CORONARY VASOCONSTRICTOR AND VASODILATOR ACTIONS OF ARACHIDONIC ACID IN THE ISOLATED PERFUSED HEART OF THE RAT

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1 The administration of arachidonic acid (AA) to the isolated perfused heart of the rat usually produced biphasic coronary responses characterized by initial vasoconstriction followed by prolonged vasodilatation. However, some responses were predominantly vasoconstrictor or vasodilator.

2 The non-steroidal anti-inflammatory agents (NSAA) indomethacin (1–5 mg/l) and naproxen (12.5–25 mg/l) reversibly inhibited both phases of the response induced by AA.

3 Pretreatment of animals with indomethacin (5 mg/kg) or naproxen (25 mg/kg) daily, resulted in unaltered coronary response to AA. Subsequent addition of NSAA to the perfusate produced inhibition of the AA effect.

4 Short infusions of acetylsalicylic acid at low concentrations (2.9 μg/ml), dipyridamole (0.6 μg/ml) and sulphinpyrazone (28.7 μg/ml) selectively inhibited the vasoconstrictor phase of the response to AA.

5 It was confirmed that metabolic coronary dilatation induced by cardiostimulation was inhibited by prolonged AA administration; this effect was prevented by NSAA pretreatment. Reactive hyperaemic responses to short lasting occlusions of coronary inflow were unaffected by NSAA.

6 Linolenic, linoleic, dihomo-γ-linolenic and oleic acid usually produced decreases in coronary flow which were unaffected by NSAA, dipyridamole or sulphinpyrazone.

7 Intra-aortic injections of AA, prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) in the intact rat produced a dose-dependent decrease in blood pressure with the AA response inhibited by indomethacin. PGI_2 and PGE_2 produced long lasting coronary vasodilatation in the isolated heart.

8 The coronary actions of AA appear to be due to its transformation, within the easily accessible vascular wall, into prostaglandin and thromboxane-like substances. We suggest that a vasoconstrictor thromboxane A_2-like substance may be responsible for coronary vasospasm.

9 Coronary insufficiency may also result from an inhibition of compensatory metabolic coronary dilatation by increased synthesis of PGE_2 within the myocardial cell.

Introduction

It is well established that the administration of arachidonic acid (AA) into the coronary circulation is followed by the formation of prostaglandin-like substances (PGL) (Needleman, 1978), biosynthesized by an enzyme system found in the microsomal fraction of mammalian hearts (Limas & Cohn, 1973). The formation of cardiac PGL may be prevented by the administration of non-steroidal anti-inflammatory drugs (NSAA), particularly indomethacin (Flower, 1974; Kulkarni, Roberts & Needleman, 1976; Needleman, 1976). It has been postulated that prostacyclin (PGI_2) is the major product of AA metabolism in the heart (Isakson, Raz, Denny, Puré & Needleman, 1977; de Deckere, Nugteren & Ten Hoor, 1977). The identification and properties of PGI_2 described by Dusting, Moncada & Vane (1977) led these investigators to suggest that it is involved in the local regulation of the coronary vascular bed. In addition to its potent vascular smooth muscle relaxing properties, PGI_2 also possesses the most potent blood platelet anti-aggregatory function so far described; thus de Deckere *et al.* (1977) proposed that this would be the main function of PGI_2. The enzyme that converts AA into endoperoxides leading to PGL, including PGI_2, is located in the vascular wall (Needleman, Raz, Kulkarni, Puré, Wyche, Denny & Isakson, 1977; Hyman, Kadowitz, Lands, Crawford, Fried & Barton, 1978) particularly in the endothelial cells (Weksler, Marcus & Jaffe, 1977; MacIntyre, Pearson & Gordon, 1978)

and subendothelium (Silberbauer, Sinzinger, Winter, Feigl & Ring, 1978). This would reinforce the original proposal that vascular PGI_2 production could be an important regulatory mechanism in haemostasis (Bunting, Gryglewski, Moncada & Vane, 1976). The transformation of exogenous AA into vasoactive metabolites has been confirmed in guinea-pig isolated perfused hearts, in which several prostaglandins were found in the venous effluent. Furthermore, an infusion of indomethacin produced blockade of the coronary actions as well as the PGL efflux (Schrör, Moncada, Ubatuba & Vane, 1978). These data corroborate previous experiments in which NSAA was administered to inhibit prostaglandin synthetase in isolated perfused or intact hearts (Minkes, Douglas & Needleman, 1973; Needleman, 1976; Hintze & Kaley, 1977). Other polyunsaturated fatty acids have also been reported to produce changes in coronary flow and in the output of PGL (Mentz & Förster, 1977). The effects of these polyunsaturated fatty acids were also said to be inhibited by indomethacin (Mentz, Blass & Förster, 1976). These observations conflict with the results obtained by Kulkarni *et al.* (1976) who demonstrated that indomethacin abolished only the effects of AA and not of other fatty acids on the coronaries. Thus, we reinvestigated the actions of different polyunsaturated fatty acids including AA on the coronaries in isolated perfused hearts and the influence of NSAA in this experimental model.

The isolated perfused heart model effectively provides information on the behaviour of the resistance vessels of the coronary circulation in which small changes in smooth muscle tone produce marked changes in coronary flow (Schaper & Schaper, 1977) and should be regarded as one of the most important factors in the regulation of the overall coronary flow. This experimental design allowed us to study the mechanisms involved in the regulation of coronary flow and in the processes that may induce coronary insufficiency (Sunahara & Talesnik, 1979). The data provided by the action of prostaglandins and/or related substances on the smooth muscle of isolated strips of large vessels does not necessarily indicate that the resistance vessels will respond in a similar manner. For instance, it has repeatedly been shown that PGE_2 causes contraction of isolated coronary arteries (Kalsner, 1975; Kulkarni *et al.*, 1976) while it produces relaxation of arterioles in the coronaries of intact hearts (Hintze & Kaley, 1977; Dusting & Vane, 1980).

We have previously shown that inhibition of cardiac cyclo-oxygenase does not prevent the metabolic coronary dilatation (MCD) that follows the cardiostimulation induced by different agents. The present experiments support our concept that PGL synthesized in the myocardium may act as modulators of

MCD responses while prostaglandins, and related substances, formed in the vascular compartment would have local effects but would not participate in the regulation of the arteriolar tone when there is an increase in myocardial O_2 demands.

We have also included data from isolated perfused hearts of guinea-pig to establish whether there are species differences in the biological activation of AA in the heart as has often been reported for other tissues (Al-Ubaidi & Bakhle, 1980). In some experiments we also tested the coronary reactions of the rat heart to authentic PGE_2 and PGI_2. Lastly we checked the effectiveness of AA, PGE_2 and PGI_2 in inducing blood pressure responses in anaesthetized rats.

Methods

Experiments were carried out in isolated perfused hearts from male Sprague-Dawley rats obtained from Canadian Biobreeding Laboratories. For the perfusion, the Langendorff technique was used with Krebs-Henseleit-bicarbonate solution at 36°C, modified to contain half the normal Ca^{2+} (Zachariah, 1961), insulin 2 units/l (Bleehan & Fisher, 1954; Weissler, Atschuld, Gibb, Pollack & Kruger, 1973), Na lactate 4 mM (Drake, Haines & Noble, 1980) and glycerol 10 mM (de Kock, Lochner, Kotzé & Gevers, 1978). Continuous measurement of coronary flow (CF) was calculated from a differential pressure transducer tracing (Sen, Sunahara & Talesnik, 1977) and calibrations of CF were performed after each experiment. Heart activity was often recorded with a force displacement transducer (Grass FT .03) at an initial diastolic tension of 2 g. In order to obtain a more accurate evaluation of cardiac performance we also used a modification of the system described by Opie (1965) and Fallen, Elliott & Gorlin (1967), in which the left ventricular pressure development was continuously measured. This method consisted essentially of a vinyl water-filled balloon attached to a hypodermic needle (25 mm long, gauge 16) that was introduced into the left ventricle through the left atrium. After piercing the heart apex, the needle was connected to a Windkessel (air capacity 0.5 ml) and the latter to a pressure transducer (Statham P23Db); therefore, the recorded left ventricular pressure was not measured in isovolumic conditions as in Opie's (1965) or Fallen *et al.*'s (1967) methods. The volume of fluid in the balloon (about 0.1 ml) was adjusted with a micrometer drive to obtain zero diastolic pressure. The product of peak systolic pressure and heart rate ['total pressure developed' (TPD)] was used as an index of left ventricular work (Opie, 1965). Spontaneous rhythm was suppressed by eliminating the right atrium and, when necessary, clamping the interventricular septum. The heart was

