–100 μl over a period of 4–12 sec into the blood conducting rubber tubing connected with an arterial cannula by the use of microsyringes. A single injection of this volume of 0.9% saline exerted no effect on the developed tension or on the blood flow rate of the papillary muscle preparations. A single injection of the vehicle of nifedipine in 100 μl which corresponded to 10 μg of nifedipine produced no change in the blood flow rate but caused a transient negative inotropic effect amounting to 9.5±1.3 (mean±S.E., n=4)% of the basal developed tension. A single i.a. injection of the vehicle of dipyridamole in 60 μl which corresponded to 0.3 mg of dipyridamole produced only a slight increase (6.4±1.8%, n=4) in blood flow rate, and a slight negative inotropic effect (5.8±1.0%, n=4). Both effects disappeared in about 1 min. All doses were expressed in terms of their bases.

RESULTS

Effects on the blood flow rate

The basal rate of the blood flow through the anterior septal artery of 28 preparations at a constant perfusion pressure of about 100 mm Hg was 5.4±0.4 (mean±S.E.) ml/min. Single injections of nifedipine (0.01–10 μg), verapamil (0.3 μg–0.1 mg), diltiazem (0.3 μg–0.3 mg), dilazep (1 μg–1 mg), dipyridamole (3 μg–0.3 mg) and carbochromen (10 μg–3 mg) into the anterior septal artery produced dose-dependent increases in blood flow rate, viz., vasodilation. Figs. 2 and 3 are typical of such experiments and Fig. 4 shows dose-response curves for a peak increase in blood flow rate as a percentage of the basal blood flow rate in each preparation. The mean values of doses producing a 100% increase in blood flow rate (ED100) and relative potencies of the 6 coronary vasodilators as determined on the basis of ED100 are tabulated in Table 1. The relative potencies of nifedipine, verapamil, diltiazem, dilazep, dipyridamole and carbochromen were in the descending order approximately 1 : 1/12 : 1/26 : 1/100 : 1/300 : 1/500. The relative potencies of them determined on the basis of doses producing a 50% increase in blood flow rate were approximately equal to those de-
FIG. 2. Effects of nifedipine (Nif), verapamil (Ver) and diltiazem (Dilt) injected into the anterior septal artery on the blood flow rate and developed tension of the blood-perfused canine papillary muscle. The papillary muscle was stretched with a weight of 1.5 g and stimulated by rectangular pulses of 0.6-1.2 V and 5-msec duration at a rate of 120 beats/min.

FIG. 3. Effects of dilazep (Dila), dipyridamole (Dipy) and carbochromen (Car) on the blood flow rate and developed tension of the blood-perfused papillary muscle of the dog. All else as in Fig. 2.

terminated on the basis of ED100.

As shown in Figs. 2 and 3, the vascular responses to nifedipine, verapamil and diltiazem were similar in time course and rather long-lasting. The half-duration of the response to ED100 of the 3 vasodilators, that is the period from the onset to the point of half recovery of the response, was equally about 4 min. The vasodilator action of dipyridamole was conspicuously long-lasting in higher doses (0.1-0.3 mg) and at ED100 lasted about 15 min (the half-duration). Dilazep produced only a transient increase in blood flow rate in 4 of 6 preparations, as shown in Fig. 3, but in the other 2 preparations caused a relatively long-lasting increase. Carbochromen, in lower doses (10 μg-0.1 mg), increased the blood flow rate only for a short period, but in higher doses (0.3-3 mg) this brief increase was followed
by a long-lasting increase (Fig. 3) and at ED100 the vascular response as a whole lasted about 11 min (the half-duration).

Effects on the developed tension

The basal developed tension of the papillary muscle driven with electric pulses of about twice the threshold voltage and 5-msec duration at a rate of 120 beats/min was 4.9 ± 0.4 (mean ± S.E., n=28) g. Single i.a. injections of nifedipine (0.1–10 μg), verapamil (1 μg–0.1 mg), diltiazem (10 μg–0.3 mg), dilazep (10 μg–1 mg) and carbochromen (30 μg–3 mg) produced dose-dependent decreases in developed tension, viz., negative inotropic effects. Doses of these drugs were increased until a decrease in developed tension expressed as a percentage of the basal developed tension reached about 75% in each preparation. The mean doses producing a 50% decrease in the developed tension (ID50) of nifedipine, ve-
rapamil, diltiazem, dilazep and carbochromen are shown in Table 1. Their relative negative inotropic potencies determined on the basis of ID50 were in the descending order approximately 1 : 1/13 : 1/40 : 1/100 and 1/270. However, with lower doses of verapamil (0.3 μg) in 3 of 8 preparations, with those of nifedipine (0.01–0.3 μg) in 6 of 8 preparations, and with those of diltiazem (0.3–3 μg) in all 8 preparations, a slight but definite increase in developed tension appeared singly or was superimposed on the negative effect. An increase in developed tension by 3 μg of diltiazem amounted to about 13% of the basal developed tension. With 30 μg to 3 mg of carbochromen, an increase in developed tension followed the decrease and at 3 mg the increase was about 19% of the basal developed tension. Such results are exemplified in Figs. 2 and 3, and dose-response curves to these drugs for percent changes in developed tension are shown in Fig. 4 (lower panel).

