EFFECTS OF ARACHIDONIC ACID AND BRADYKININ ON THE CORONARY FLOW, RELEASE OF PGI₂ AND CARDIAC FUNCTIONS IN THE PERFUSED GUINEA-PIG HEART

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Abstract—The contribution of prostacyclin (PGI₂) to the coronary vasodilating action of arachidonic acid (AA) and bradykinin (BK) was examined in isolated perfused guinea-pig hearts. The injection of AA (100 to 1,000 ng) and BK (1 to 100 ng) into the heart resulted in a dose-dependent increase in the total amount of coronary flow and a release of 6-keto-prostaglandin F_{1α}, a stable metabolite of PGI₂. Both AA and BK showed weak positive chronotropic effects. In addition, higher doses (300 and 1,000 ng) of AA caused a transient reduction in the coronary flow rate, left ventricular systolic pressure, and left ventricular dp/dt. The changes in coronary flow, release of PGI₂, and all cardiodynamic parameters induced by AA were abolished by pretreatment of the preparation with diclofenac-Na. On the other hand, the BK-induced increase in coronary flow rate was only partially reduced by diclofenac-Na when the release of 6-keto-prostaglandin F_{1α} was completely inhibited. It is concluded that in isolated perfused guinea-pig hearts, BK has both PGI₂-independent and PGI₂-dependent coronary vasodilating actions; the latter action is less than 25%, and the coronary vasodilating action of AA is mainly mediated *via* PGI₂.

Arachidonic acid (AA) and bradykinin (BK) have potent coronary vasodilating actions in isolated mammalian hearts (1, 2). The vasodilating action of AA is attributed to a metabolic product of AA, prostacyclin (PGI₂) (3), which has been described as being a prostaglandin-like substance (PLS). On the other hand, the mechanism for the coronary vasodilating action of BK remains obscure. Needleman et al. (2) suggested that the BK-induced coronary vasodilation seen in isolated rabbit heart preparations was mediated *via* the release of prostaglandins. Later, it was argued that PGI₂ might play a main role in the coronary action of BK in isolated rabbit hearts (4). Recently, however,

Schorr et al. provided the evidence for an additional PGI₂-independent mechanism of coronary vasodilation, as induced by BK in guinea-pig hearts (5). In these studies, the release of PGI₂-like substance was estimated by bioassay, and a quantitative relation between coronary vasodilation and PGI₂ release following injection of BK was not studied in detail.

We now report our findings of the quantitative relation between coronary vasodilation and release of PGI₂ as induced by AA or BK. PGI₂ was measured by the radioimmunoassay of 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}), a stable derivative of PGI₂. The cardio-hemodynamic effects of both compounds

were also studied.

MATERIALS AND METHODS

The isolated guinea-pig heart preparation was made according to the method of Langendorff with a slight modification (6). In brief, male guinea pigs, weighing about 300 g, were sacrificed by a blow on the head about 20 min after i.p. administration of 1,000 U/kg of heparin. A thoracotomy was done, and the heart immediately placed in ice-cold Krebs-Ringer bicarbonate solution composed of (mM) 127 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 25 NaHCO₃, 2.0 sodium pyruvate and 5.5 glucose, and oxygenated with 97% O₂-3% CO₂ (pH 7.4). After removing perivascular tissues, a polyethylene cannula was inserted retrogradely into the aorta. A urethane polymer balloon connected to a glass tube was inserted into the left ventricular cavity through an incision of the left auricle. The heart was suspended in a chamber maintained at 37°C. To measure the coronary flow rate, a cannulating type flow probe (inner diameter of 2 mm) of an electromagnetic flowmeter (Nihon Kohden, MF-26) was placed just above the aortic cannula. The left ventricular isovolumetric pressure was recorded with a pressure transducer (Nihon Kohden, MPU-0.5) connected to the intraventricular balloon which was filled with physiological saline. The diastolic pressure was adjusted to lower than 10 mmHg so that the pulse pressure would attain the maximum. The heart rate was recorded from left ventricular pressure pulses with a cardi tachometer (Nihon Kohden, RT-2). The left ventricular pressure dp/dt was recorded through an electronic differential circuit with a time constant of 2.5 msec. The preparation was perfused at a constant perfusion pressure of 750 mm H₂O with the modified Krebs-Ringer bicarbonate solution described above, warmed to 37°C, and aerated with 97% O₂-3% CO₂. Experi-