electrically paced at 210 beats/min through bipolar electrodes with square wave pulses at a current about 20% above threshold. Single injections were given by means of an injector built to administer drugs at a speed self-controlled by the coronary flow; slow infusions were applied through a side arm of the injector (Sunahara & Talesnik, 1979). Perfusion with constant concentrations of drugs was carried out by adding them to the reservoir in which the perfusate was stored. The perfusion apparatus was constructed so that control and drug containing perfusion fluids flowed independently through a water jacketed heat exchanger from levelling chambers with overflow systems to maintain a constant height throughout the experiment. The fluid was continuously pumped through an on-line millipore filter ($5 \mu m$) towards the levelling chambers from where the overflow returned to the reservoirs in which the perfusion fluid was oxygenated (95% O_2; 5% CO_2). This system could be used as an open or closed circuit; in the latter case the coronary outflow was collected and returned to the corresponding reservoir. Reactive hyperaemic responses were induced by short lasting (5, 10, 15 s) occlusions of coronary inflow.

Changes in CF, force of contraction (measured with the force transducer) or TPD were assessed by integrating the areas under the recordings. At the end of each experiment, the hearts were blotted and weighed. With these data, the changes in CF were calculated in $ml\,min^{-1}\,g^{-1}$ tissue. Statistical analysis was carried out by methods indicated in results.

In the experiments in guinea-pigs, a similar method was used but with full Ca^{2+} concentration in the Krebs-Henseleit solution. In some cases the coronary flow was recorded with an electromagnetic flowmeter (Biotronex BLI-610).

Blood pressure in anaesthetized rats (Inactin i.p., 100 mg/kg) was recorded from the left carotid artery in which a vinyl catheter was introduced as far as its origin from the aorta. On line with the catheter a thick membrane was arranged on an acrylic micro T tube allowing intra-aortic injections of the substances to be tested in constant volumes of 0.1 ml. The rats were maintained at $37-37.5°C$ and the trachea cannulated to avoid obstruction of the respiratory tract. Heparin (4 mg/kg, i.v.) was administered at the time when the carotid was catheterized; further administration of half the initial dose of heparin was repeated every 60 min. If needed, more Inactin was administered during the experiment.

Drugs

The compounds used and the sources from which they were obtained were as follows: noradrenaline bitartrate monohydrate (Levophed, Winthrop); heparin and insulin (Connaught Laboratories); in-domethacin (Merck Frosst Laboratories); naproxen (Syntex Laboratories Inc.); arachidonic, linolenic, linoleic, dihomo-γ-linolenic and oleic acids (Sigma Chemical Co. or Nu Chek Prep. Inc.); acetylsalicylic acid (Sigma Chemical Co.); sulphinpyrazone (Anturan, Ciba-Geigy); dipyridamole (Persantine, Boeringer-Mannheim); prostaglandin E_2 and I_2 sodium salt (Upjohn); Inactin (BYK). All reagents used were of analytical grade and drug solutions freshly prepared. Doses are expressed in terms of their respective base or acid. Stock solutions of noradrenaline were prepared in 0.9% w/v NaCl solution (saline) with added ascorbic acid (20 mg/l; 1.14×10^{-4} M; Samuelsson & Wennmalm, 1971). In-domethacin was prepared in ethanol (10 mg/ml) and diluted with phosphate-buffered saline at pH 7.2 (Owen, Ehrhart, Weidner, Scott and Haddy, 1975) and naproxen in phosphate buffer. Arachidonic acid was stored at $-10°C$ in ethanol 95% (10 mg/ml) or in hexane-methanol mixture (1:3). The other fatty acids were also kept in hexane-methanol at $-10°C$ at different concentration (5–10 mg/ml). Fresh stock solutions of fatty acids were prepared every week. Authentic PGE_2 and stock solutions were made in 95% ethanol and refrigerated at $-10°C$ until dilution was prepared. PGI_2 was prepared shortly before use in sodium carbonate (2 mM) at a concentration of 0.5 mg per ml; further dilutions were made in Krebs-Henseleit buffer.

The dilutions of arachidonic acid measured with Hamilton syringes were obtained either by emulsion of the ethanol solution into the Krebs-Henseleit, as used by some investigators (Carlson, Wentland, Leonard, Ruder & Reeve, 1979) or by 10 fold dilution in 2 mM sodium carbonate solution (Kulkarni *et al.*, 1976). In other experiments aliquots of the hexane-methanol stock solution of fatty acids, including AA, were evaporated, protected from light in nitrogen and the sodium salts were obtained by dissolving them in 100 mM sodium carbonate; these solutions were freshly prepared and used only if they were 'crystal clear' (Wicks, Rose, Johnson, Ramwell & Kot, 1976). Further dilutions of the fatty acids were made with Krebs-Henseleit buffer. The dilutions of AA from the ethanol solution with Krebs-Henseleit buffer resulted in a suspension found unsuitable for our investigations. The preparation of the arachidonate in 2 mM sodium carbonate that contain more than enough base to give clear solutions, appeared to be appropriate for slow infusions. The pH of a solution in Krebs-Henseleit containing AA $100 \mu g/ml$ was approximately 7.75; when slowly infused at a rate of 0.1 ml/min the dilution in the perfusate resulted in a lowering of the pH to 7.4 which was the normal value of our Krebs-Henseleit-bicarbonate solution. When the arachidonate was prepared in 100 mM sodium carbonate and further

diluted in Krebs-Henseleit buffer to contain AA 20 μg/ml, the crystal clear solution obtained had a pH of approx. 8.0. This solution slowly infused into the perfusate (0.1 ml/min) resulted in a drop of pH to 7.4. Bolus doses of AA were given when prepared in 100 mM carbonate. Control administration of the solvents were routinely carried out at equivalent concentrations used with AA and they were ineffective on the coronaries (data not shown). The type of solutions used are indicated in results. Acetylsalicylic acid was dissolved in 95% ethanol (10 mg/ml) and further dilutions in isotonic glucose-saline solution (8:8 g/l). Sulphinpyrazone and dipyridamole were prepared in glucose-saline solution.

Results

The coronary reactions elicited by slow infusions of AA in the rat isolated perfused heart could be classified, according to the predominant type of response, into three major groups; (a) one characterized by a biphasic response; (b) another in which vasoconstriction predominated, and (c) a group in which vasodilatation was preponderant. The coronary reactions were usually easily converted into a predominant vasoconstriction at higher concentrations of AA. Initially it appeared that the method by which the AA was prepared was a contributing factor in determining the type of coronary response (Table 1), nevertheless, more recently we have observed that AA prepared by different methods produces similar types of responses in the same heart preparation (data not shown). However, it became obvious that the amounts of AA required to produce the effects were lower if the AA solutions were prepared with 100 mM sodium carbonate, probably due to an increased availability of sodium arachidonate to its metabolic sites. Examples of the three types of responses obtained in different rat hearts are shown in Figure 1. For this illustration we selected 3 cases in which the sodium AA was obtained by treatment of the fatty acid with 100 mM sodium carbonate.
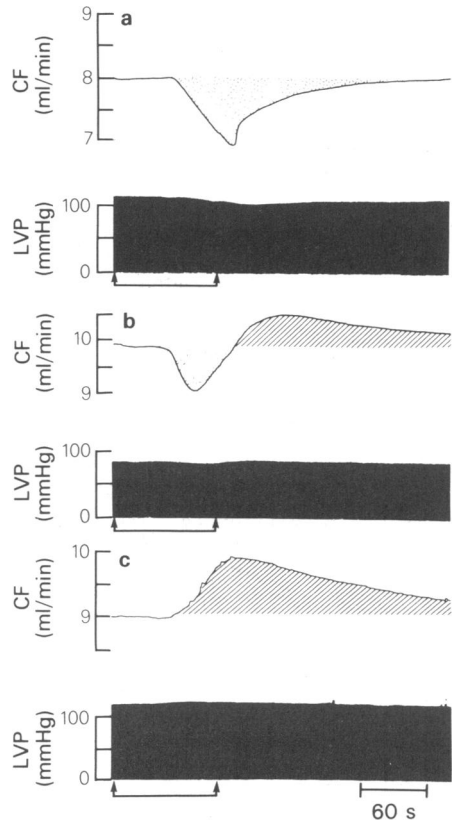

Figure 1 Effect of a slow infusion (1.5 min) of arachidonic acid (AA) prepared in 100 mM Na_2CO_3, on coronary flow (CF) and left ventricular pressure (LVP) in different isolated rat hearts paced at 240 beats/min. AA infusion of 2 μg/min indicated by ◆——◆. (a) Predominant vasoconstrictor response; (b) biphasic response; (c) predominant vasodilator response.