Dipyridamole (10 μg–0.3 mg) produced a monophasic positive inotropic effect in a dose-dependent manner (Fig. 3), and at 0.3 mg the mean increase amounted to 45.3±9.7 (mean±S.E., n=5)%.

Comparison of the effects on the blood flow rate with those on the developed tension

Ratios of a dose causing a 50% decrease in the developed tension (ID50) to that producing a 100% increase in the blood flow rate (ED100) were calculated for the 5 coronary vasodilators producing the negative inotropic effect and such was used as a criterion for determining the predominance of actions on the coronary blood vessels over the ventricular myocardium in their actions. As shown in Table 1, ratios (ID50/ED100) for nifedipine, verapamil, diltiazem, dilazep and carbochromen were 3.5, 3.5, 5.2, 2.5 and 1.8, respectively.

**DISCUSSION**

All 6 coronary vasodilators examined produced a dose-dependent increase in blood
flow rate through the preparation and their relative potencies determined on the basis of doses producing a 100% increase in blood flow rate were approximately as follows: nifedipine : verapamil : diltiazem : dilazep : dipyridamole : carbochromen = 1 : 1/12 : 1/26 : 1/100 : 1/300 : 1/500. All values but those for diltiazem and dilazep were approximately equal to those obtained in the canine Langendorff’s preparation (16) in which the effects of diltiazem and dilazep had not been examined. Thus, the assessment of the coronary vasodilator potency by utilizing the blood-perfused papillary muscle preparation is valid. However, the values are different from the relative potencies estimated on the basis of i.v. doses producing about a 100% increase in the coronary sinus outflow in the in situ canine heart. Nifedipine in an i.v. dose of 3 μg/kg almost doubles the coronary sinus outflow (5, 6). Such i.v. doses of verapamil (2), diltiazem (9), dilazep (17) and dipyridamole (13) are roughly 0.1 mg/kg. A reduction in the potency of i.a. potent vasodilators like nifedipine, verapamil and diltiazem given i.v. can be attributed to the reduction in the systemic blood pressure which is the perfusion pressure for the coronary circulation. Alternatively, an increase in the potency of i.a. less-potent dilazep and dipyridamole given i.v. can be ascribed to the lesser decrease in the systemic blood pressure (unpublished observations).

All 3 calcium-antagonistic vasodilators, nifedipine, verapamil and diltiazem, produced a negative inotropic effect on the papillary muscle but in lower doses, verapamil in some, nifedipine in the majority and diltiazem in all preparations examined produced a slight but definite positive inotropic effect. The positive inotropic effect was the most obvious with diltiazem. Thus, calculation of a ratio of a dose causing a 50% reduction in the developed tension, ID50, to a dose doubling the blood flow rate, ED100 gave 5.2 to diltiazem and 3.5 to nifedipine and verapamil. This means that to produce a 50% decrease in the myocardial contractile force, a dose of diltiazem as large as 5.2 times the ED100 is needed. In other words, at ED100 the negative inotropic effect of diltiazem was about 20% and those of nifedipine and verapamil were about 30%. Thus, for these drugs the coronary vasodilation is more predominant than the reduction in myocardial contractile force. Although the negative inotropic action of the calcium-antagonistic coronary vasodilators is claimed to be primarily important as a mechanism of action responsible for relieving the ischemic heart disease (4), the results of the present experiments suggest that the coronary vasodilator action is a principal action of these drugs.