ments were started after a 20-30 min equilibration period. Only one dose-response relation for either AA or BK was determined in each preparation. AA and BK were dissolved in saline (200 μ l) and injected in a bolus fashion (0.2 ml) into the coronary perfusion system. About 20 min after the injection of the highest dose of AA or BK, the heart preparation was treated for 5 min with diclofenac-Na (final concentration, 1 μ g/ml), a cyclooxygenase inhibitor (7), and then AA or BK was injected again under the continuous infusion of diclofenac-Na. Diclofenac-Na induced no changes in the basal coronary flow rate.

Effects of both agents on coronary flow were expressed by two ways as follows: 1) The changes from the basal coronary flow rate were shown as delta coronary flow rate (ml/min). 2) Total increase in coronary flow (ml) was estimated by determining the area under the curve of coronary flow increased by both agents.

Preparation of the antiserum for 6-keto-PGF_{1 α} : Three mg of 6-keto-PGF_{1 α} was conjugated to 4 mg of bovine serum albumin (BSA) using water soluble carbodiimide following a method similar to that used in the case of PGF_{2 α} (8, 9). The conjugate was purified by repeated dialysis against distilled water, lyophilized, and stored at -20°C until use. An emulsion of the conjugate (1 mg in 1 ml of saline) in an equal volume of Freund's complete adjuvant was given s.c. at various sites on the back of one male albino rabbit once a week for 4 weeks, and thereafter, it was injected once a month for 3 months. The antiserum was prepared one month after the last immunization. The dilution of the antiserum which gives about 50% binding to ³H-6-keto-PGF_{1 α} was 1:1,000. Radioimmunoassay with this antiserum enabled detection of 10 to 1,000 pg of 6-keto-PGF_{1 α} . Cross reactivities of the antiserum with various prostaglandins and thromboxane B₂

(TXB₂) tested were less than 0.1% for PGA₂, PGD₂ and TXB₂, 1.8% for PGE₁, 1.3% for PGE₂ and 4.5% for PGF_{2α}.

Radioimmunoassay of 6-keto-PGF_{1α} in the perfusate: The perfusate was serially collected for 30 sec just before and every 30 sec starting just after injection of test agents and until the coronary flow rate returned to the initial level. The volume of the perfusate was measured with a graduated glass cylinder. After mixing well, 1 ml of the effluent was adjusted to pH 3.5 with 1N HCl, extracted with 4 volumes of ethyl acetate, and 6-keto-PGF_{1α} determined by radioimmunoassay. The rabbit antiserum to 6-keto-PGF_{1α} was diluted 1:1,000 with buffer I (0.1 M phosphate buffer at pH 7.5 containing 0.1% gelatin, 0.9% NaCl and 0.01% NaN₃). The authentic 6-keto-PGF_{1α} (10–1,000 pg) for the standard curve was dissolved in 50 μl of buffer I. A 0.25 ml aliquot of the extract was dried with N₂ gas and dissolved in 50 μl of buffer I. ³H-6-Keto-PGF_{1α} in buffer I (10,000 cpm/100 μl) was added to the extract solution, after which the 50 μl of diluted rabbit antiserum was added. The tubes were shaken for 15 sec and incubated for 1 hr at 25°C and then for 16–20 hr at 4°C.

To separate the antiserum-bound and -free ³H-6-keto-PGF_{1α}, dextran-coated charcoal (0.1 ml of buffer I containing 2.5 mg charcoal and 0.25 mg dextran) was added to each tube and mixed for 15 sec. The tubes were left standing on ice for 10 min followed by centrifugation for 5 min at 3,000 rpm at 0–4°C. The radioactivity of the supernatant (180 μl) was measured in 4 ml of ACS II scintillator with a scintillation counter (LKB-1216, Rackbeta). The counts of antiserum blank tubes, containing no antiserum, were subtracted from the counts of all other tubes as nonspecific binding. The standard curve was plotted as a function of logit versus the amount of authentic 6-keto-PGF_{1α} and the

binding in each sample was compared to the standard curve. The recovery of 6-keto-PGF_{1α} in this method was 77.6±1.7% (n=3).