Coronary reactions to arachidonic acid prepared with 2 mM Na_2CO_3

Rats weighing 284 ± 6.0 g (mean ± s.e.) in which the

Table 1 Frequency of type of coronary response elicited by slow infusions of arachidonic acid (AA) (1.5 min) at suprathreshold concentrations

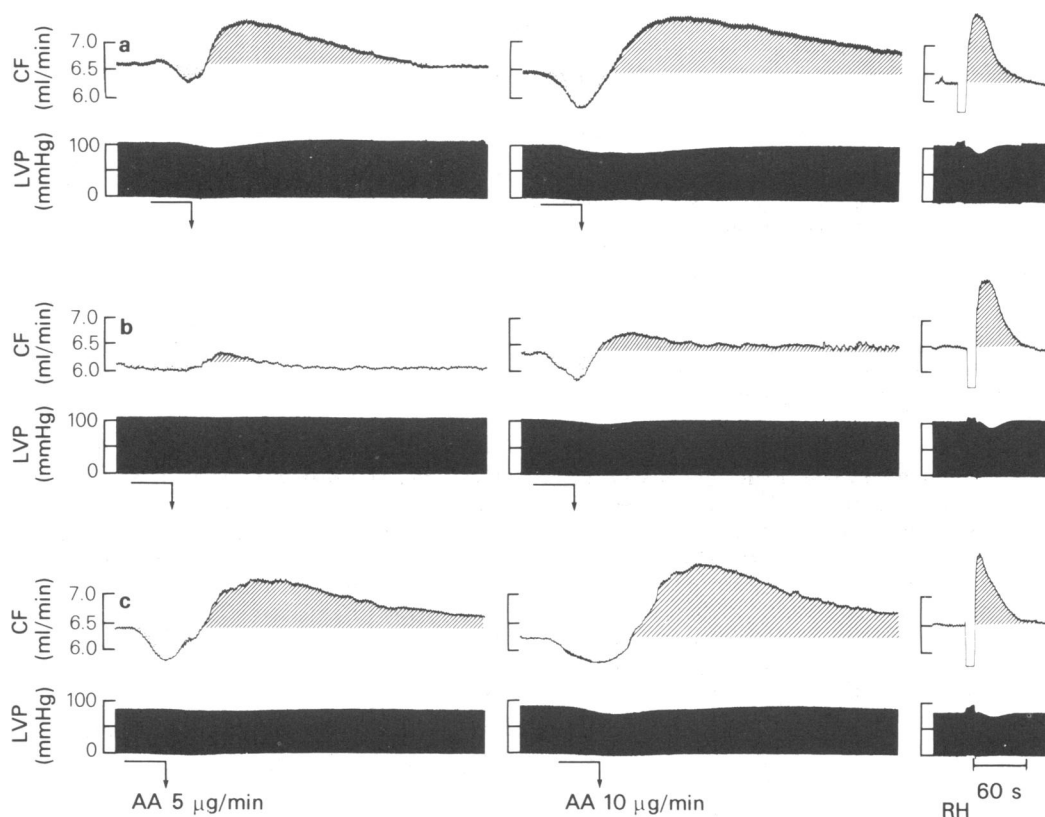| AA preparation	n	Concentration range (μg/min)	Predominant response		
			Constriction	Biphasic	Vasodilatation
Na_2CO_3 2 mM	59	5–20	4	35	20
Na_2CO_3 100 mM	76	0.5–4	39	29	8
	135		43	64	28

Figure 2 Effect of arachidonic acid (AA) on coronary flow (CF) and left ventricular pressure (LVP) in the isolated, rat heart paced at 240 beats/min. Doses of AA, prepared in 2 mM Na$_2$CO$_3$, indicated at bottom of figure were infused during 1.5 min terminating at ⌐↓. The stippled and hatched portions indicate the areas measured for quantitation of the CF changes. Reactive hyperaemia (RH) was induced by interruption of coronary inflow for 10 s. (a) Control AA and RH responses; (b) responses after 15 min of indomethacin administration (1 mg/l); (c) responses after 20 min withdrawal of indomethacin.

basal CF was 7.5 ± 0.2 ml min^{-1} g^{-1} were used. AA prepared in 2 mM Na$_2$CO$_3$ usually elicited a biphasic response (Table 1) when infused at 0.1 ml/min during 1.5 min. This reaction was characterized by a phase I of diminution in CF that always preceded a phase II of enhanced CF (Figure 2). These changes were obviously due to vasoconstriction or to vasodilatation of the coronary resistance vessels. The vasoconstrictor phase usually started about 45 s after beginning the AA infusion and lasted from 40 to 90 s. The diminution in CF was relative to the amount of AA administered (Table 2); the peak of this phase often coincided with the interruption of the AA administration. The phase II of vasodilatation that followed, developed very slowly but lasted for a prolonged period of time (Table 2). The vasodilator phase started about 100–120 s after the beginning of the infusion, peaked between 50 to 100 s and lasted from 300–400 s; in some cases the CF remained elevated

for more than 10 min. When this pattern of response was obtained, the entire diminution of CF was evaluated while the vasodilator phase was measured for a fixed interval of 5 min. Table 2 also summarizes the duration of phase II and it may be seen that it is relatively unrelated to the amount of AA administered although the magnitude of the vasodilatation appeared to be dose-dependent (Figure 2).

When vasodilatation was the predominant response to AA it was found that it was either preceded by a minor constrictor phase, lasting 40–60 s, or it developed after a delay of approximately the same duration. Furthermore, in several hearts of this group it was found that higher concentrations of AA became effective in inducing a vasoconstrictor response.

In each experiment only 2 to 3 infusions of AA were tested during the control period and only after restoration of the normal CF level.

Table 2   Coronary responses to arachidonic acid (AA) in the rat isolated heart and its inhibition by non-steroidal anti-inflammatory agents (NSAA)

AA (μg/min)	n	Control ΔCF (ml) Phase I	Phase II	Duration of phase II (s)	n	NSAA ΔCF (ml) Phase I	Phase II	Duration of phase II (s)
5	8	0.09 ± 0.04	1.98 ± 0.26	402 ± 74				
10	18	0.25 ± 0.07	2.86 ± 0.66	347 ± 41	10	0.08 ± 0.03	0.28 ± 0.05	184 ± 56
20	19	0.35 ± 0.12	3.36 ± 0.68	321 ± 38	13	0.19 ± 0.04	0.48 ± 0.15	192 ± 44
40	8	0.40 ± 0.17	2.76 ± 0.53	313 ± 40	5	0.35 ± 0.04	1.88 ± 0.85	113 ± 78

Arachidonic acid (AA), prepared in Na_2CO_3 2 mM, infused for 1.5 min induced vasoconstriction (Phase I) followed by vasodilatation (Phase II). The changes in coronary flow (ΔCF $\pm$ s.e.mean) for vasoconstriction represents total diminution in volume while vasodilatation volume was measured over an interval of 5 min. Non-steroidal anti-inflammatory agents (NSAA) (indomethacin 1–5 mg/l or naproxen 12–25 mg/l) produced inhibition of both phases.

In all experiments in which substantial vasoconstriction was produced by AA, it was associated with a depression of the left ventricular pressure development (Figure 2). However, contractility recovered rapidly with the return of CF to control levels. Conversely, marked coronary vasodilatation was often associated with an increase in left ventricular pressure. Since these alterations in inotropy were elicited only with marked modifications in coronary flow, we suggest that they should not be attributed to a direct effect of AA on myocardial contractility.