Although the mechanism of vasodilator action of dipyridamole (13), dilazep (12, 17) and carbochromen (14, 15) has been attributed to potentiation of the vasodilator action of endogeneous adenosine (18), unlike dipyridamole, dilazep and carbochromen produced a negative inotropic effect in the blood-perfused papillary muscle preparation. Since adenosine even in a dose markedly increasing the blood flow rate in the present preparation exerts only a slight negative inotropic effect (unpublished observations), the observed negative inotropic effect of dilazep and carbochromen may be due to their calcium-antagonistic action. A low ID50 to ED100 ratio for dilazep cannot explain the virtual absence of the cardio depressant action at an i.v. dose almost doubling the coronary sinus outflow in the anesthetized dog (17). When dilazep is given i.v. its adenosine-potentiating action may play an important
role in increasing the coronary blood flow. Indeed, the increase in the coronary sinus outflow caused by i.v. dilazep is far more long-lasting in comparison with the increased blood flow rate by i.a. dilazep as observed in the present preparation (unpublished observations).

Among the 6 coronary vasodilators examined, dipyridamole alone produced a monophasic positive inotropic effect and at 0.3 mg an increase in developed tension reached about 45% of the basal developed tension. This is consistent with the finding that in dogs i.v. dipyridamole tended to increase myocardial oxygen consumption (13). The mechanism of the positive inotropic action of dipyridamole is to be described in another paper (19). The mechanism of the positive inotropic action of lower doses of diltiazem has been discussed in a previous paper (10). With higher doses of carbochromen, a positive inotropic effect followed a negative one. At present no explanation can be given for the mechanism of the positive inotropic effect. However, this positive inotropic effect may give an answer to the question why carbochromen rather increased myocardial oxygen consumption in the canine Langendorff’s preparation (16). Indeed, in the present experiments with higher doses of carbochromen, a long-lasting increase in blood flow rate occurred over a period in which a rather positive inotropic effect also was observed.

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REFERENCES

1) KOKUBUN, M., Taira, N. and Hashimoto, K.: Japan. Heart J. 15, 126 (1974)
2) Haas, H. and Hartfelder, G.: Arzneim. -Forsch. 12, 549 (1962)
3) Fleckenstein, A.: Verh. Dt. Ges. Inn. Med. 70, 81 (1964)
4) Fleckenstein, A.: Calcium and the Heart, Edited by Harris, P. and Opie, L., p. 135, Academic Press, London and New York (1971)
5) Vater, W., Kroneberg, G., Hoffmister, F., Kaller, H., Meng, K., Oberdorff, A., Puls, W., Schloßmann, K. and Stoepel, K.: Arzneim.-Forsch. 22, 1 (1972)
6) Hashimoto, K., Taira, N., Chiba, S., Hashimoto, K., Endoh, M., Koku-bun, M., Koku-bun, H., Iijima, T., Kimura, T., Kubota, K. and Oguro, K.: Arzneim.-Forsch. 22, 15 (1972)
7) Fleckenstein, A., Trippthart, H., Döring, H.-J. and Byon, K.Y.: Arzneim.-Forsch. 22, 22 (1972)
8) Endoh, M. and Hashimoto, K.: Am. J. Physiol. 218, 1459 (1970)
9) Sato, M., Nagao, T., Yamaguchi, I., Nakajima, H. and Kidomiito, A.: Arzneim.-Forsch. 21, 1338 (1971)
10) Himori, N., Ono, H. and Taira, N.: Japan. J. Pharmacol. 25, 350 (1975)
11) Nakajima, H., Hoshiyama, M., Yamashita, K. and Kidomito, A.: Japan. J. Pharmacol. 25, 383 (1975)
12) Buyyniski, J.P., Losada, M., Bierwagen, M.E., and gardner, R.W.: J. Pharmacol. exp. Ther. 181, 522 (1972)
13) Bretschneider, H.J., Frank, A., Bernard, U., Kochsieck, K. and Scheler, F.: Arzneim.-Forsch. 9, 49 (1959)
14) Bretschneider, H.J., Eberlein, H.J., Kabus, H.-M., Nefele, G. and Reichmann, W.: Arzneim.-Forsch. 13, 255 (1963)
15) Sano, N., Sato, S. and Hashimoto, K.: Japan. J. Pharmacol. 22, 857 (1972)
16) Oguro, K., Kubota, K., Kimura, T. and Hashimoto, K.: Japan. J. Pharmacol. 23, 459 (1973)

17) Hensel, I., Bretschneider, H.J., Kettler, D., Knoll, D., Kochsieck, K., Reploh, H.D., Speckermann, P.G. and Tauchert, M.: Arzneim.-Forsch. 22, 652 (1972)

18) Rubio, R. and Berne, R.M.: Circulation Res. 25, 407 (1969)

19) Himori, N. and Taira, N.: Arch. Pharmacol. (in press)