Materials: Materials used were ³H-6-keto-PGF_{1α} (100 Ci/mmol, New England Nuclear, U.S.A.), arachidonic acid (Sigma, U.S.A.), 6-keto-PGF_{1α} (synthesized in this Chemical Laboratories), bradykinin (Protein Research Foundation, Japan), diclofenac-Na (extracted from Voltaren®, Fujisawa, Japan), dextran (Pharmacia Fine Chemicals, U.S.A.), charcoal (Norit A, American Norit, U.S.A.), gelatin (Merck, Germany), NaN₃ (Wako Pure Chemicals, Japan), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (Nakarai Chemical, Japan), BSA (Miles Laboratories, U.S.A.), ACS II (Amersham, U.S.A.), and ethyl acetate (Wako Pure Chemical, Japan).

Statistical analysis: Values presented in this paper are the mean±S.E.M. The Student's *t*-test (paired) was used. Differences with *P*<0.05 were considered to be statistically significant: **P*<0.05, ***P*<0.01.

RESULTS

Effects of arachidonic acid on coronary flow, release of 6-keto-PGF_{1α} and cardiac parameters: The time course of the AA-induced increase in the coronary flow (delta coronary flow) and the release of 6-keto-PGF_{1α} are shown in Fig. 1 and Table 1. The basal coronary flow rate was about 7 ml/min. AA at doses of 100 and 300 ng caused a dose-dependent increase in the maximum delta coronary flow, but the maximum delta coronary flow induced by 1,000 ng of AA was lower than that by 300 ng of AA. The higher doses (300 and 1,000 ng) induced a transient, dose-dependent reduction in the coronary flow rate. The maximum delta coronary flow induced by 1,000 ng of AA was less but longer-lasting than that by 300 ng of AA. Thus, total increases in

coronary flow induced by these 3 doses of AA were dose-dependent. The basal release of 6-keto-PGF_{1α} was negligible. When the heart was stimulated with AA, 6-keto-PGF_{1α} was released immediately following AA injection in a dose related manner, and its maximum release was seen in the first 30 sec-collection period. A highly significant cor-

relation was observed between the total increase in coronary flow and the total amount of 6-keto-PGF_{1α} released (Fig. 2). Diclofenac-Na significantly inhibited both the transient reduction and the following increase in coronary flow rate and the release of 6-keto-PGF_{1α} induced by 1,000 ng of AA. The total increase in coronary flow induced by 1,000 ng of AA under the continuous infusion of diclofenac-Na was very small (Table 1).

Fig. 1. Effects of arachidonic acid (AA) on delta coronary flow and release of 6-keto-PGF_{1α} in the isolated perfused guinea-pig heart with or without diclofenac-Na. ○—○ Δ coronary flow without diclofenac-Na, ●—● Δ coronary flow with diclofenac-Na (1 μg/ml), ▨▨▨ the release of 6-keto-PGF_{1α} without diclofenac-Na, ▨▨▨ the release of 6-keto-PGF_{1α} with diclofenac-Na.

Fig. 2. The correlation between the increase in coronary flow and the release of 6-keto-PGF_{1α} in the isolated perfused guinea-pig heart following stimulation with arachidonic acid (AA) or bradykinin (BK). AA (○): $y = 1.35x - 0.59$, $r = 0.89$, $P < 0.01$, BK (×): $y = 1.17x - 0.50$, $r = 0.77$, $P < 0.01$.

Table 1. Effects of arachidonic acid and bradykinin on the coronary flow and the release of 6-keto-PGF_{1α} in isolated perfused guinea pig hearts

	Arachidonic acid (ng) (n=4)			Bradykinin (ng)		
	100	300	1,000	1 (n=3)	10 (n=4)	100 (n=5)
Maximum delta coronary flow (ml/min)	3.5±1.4	5.5±0.8	4.5±0.3	4.4±0.8	5.7±0.7	4.9±0.6
Total increase in coronary flow (ml)	3.0±1.2	11.3±2.3	14.3±1.8	3.5±0.1	13.4±2.0	22.9±2.7
(after diclofenac-Na)			(0.9±0.6)	(3.4±0.2)	(10.2±2.0)	(18.2±3.0)
Total amounts of released 6-keto-PGF _{1α} (ng)	0.52±0.16	7.24±2.71	32.16±3.65	0.09±0.09	3.36±1.53	17.33±4.78
(after diclofenac-Na)			(0.20±0.12)			(0.34±0.22)