Effect of non-steroidal anti-inflammatory agents on the coronary response to arachidonic acid

Indomethacin or naproxen (NSAA) were used since they are known to inhibit the formation of PGL. Usually NSAA were perfused at constant concentrations but in some instances they were infused at a rate such that, according to the coronary flow, they were equivalent to the constant concentration experiments. Indomethacin was administered at 1 mg/l ($n = 6$) or 5 mg/l ($n = 6$) (2.8×10^{-6} or 1.4×10^{-5} M), respectively) and naproxen ($n = 4$) at 12.5, 15 or 25 mg/l (5×10^{-5} to 1×10^{-4} M). Because the results were similar with either drug at different concentrations, they were tabulated or plotted together in Table 2 and Figure 3. The basal CF before NSAA was 7.2 ± 0.6 ml min^{-1} g^{-1} and during NSAA (stabilization allowed for about 15–20 min) remained about the same (7.1 ± 0.57 ml min^{-1}g^{-1}). As can be seen in Figure 2, NSAA markedly reduced both phases of AA action on the coronaries. The inhibitory effect of NSAA was almost complete for low concentrations of AA while diminished responses were obtained with greater amounts of AA. We would like to emphasize (Table 2) that the inhibition of the vasoconstriction was as marked as the inhibition of the vasodilatation. The latter is represented in Figure 3 in which paired analysis of phase II is shown.

It can be seen in this figure that the increased CF induced by AA is a dose-dependent reaction and that the administration of NSAA, for about 30 min, produced a marked inhibition of the vasodilator response. Furthermore, the inhibition due to NSAA reduced the enhancement of CF to a similar level whether 5, 10 or 20 μg/min of AA were given. It was also noted that during NSAA administration, when the coronaries ceased to react to AA, the LVP remained unaltered.

Withdrawal of non-steroidal anti-inflammatory agents from the perfusate

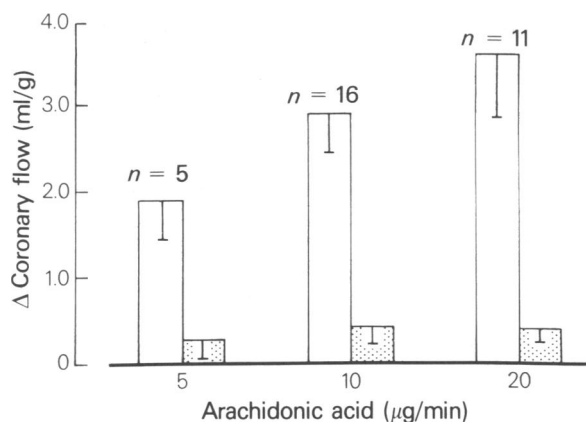In 9 of the 16 experiments in which a NSAA was

Figure 3   Mean increases in coronary flow induced by arachidonic acid (AA, prepared in 2 mM Na_2CO_3) before (open columns) and during the administration of indomethacin (1–5 mg/l) or naproxen (12–25 mg/l) (stippled columns). Vertical lines indicate s.e.mean. Paired analysis showed significant difference between control response and response during indomethacin or naproxen ($P < 0.001$). Doses of AA indicated at bottom of figure.

Table 3 Recovery of arachidonic acid (AA)-induced vasodilatation after withdrawal of non-steroidal anti-inflammatory agents (NSAA)

Exp. No.	AA (μg/min)	ΔCF (ml)				
		Control	NSAA	% from control	NSAA withdrawal	% from control
7	5	1.47	0.13	−91.2	2.45	+66.6
7	10	3.90	0.39	−90.0	4.00	+ 2.6
8	10	7.37	0.17	−97.7	0.30	−95.5
15	10	2.29	0.11	−95.2	5.16	+125.3
16	10	1.44	0.35	−75.7	0.75	−47.9
20	10	1.44	0.04	−97.2	0.86	−40.3
15	20	4.85	1.04	−78.5	4.09	−15.6
22	20	5.84	0.36	−93.8	3.78	−35.3
24	20	2.62	0.22	−91.6	0.69	−73.6
14	40	3.18	0.25	−92.1	5.68	+78.6
13	40	1.33	0.00	−100.0	1.46	+ 9.8

The inhibition of arachidonic acid (AA prepared in 2 mM Na_2CO_3)-induced coronary dilatation (ΔCF) by non-steroidal anti-inflammatory agents (NSAA) (indomethacin 1–5 mg/l; naproxen 12–25 mg/l) was reversed after 20 min perfusion without NSAA.

used, the administration of the drug was stopped. About 15–30 min later, the basal CF was 7.07 ± 0.32 ml min^{-1} g^{-1} and infusions of AA were repeated. There was full recovery of phases I and II similar to the example shown in Figure 2. Sometimes the coronary reactions became more marked following the inhibition produced by NSAA while in some experiments the recovery was only partial as though not enough time had been given for the NSAA washout to be completed. The results obtained for the vasodilator phase are tabulated in Table 3. We think that the observation of paired cases provides a better indication of the recovery of the coronary reactions after withdrawal of NSAA because of the variation in the data. By normalizing these data it became obvious that NSAA produced a similar degree of blockade in all cases studied ($91.18 \pm 2.3\%$), irrespective of the amount of AA given or the NSAA used. Regardless of the great scatter of values during NSAA withdrawal, the statistical analysis of paired data clearly showed that the recovery was significant ($t = 4.85$, $P < 0.001$).

Coronary reactions to arachidonic acid in hearts from rats pretreated with non-steroidal anti-inflammatory agents

Hearts isolated from 8 rats treated for 5 days with

Table 4 Effect of arachidonic acid (AA) in hearts from rats pretreated with non-steroidal anti-inflammatory agents (NSAA)

Exp. No.	AA (μg/min)	ΔCF (ml)					
		Control		NSAA		% inhibition	
		Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
11	5	0.08	1.48				
9	5	0.40	3.72	0.07	1.37	83.5	63.1
9	10	0.00	7.20	0.00	2.09	—	29.0
5	10	0.07	1.55				
13	10	0.00	3.75				
14	10	0.00	2.41				
14	20	0.47	3.35				
5	20	0.02	3.59				
1	20	0.14	2.12	0.05	0.52	64.3	75.4
2	20	0.03	3.75	0.02	1.74	33.5	53.6
3	20	0.79	2.73	0.03	0.84	96.2	69.2

Pretreatment of rats with non-steroidal anti-inflammatory agents (NSAA) (indomethacin or naproxen) did not alter vasoconstriction (Phase I) or vasodilatation (Phase II) in response to arachidonic acid (AA) prepared with 2 mM Na_2CO_3. Perfusion of the hearts with NSAA for 15–20 min produced inhibition of both phases.

indomethacin (5 mg/kg i.p.) ($n = 6$) or naproxen (25 mg/kg i.p.) ($n = 2$) showed similar basal CF after stabilization in the perfusion apparatus; thus, the mean CF was calculated together for all NSAA-treated hearts; the value 7.6 ± 0.97 ml min^{-1} g^{-1} was not significantly different from controls (7.5 ± 0.2 ml min^{-1} g^{-1}). Challenging these hearts with AA produced biphasic responses as in the hearts from untreated rats. The variation obtained in the untreated hearts was also found in the NSAA-treatedgroup and the results are given in Table 4. In some experiments in which a clear biphasic response was obtained, further addition of indomethacin to the perfusate produced inhibition of both the vasoconstrictor as well as the vasodilator actions elicited by AA.

Coronary reactions to arachidonic acid prepared with 100 mM Na₂CO₃

In this series of experiments AA prepared in 100 mM Na$_2$CO$_3$ usually produced either a biphasic or predominantly vasoconstrictor response to one fifth the concentrations used in the previous series (Table 1).

Hearts weighing 1.09 ± 0.02 g were obtained from

rats weighing 311.7 ± 5.0 g after equilibration for about 20 min, the CF was 8.84 ± 0.13 ml min^{-1} g^{-1} and after 3 h (usual duration of experiment) the CF was 8.66 ± 0.14 ml min^{-1} g^{-1}. An infusion of AA at 2 μg/min, that always produced a coronary reaction, reached, according to the flow rate, a concentration of about 0.74 μM. Figure 4 illustrates representative experiments in which biphasic responses were predominant. An initial diminution in coronary flow developed after a delay of a few seconds and lasted until the infusion was interrupted. It may also be seen in Figure 4 that a marked vasoconstriction was usually associated with a transient decrease in left ventricular systolic pressure. After the vasoconstriction subsided, a slow developing vasodilatation often lasting over a 10 min period, ensued.

Influence of indomethacin (Indo), acetylsalicylic acid (ASA), sulphinpyrazone (Spyz) and dipyridamole (Dpy) on the coronary reactions to arachidonic acid

The administration of ASA by slow infusion for 10–15 min at a low concentration (2.9 μg/ml) produced blockade of the vasoconstrictor phase primari-
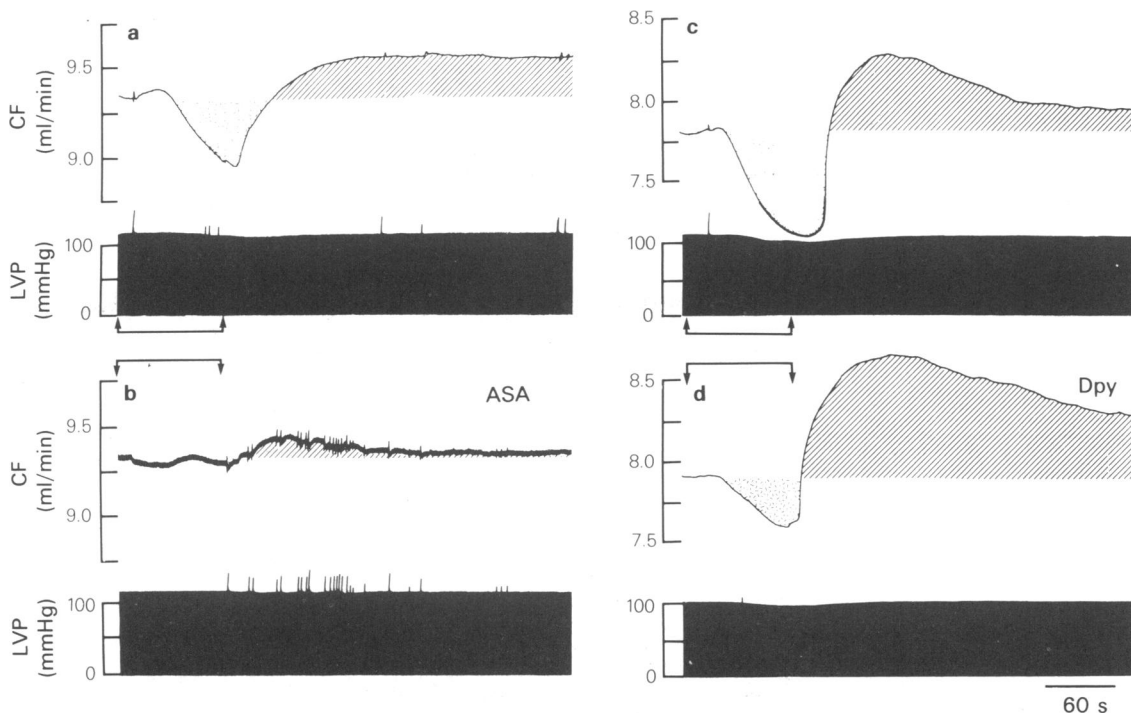

Figure 4 Effect of slow infusions of arachidonic acid (AA, prepared in 100 mM Na$_2$CO$_3$) on the coronary flow (CF) and left ventricular pressure (LVP) in rat isolated hearts. The stippled and hatched portions indicate the areas measured for quantitation of the CF changes. Isolated spikes in the LVP recording indicate premature ventricular contractions. (a) Control response; (b) response after acetylsalicylic acid (ASA) 5.4 μg/ml for 15 min; (c) control response; (d) response after dipyridamole (Dpy) 0.06 μg/ml for 30 min.

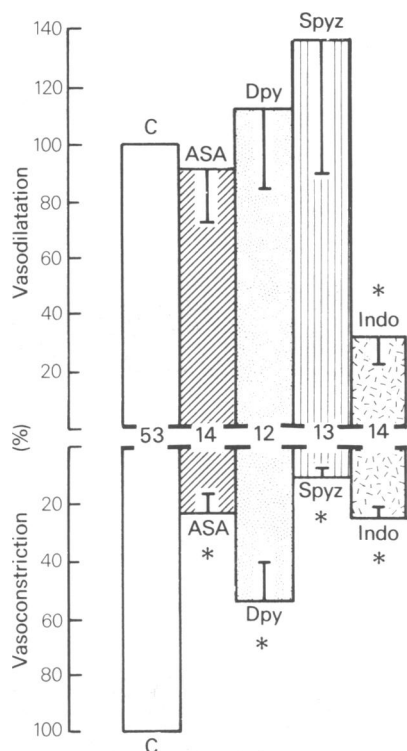**Figure 5** Influence of acetylsalicylic acid (ASA, 2.9 μg/ml for 10 min), dipyridamole (Dpy, 0.6 μg/ml), sulphinpyrazone (Spyz, 28.7 μg/ml) and indomethacin (Indo, 0.1 μg/ml) on the vasoconstriction and vasodilatation (mean values, vertical lines indicate s.e.) produced by slow infusions of arachidonic acid (AA), prepared in 100 mM Na_2CO_3. Quantitation of changes in coronary flow (CF) were obtained by measuring the appropriate areas under the recordings of CF. C refers to pooled control responses and number of experiments in each group is indicated at the base of the columns. (*$P < 0.001$).

ly while at slightly higher concentrations (5.4 μg/ml) the vasodilator phase was also inhibited, as shown in Figure 4. The administration of Spyz (28.7 μg/ml) or Dpy (0.6 μg/ml) for over 30 min was effective in producing a blockade of the vasoconstrictor phase primarily while the vasodilatation was unaffected or even potentiated by these agents. Indo perfused at 0.1 μg/ml for over 30 min inhibited both the vasoconstriction and the vasodilatation induced by AA. The results of this experimental series are summarized in Figure 5.

Single injections of AA at doses of 1 to 4 μg caused on all occasions, a biphasic reaction characterized by a marked vasoconstriction, occurring seconds after the injection and lasting only 15 to 30 s; this was followed by a vasodilator phase that often lasted several minutes before restoration to control levels.
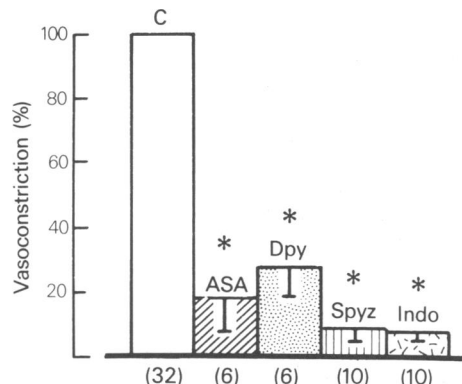

Figure 6 Effect of acetylsalicylic acid (ASA, 2.9 μg/ml for 10 min), dipyridamole (Dpy, 0.6 μg/ml), sulphinpyrazone (Spyz, 28.7 μg/ml) and indomethacin (Indo, 0.1 μg/ml) on the vasoconstriction (mean values, vertical lines indicate s.e.) induced by single injections of arachidonic acid (AA) prepared in 100 mM Na_2CO_3. C refers to pooled control responses and number of experiments in each group is indicated at the bottom of the columns (*$P < 0.001$).

In this series we will refer only to the vasoconstrictor phase induced by AA. This reaction was markedly inhibited by ASA, Spyz, Dpy or Indo when administered for 30–40 min to the perfused heart, as shown in summarized form in Figure 6.