Fig. 3. Effects of arachidonic acid (AA) on cardio-hemodynamic parameters in the isolated perfused guinea-pig heart with or without diclofenac-Na. $\circ$ — $\circ$ without diclofenac-Na, $\bullet$ — $\bullet$ with diclofenac-Na (1 μ g/ml). Abbreviations: HR, heart rate; LVSP, left ventricular systolic pressure; LVDP, left ventricular diastolic pressure; LVdp/dt, left ventricular dp/dt.

Figure 3 shows the effect of AA on the heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDS), and LVdp/dt. Three hundred and 1,000 ng of AA showed positive chronotropic effects. The AA-induced transient decrease in the LVSP and LVdp/dt paralleled the decrease in the coronary flow in the early phase as seen in Fig. 1. LVDP was slightly increased with 1,000 ng of AA. These changes in cardiac parameters induced by 1,000 ng of AA were completely inhibited by pretreatment with diclofenac-Na.

Effects of bradykinin on coronary flow, release of 6-keto-PGF_{1 α} and cardiac parameters: Figure 4 shows the effect of BK on the coronary flow rate (delta coronary flow) and the release of 6-keto-PGF_{1 α} . The maximum delta coronary flow with 100 ng of BK was lower than that with 10 ng of BK, but the coronary flow increasing action lasted longer with 100 ng of BK than with 10 ng of

Fig. 4. Effects of bradykinin (BK) on delta coronary flow and release of 6-keto-PGF_{1 α} in the isolated perfused guinea-pig heart. $\triangle$ — $\triangle$ Δ coronary flow without diclofenac-Na, $\blacktriangle$ — $\blacktriangle$ Δ coronary flow with diclofenac-Na (1 μ g/ml), hatched bar the release of 6-keto-PGF_{1 α} without diclofenac-Na, solid bar the release of 6-keto-PGF_{1 α} with diclofenac-Na.

BK. Thus, the total increase in coronary flow induced by these doses of BK were dose-dependent (Table 1). 6-keto-PGF_{1 α} was not significantly released by 1 ng of BK, but was released dose-dependently by 10 and 100 ng of BK (Table 1). The relationship between the total increase in coronary flow and total amounts of 6-keto-PGF_{1 α} released is shown in Fig. 2. The release of 6-keto-PGF_{1 α} induced by BK was less than that by AA, but the total increase in coronary flow induced by BK was more than that by AA. The patterns of 6-keto-PGF_{1 α} release and the increase in coronary flow differed between the administration of AA and BK. Following stimulation with BK, the release of 6-keto-PGF_{1 α} was scanty in the first 30 sec, but the coronary flow increased rapidly and reached the submaximum flow in the first 30 sec. Namely, the increase in the coronary flow preceded increases in the release of 6-keto-PGF_{1 α} . The difference in the time course of the increase in coronary flow and the release of 6-keto-PGF_{1 α} was most apparent with the highest doses of AA and BK. The release of 6-keto-PGF_{1 α} induced by 100 ng of BK was completely inhibited by diclofenac-Na, whereas the increase in the delta coronary flow was only partially but significantly

Fig. 5. Effects of bradykinin (BK) on cardiohemodynamic parameters in the isolated perfused guinea-pig heart. $\triangle$ — $\triangle$ with diclofenac-Na, $\blacktriangle$ — $\blacktriangle$ without diclofenac-Na. Abbreviations: HR, heart rate; LVSP, left ventricular systolic pressure; LVDP, left ventricular diastolic pressure; LVdp/dt, left ventricular dp/dt.

reduced by diclofenac-Na at the highest dose of BK (Table 1).

Figure 5 shows the effect of BK on cardiac function. The lowest dose (1 ng) of BK had little effect on HR, LVSP, LVDP, and LVdp/dt. At doses of 10 and 100 ng, BK caused a sustained increase in HR and a slight increase in LVdp/dt. Only 100 ng of BK induced a slight and transient increase of LVSP. Diclofenac-Na did not affect the positive chronotropic action induced by 10 ng of BK but partially inhibited that induced by 100 ng of BK.