Influence of non-steroidal anti-inflammatory agents and arachidonic acid on metabolic coronary dilator responses induced by noradrenaline (NA)

We confirmed our previous observation that single doses or slow infusions of NA induced inotropic responses that were followed by MCD reactions which correlated directly with the intensity of the inotropic effect. Furthermore, an infusion of NSAA resulted in an enhancement of MCD, without affecting the degree of cardiostimulation. The inhibitory action of AA on the MCD could be induced in the naive or in hearts infused with NSAA. After inhibition of the MCD by AA, its withdrawal from the perfusion fluid determined a fast recovery of the increased levels recorded during indomethacin. When no indomethacin was used prior to AA, the recovery of MCD proceeded very much in the same manner.

The MCD elicited by NA in hearts from rats pretreated with NSAA was similar to the response obtained in control hearts thus confirming our previous results (Sunahara & Talesnik, 1979). We also confirmed that a prolonged infusion of AA, which blocked the MCD in untreated hearts, was ineffective in altering the MCD in rats previously treated with NSAA.

Influence of non-steroidal anti-inflammatory agents and arachidonic acid on the reactive hyperaemia induced by coronary occlusion

We have previously reported that in hearts from NSAA-treated rats, the post-occlusive hyperaemia was not significantly different from the reactions obtained in control hearts from untreated rats. In the present study, normal control and NSAA perfused hearts (Figure 2), as well as hearts from rats pre-treated with NSAA and further infused with AA, were tested for the reactive hyperaemic response to coronary occlusions that lasted from 5 to 10 s. The evaluation of the reactive hyperaemia was carried out by measuring the post-occlusive peak flow and also by measuring the total volume of the reactive hyperaemic response until the flow returned 90% back to the initial level. Briefly, the post-occlusive hyperaemia was similar whether the NSAA was administered to the rats for several days prior to the experiment or was administered to the heart with the perfusion fluid.

Coronary reactions to linolenic, linoleic, di-homo γ linolenic and oleic acids

Slow infusions of linolenic ($n = 9$, at $8-16 \mu g/min$) or linoleic ($n = 3$, at $2-4 \mu g/min$) acids produced reactions characterized by a predominant coronary constriction followed by a transient increase in flow when large doses were administered. The fatty acids linolenic ($n = 2$, $4-8 \mu g$), linoleic ($n = 6$, $1-4 \mu g$), di-homo-γ-linolenic ($n = 4$, $1-2 \mu g$) and oleic acid ($n = 4$, $1-2 \mu g$) were also injected in single doses. In all but two experiments in which biphasic responses were observed with linolenic acid, there was only a reduction in coronary flow. The doses used were changed in each experiment to match vascular effects elicited by AA which was always used as a basis of comparison.

The same concentrations of Indo, ASA, Spyz or Dpy, effective in modifying the coronary reactions to AA, failed to alter the responses to the other fatty acids.

Coronary reactions induced by prostaglandin I_2 (PGI$_2$) and prostaglandin E_2 (PGE$_2$)

Slow infusions of PGI$_2$ or PGE$_2$ regularly produced a dose-dependent coronary vasodilatation in the isolated heart. PGI$_2$, whether administered by slow infusion ($0.05-1 \mu g/min$), or in single doses was approximately 10 times more potent than PGE$_2$ (Figure 7). The vasodilator actions of PGE$_2$ and PGI$_2$ are long lasting although PGI$_2$ tended to produce a more prolonged effect.
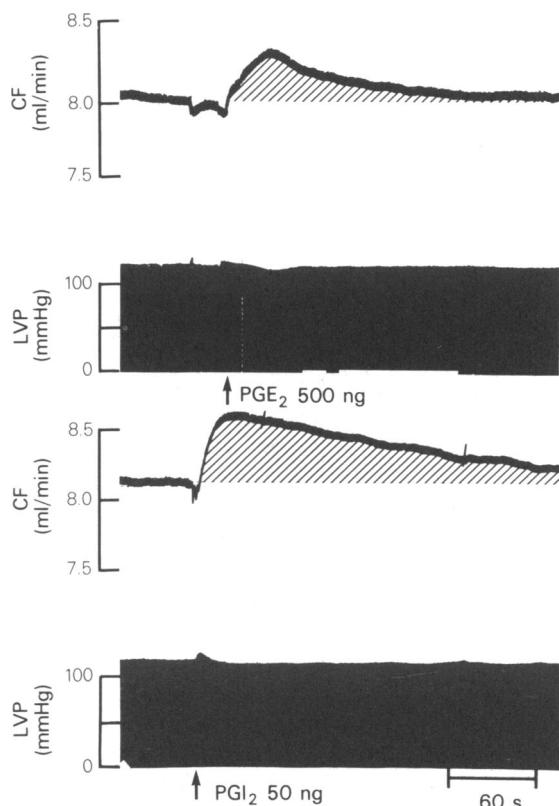

Figure 7 Effects of single injections of prostacyclin (PGI$_2$, 50 ng) or prostaglandin E$_2$ (PGE$_2$, 500 ng) on coronary flow (CF) and left ventricular pressure (LVP) in an isolated heart of rat paced at 240 beats/min.

Blood pressure responses to intra-aortic injections of arachidonic acid, prostaglandin I_2 and E_2

To test further the vascular response to AA, single doses were administered in volumes of 0.1 ml directly into the thoracic aorta. AA produced a dose-dependent ($40-80-100 \mu g$) lowering of blood pressure in all cases ($n = 6$). These effects were blocked after about 30 min of intravenous Indo (5 mg/kg) administration. Dose-dependent depression of blood pressure was also obtained by injecting PGE$_2$ or PGI$_2$ ($0.1-0.5 \mu g$). The fall in blood pressure was greater and longer lasting for PGI$_2$ than for PGE$_2$.

Coronary responses to arachidonic acid in guinea-pig isolated perfused hearts

Isolated perfused hearts obtained from 9 male guinea-pigs (body wt. 596 ± 25 g) were studied under similar conditions to the rat experiments. The mean

heart weight and coronary flow were 2.1 ± 0.14 g and 17.3 ± 2.0 ml/min, respectively; therefore the coronary flow per g of tissue (8.25 ml min^{-1} g^{-1}) was similar to the values recorded in the rat hearts. AA administered by slow infusion (1 to $4\,\mu$g/min) was effective in inducing a predominant concentration-dependent coronary vasodilatation, which as in the rat, normalized several minutes after the infusion was stopped. At $2\,\mu$g/min, a dose that always elicited a coronary response, the concentration of AA, according to the actual coronary flow was approximately, $0.38\,\mu$M. In 3 experiments, AA was also administered in single bolus doses ranging from 1 to $10\,\mu$g. In these cases an immediate dose-dependent and short lasting (12–15 s) vasoconstriction was recorded before the vasodilatation occurred. The vasodilator reaction was also dose-dependent and lasted for about 3–5 min before the flow returned to normal.

Discussion

The mammalian heart synthesizes prostaglandin-like substances (PGL) (Sivakoff, Puré, Hsueh & Needleman, 1979) from the precursor arachidonic acid (AA) (Samuelsson, 1976; Lands, 1979). Among the products of AA the most effective vasodilator formed is PGI$_2$ by enzymes located in the vascular wall (Needleman et al., 1977; Hyman et al., 1978) particularly in the endothelial cells (Weksler et al., 1977; MacIntyre et al., 1978) and in the subendothelium (Silberbauer et al., 1978). Thus, an infusion of [^{14}C]-AA in protein-free media into isolated hearts results in selective labelling of arteries and, most significantly, arterioles (Hsueh & Needleman, 1978). The endothelial cells of the coronaries are important sites of PGI$_2$ formation (Bunting et al., 1976; Armstrong, Dusting, Moncada & Vane, 1978); inclusive cultured human vascular endothelial cells generate and release PGI$_2$ from endogenous precursors (Willems, Van Aken, Peuscher-Prakke, Van Mourik, Dutilh & Ten Hoor, 1978). Therefore PGL (specifically PGI$_2$) would be involved in the regulation of the coronary circulation (Dusting et al., 1977).