DISCUSSION

Schrör et al. (5) presented the evidence for an additional PGI₂-independent mechanism for the coronary vasodilating action of BK. In these studies, however, the contribution of PGI₂ to the BK-induced coronary vasodilation was not examined quantitatively. In the present study, we attempted to clarify the quantitative contribution of PGI₂ to the BK-induced coronary vasodilation in isolated perfused guinea-pig heart using radio-immunoassay of 6-keto-PGF_{1 α} . Although PGs other than 6-keto-PGF_{1 α} were not

measured in the present experiments, it has been known that both PGD₂ and PGF_{2 α} decrease coronary flow, PGE₂ has less potent coronary dilating action than PGI₂ (10), and PGI₂ is the main metabolite of AA in the heart (4). Thus, we could focus on the contribution of the released PGI₂ to coronary vasodilation induced by AA and BK in isolated guinea-pig hearts. As shown in Fig. 4, diclofenac-Na completely inhibited the release of PGI₂, but only partially inhibited the delta increase in coronary flow induced by 10 and 100 ng of BK. The degrees of inhibition of the BK-induced total increase in coronary flow with diclofenac-Na were 2.4 ± 9.1 , 25.7 ± 9.9 and $19.5 \pm 7.8\%$ for 1, 10 and 100 ng of BK, respectively. These results suggest that though higher doses of BK (10, 100 ng) certainly release PGI₂ from guinea-pig hearts, the contribution of the released PGI₂ to the BK-induced coronary vasodilation is relatively small (less than 25%). Thus, the PGI₂-independent mechanism may play a main role in the BK-induced coronary vasodilation in isolated guinea-pig hearts. The PGI₂-independent mechanism seems to act faster than the PGI₂-dependent one in the coronary vasodilation since the increase in coronary flow preceded the release of 6-keto-PGF_{1 α} (Fig. 4).

The maximum delta coronary flow induced by 100 ng of BK was lower than that by 10 ng of BK. The mechanism of this phenomenon is unclear. The increase in heart rate induced by 100 ng of BK was partially but significantly inhibited by diclofenac-Na (Fig. 5). This positive chronotropic action of BK may be mediated *via* PGE₂ since in isolated guinea-pig hearts, the positive chronotropic effect of PGE₂ is much more potent than that of PGI₂, and both PGF_{2 α} and PGD₂ have negative effects (10).

The AA-induced increase in coronary flow seems to be due to PGI₂ synthesized from the injected AA since there was a highly

significant correlation between the total increase in coronary flow and the total amount of 6-keto-PGF_{1α} released following injection of 3 doses of AA. The maximum delta coronary flow induced by 1,000 ng of AA was lower than that by 300 ng. This phenomenon might have resulted from the masking of the action of PGI₂ to increase coronary flow by a marked dose-dependent, transient reduction in coronary flow seen with higher doses of AA. The transient reduction in coronary flow at higher doses of AA, as inhibited by diclofenac-Na, may be due to a coronary vasoconstricting action of PG endoperoxides synthesized from the injection of AA (3). We have already reported that in isolated guinea-pig hearts, exogenous PGH₂ (one of the PG endoperoxides) reduces coronary flow rate (11). The AA-induced positive chronotropic effects may also be due to PGE₂ and/or PG endoperoxides.

A difference in the time course for 6-keto-PGF_{1α} release between AA and BK suggests that AA is directly transformed to PGI₂ and other PGs through PG endoperoxides, whereas the release of PGs induced by BK needs an additional step, probably an activation of phospholipase A₂ (12).

AA produced the decrease in the LVSP and LVdp/dt in parallel with the decrease in the coronary flow, suggesting that the depression of both parameters might be secondary to the decrease in the coronary flow in the early phase following injection of AA. LVDP was slightly increased following 1,000 ng of AA. This mechanism is unclear, but the increase in LVDP might be the extravascular component of the coronary resistance.

In conclusion, BK has both the PGI₂-independent and PGI₂-dependent coronary vasodilating actions in isolated perfused guinea-pig hearts, but the contribution of the latter mechanism is small (less than 25%), whereas the coronary vasodilating action of

AA is mediated mainly *via* PGI₂.

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