Our results show that slow infusions of AA usually produce a biphasic coronary response characterized by an initial vasoconstriction followed by a slow developing and long lasting vasodilatation. However, in some experiments the predominant reaction was vasoconstriction, while only vasodilatation was observed at other times. At this point we do not have a suitable explanation for this variability in the responses. The coronary reactions to AA appeared to be dose-dependent and since they were blocked by NSAA it would imply that they were due to its biotransformation into vasoactive PGL. The delayed response, particularly for the lower concentrations, may be due to the time for penetration of AA into the endothelial and subendothelial layers, its further metabolism in the vascular wall, and diffusion of the vasoactive metabolites towards the vascular smooth muscle. Obviously, in isolated hearts perfused at a constant pressure, changes in vasomotor tone affecting the coronary flow, occur if this action is exerted at the level of the resistance vessels of the microcirculation (Schaper & Schaper, 1977). Since the effects of PGL occur at the site of its synthesis (Moncada & Vane, 1978) we conclude that the administration of AA activated the formation of vasoactive metabolites in the coronaries as confirmed in rabbit isolated perfused hearts prepared with [^{14}C]-AA (Wennmalm, 1979). Furthermore, the coronary reactions obtained with AA, correlated with the release of PGL into the perfusate from rabbit and rat isolated hearts (de Deckere et al., 1977) as well as guinea-pig hearts (Schrör et al., 1978); in addition, the metabolite ceased to be released when cyclo-oxygenase inhibitors were added to the perfusate. Thus, in our experiments, the coronary responses to exogenous AA agree with the basic guidelines for evaluation of data in which synthesis of PGL are believed to be involved (Needleman, 1978).

Nevertheless, the dual expression of PGL formation, i.e. vascular smooth muscle action as well as efflux into the perfusion fluid in 'intact vascular beds' remains to be explained. It may be hypothesized that the endothelial cells where the major transformation of AA occurs (Weksler, Ley & Jaffe, 1978) would deliver the active metabolites bidirectionally: towards the vascular smooth muscle and towards the lumen of the vessels. If that were the case, the detected PGL in the coronary effluent does not necessarily represent the same substance nor its concentration acting on the vasculature. Accordingly, we agree with Parratt & Marshall's (1978) conclusion that increases in the concentration of PGL in coronary venous blood, for instance during augmented blood flow, is not necessarily a cause and effect phenomenon.

We do not have direct evidence permitting the identification of the mediators of the vasoconstriction or the vasodilatation induced by AA; however, since both phases were blocked by inhibitors of the cyclo-oxygenase (NSAA) we assume that we are dealing with different metabolites originating from the same endoperoxide intermediate. One possible mediator of the vasoconstriction may be thromboxane A$_2$ (TxA$_2$). Schrör et al. (1978) who also observed the first phase of vasoconstriction and its inhibition by NSAA do not favour the idea that TxA$_2$ generated in the vascular wall is the vasocon-

strictor agent, arguing that vascular tissue does not contain a thromboxane synthetase (Moncada, Gryglewski, Bunting & Vane, 1976; Gryglewski, Bunting, Moncada, Flower & Vane, 1976; Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976). These studies were carried out in microsomal preparations obtained from rabbit and pig aortae that would not necessarily represent the biosynthetic capabilities of other areas of the vascular system, particularly the coronaries. In fact, Nugteren, Jouvenaz & Dutilh (1978) using sensitive methods of electron capture detection found that isolated perfused rat hearts released PGI_2 (detected as 6-oxo-$PGF_{1\alpha}$) as well as a small but consistent amount of TxB_2 (hydrolytic product of TxA_2) into the perfusate. TxB_2 was also found in low concentrations in the coronary effluent from isolated rabbit and rat hearts (de Deckere et al., 1977). Although platelets are the most active in synthesizing vasoactive endoperoxides and TxA_2 (Hamberg, Svensson & Samuelsson, 1975) other tissues may also form TxB_2 following the administration of AA (Hamberg, Svensson, Hedqvist, Strandberg & Samuelsson, 1976). Similarly, pressor responses to AA in the vasculature of canine and feline lungs seem to be elicited by conversion of AA into TxA_2-like substances (Wicks et al., 1976; Kadowitz, Spannhake, Greenberg, Feigen & Hyman, 1977; Hyman, Spannhake & Kadowitz, 1980). Furthermore, in isolated strips of human umbilical cord it was demonstrated that prostaglandin endoperoxides (PGG_2 and PGH_2) are potent contractors, and that TxB_2 is also formed in this preparation (Tuvemo, Strandberg, Hamberg & Samuelsson, 1976). These investigators concluded that part of the vascular activity of the endoperoxides could be attributed to the formation of TxA_2. Furthermore, the unstable compound RCS ('rabbit aorta contracting substance', Gryglewski & Vane, 1972) was also identified as TxA_2 by Hamberg et al. (1975). When we first reported vasospasm induced by intracoronary administration of AA (Belo & Talesnik, 1980) it was also shown that cultured endothelial cells from rabbit or bovine arteries produced PGI_2 as well as TxA_2 in response to AA (Ingerman, Aharony, Silver, Smith, Nissenbaum, Sedar & Macarak, 1980). Nevertheless, Ali, Barrett & Eling (1980) presented conflicting results in which cultured endothelial cells did not synthesize TxA_2. Although the data collected do not permit us to reach a conclusion on the role of TxA_2 in the coronary constriction induced by AA, the TxA_2 generated by enzymatic conversion of PGH_2 has been shown to be a potent coronary constrictor agent in the isolated perfused working heart (Terashita, Fukui, Nishikawa, Hirata & Kikuchi, 1978) as well as in isolated perfused coronary arteries of the cat (Ogletree, Smith & Lefer, 1978). The findings of Anhut,

Bernauer & Peskar (1977, 1978) support the theory that TxA_2 may be formed in isolated blood-free perfused guinea-pig hearts. Newer and more sensitive methods of investigation have shown that TxA_2 and PGI_2 synthesis occur in distal segments of rabbit pulmonary arteries (Salzman, Salmon & Moncada, 1980).

We conclude from our experiments that the inhibition of the cyclo-oxygenase by indomethacin or naproxen would be responsible for the blockade of phases I and II of the coronary reactions to AA. The reduced production of cyclic endoperoxides (PGG_2 and PGH_2) would cause a diminished formation of the vasoconstrictor TxA_2 (Ellis, Oelz, Roberts, Payne, Sweetman, Nies & Oates, 1976) and the vasodilator PGI_2 (Dusting, Chapple, Hughes, Moncada & Vane, 1978). By focussing our attention on the coronary constriction induced by AA, we inferred that the vascular effect resulted from its biotransformation in the coronary vascular wall itself. This hypothesis is supported by the fact that in hearts perfused in the absence of blood cells or platelets, NSAA, including ASA, produced blockade when 'acutely' administered and that other fatty acids, although active on the vasculature, are unaffected by blockers of cyclo-oxygenase. Furthermore, AA also elicited coronary constriction in isolated perfused guinea-pig hearts. This constrictor effect always preceded an increase in coronary flow attributed to the release of PGI_2 that was inhibited by 15-hydroperoxyarachidonic acid (Schrör et al., 1978). The immediate vasoconstrictor effect of AA would indicate a fast response of a TxA_2 synthetase when there is a sudden availability of substrate while the cessation of vasoconstriction may be ascribed to a stoppage of TxA_2 formation and its rapid disappearance from the vascular smooth muscle since its half-life is only about $30\,s$ (Moncada & Vane, 1979). A marked and longer-lasting vasoconstriction induced by AA led to a diminished left ventricular systolic pressure most probably secondary to the relative hypoxia resulting from the diminished coronary flow. Dpy, which has been shown to inhibit the formation of TxA_2 without inhibiting the synthesis of other PGL (Ally, Manku, Horrobin, Morgan, Karmazyn & Karmali, 1977; Greenwald, Wong, Rao, Bianchine & Panganamala, 1978) would prevent AA-induced coronary constriction in our experiments. TxA_2 formation is also inactivated by ASA as was shown by the cyclo-oxygenase acetylation in platelets (Burch, Stanford & Majerus, 1978). In our experiments, low concentrations of ASA infused for a short time, produced primarily a blockade of the vasoconstriction, while longer periods of ASA administration or higher concentrations, reduced the vasodilator phase as well. The selective inhibition of coronary spasm by short lasting infusions of ASA at low con-

centrations may be attributed to the fact that only a small portion of the cyclo-oxygenase was affected by the ASA, but enough to diminish the endoperoxides that eventually would be metabolized to the vasoconstrictor TxA_2; or it may be that two distinct cyclo-oxygenases exist: one leading to TxA_2 synthesis while another would provide endoperoxides to different synthetic pathways. The latter suggestion would not be unreasonable since it has been shown that cyclo-oxygenases from platelets are more sensitive to ASA inhibition than that from vessel walls (Burch et al., 1978) as well as to other NSAA (Schrör, Sauerland, Kuhn & Rösen, 1980). Indomethacin is one of the most effective cyclo-oxygenase inhibitors and the blockade of both phases of the reactions to AA could be explained by its high potency and indiscriminate cyclo-oxygenase inhibition. The vasospastic reaction of the coronaries to AA was also blocked by Spyz, which although it may inhibit PGI_2 synthesis by endothelial cells when given at high concentrations (Gordon & Pearson, 1978) has a greater potency as a competitive inhibitor of PGL synthesis in platelets (Ali & McDonald, 1977).

Vasospasm plays an important role in producing myocardial ischaemia, angina, myocardial infarction and possibly sudden death (Braunwald, 1978; Maseri, L'Abbate, Chierchia, Parodi, Severi, Biagini, Distante, Marzilli & Ballestra, 1979) and suggests that coronary synthesized TxA_2 may be implicated in the pathogenesis of coronary vasospasm, independently of, or superimposed upon, other pathological conditions. The role of TxA_2 as a vasospastic agent leading to coronary insufficiency has been examined in humans and it was shown that TxB_2 in the coronary sinus increased during angina pectoris electrically induced by tachycardia (Lewy, Wiener, Walinsky, Lefer, Silver & Smith, 1980). The coronary constriction would be located, particularly at the level of the arterioles, as it has been demonstrated in the hamster cheek pouch by the administration of the prostaglandin endoperoxide, PGH_2 (Higgs, Cardinal, Moncada & Vane, 1979). The response of the arterioles to PGH_2 was also biphasic with a long-lasting vasodilatation often preceded by a vasoconstriction, but contrary to our hypothesis, Higgs et al. (1979) suggest that PGH_2 would have a direct contractor effect on the vasculature of the arterioles. Nevertheless, AA or PGI_2 may not produce similar effects on the microcirculation in all species, or vascular areas. For instance Messina, Rodenburg, Slomiany, Roberts, Hintze & Kaley (1980) found that in the rat, AA and PGH_2 produced only vasodilatation in the cremaster microcirculation.

Another aspect of the coronary response to AA deserving comment is the prolonged duration of the vasodilator phase. This observation concurs with Higgs et al.'s (1979) report that single injections of

PGI_2, PGE_2 and PGH_2 produced a long-lasting dilatation (3–13 min). In our experiments we found that single doses of authentic PGI_2 produced an immediate and prolonged vasodilatation, in agreement with results of recent investigations in rat hearts (de Deckere, 1979; Schrör, Link, Rösen, Klaus & Rösen, 1980). The rapid vasoconstrictor reaction of the vascular smooth muscle to AA or the vasodilator response to PGI_2, would indicate that the penetration of these substances through the vascular wall occurs at a very fast rate. Furthermore, the AA pathway leading to the formation of a constrictor metabolite would react at a faster rate than the system leading to the vasodilator product since the latter has a longer latency and develops slowly before it reaches its maximal effect. The mechanism explaining the sustained vasodilatation induced by AA or PGI_2 is unknown, but we think that this observation deserves further investigation.

AA administered to hearts from rats pretreated with NSAA produced coronary responses indistinguishable from that of untreated control hearts. This was a similar situation to that in which NSAA withdrawal after the blockade of AA effects was followed by full recovery of both phases of the coronary response. The recovery of the vascular response to AA does not conform with the concept that the majority of the NSAA are 'irreversible inhibitors' (Smith & Lands, 1971; Raz, Stern & Kenig-Wakshal, 1973); however, synthetases in different locations or under varying experimental conditions may show reversibility (Hornstra, Haddeman & Don, 1979). Accordingly, NSAA infusion may produce a reversible blockade of the cyclo-oxygenase only at the level of the coronary vessels where the enzyme system would be more accessible and the rate of penetration of the drug would be faster. The myocardial cell synthetase on the other hand requires NSAA pretreatment for inhibition and acute perfusion of the heart with NSAA is ineffective in producing blockade of the enzyme system. Thus the inhibitor requires time to penetrate and reach sufficient concentrations in order to block the cyclo-oxygenase system within the myocytes. This inhibition would be 'irreversible' and prolonged AA administration would fail to induce the formation of PGL in the myocardial cell and the MCD response, which otherwise would be inhibited by the sustained supply of AA with the perfusate, would persist (Sunahara & Talesnik, 1979). Reversibility of the vascular cyclo-oxygenase, when the blood levels of available NSAA diminish would have important clinical implications (Rane, Oelz, Frolich, Seyberth, Sweetman, Watson, Wilkinson & Oates, 1978).

We postulated that the prolonged administration of PGE_2 (Sen et al., 1977) or continuous infusion of AA producing inhibition of the metabolic coronary

dilatation (MCD) following cardiostimulation, may be due to inhibition of the adenylate cyclase in the myocardial cell by PGE_2 synthesized in this compartment (Sunahara & Talesnik, 1979). It has recently been confirmed that rat heart myocytes synthesize PGE_2, but do not form PGI_2 nor TxA_2 (Ahumada, Sobel & Needleman, 1980). Thus, if the prostaglandin synthetase in the myocardial cell is blocked by NSAA pretreatment, further addition of AA to the perfused heart would fail to induce endogenous PGL formation and therefore the MCD reactions would remain unaffected, preventing the induction of 'coronary insufficiency'. Once the cyclo-oxygenase in the myocytes is 'irreversibly' inhibited (Smith & Lands, 1971), the modulation of the adenylate cyclase by endogenously synthesized PGE_2 would also be suppressed. Although the actual process that links the increase in myocardial cyclic AMP to the vascular smooth muscle relaxation remains unknown, it appears that Vapaatalo, Parantainen, Metsä-Ketelä & Kangasaho's (1978) statement that 'the possible interactions and relationships between these two systems (cyclic nucleotides and prostaglandins) have not been an object of very active interest' is not entirely accurate.

Our working hypothesis appears to conflict with the results obtained in dogs by Harlan, Rooke, Belloni & Sparks (1978) in which indomethacin, at a concentration that reduced the coronary responses to AA, failed to alter the coronary flow responses produced by an infusion of isoprenaline. The conclusion that MCD is not influenced by arachidonic acid metabolites would be expected from Harlan et al.'s (1978) experimental design since it is equivalent to our experiments in which acute NSAA produced blockade of AA actions by inhibition of prostaglandin-synthetase at the level of the vascular and not the myocardial cell compartment.

We concluded in previous experiments that post-occlusive reactive hyperaemia appeared to be independent of PGL formation, based on responses obtained in hearts from untreated versus NSAA pretreated rats (Sunahara & Talesnik, 1979). In the present experiments, reactive hyperaemia was investigated in hearts receiving continuous NSAA with the perfusion fluid. Although in the present experiments PGL synthesis is greatly inhibited, the post-occlusive hyperaemia was indistinguishable from the controls. Therefore, the coronary vasodilatation due to short lasting coronary occlusion apparently is unrelated to the formation of PGL in the heart and is a process independent of those involved in MCD. This would be in conflict with Wennmalm's (1979) contention that the increased coronary vascular formation of PGI_2 would be responsible for the coronary dilatation induced by prolonged hypoxia.

Since AA significantly decreased in a dose-dependent manner the blood pressure, we confirmed the demonstration of Gerber & Nies (1979) that this action was due to a decrease in total peripheral resistance. The inhibition of PGL synthesis by indomethacin prevented the fall in blood pressure induced by AA, without affecting the actions of PGE_2 or PGI_2. Therefore, we are confident that the effects elicited in the rat coronaries by AA were due to its biotransformation into vasoactive substances during a single passage through the coronary circulation.

Regarding species differences in the coronary reactions of isolated perfused hearts, experiments were carried out in guinea-pigs. The guinea-pig heart has a greater sensitivity to AA, which usually produced a dose-dependent vasodilatation when given by slow infusion. Thus, while in the rat the mean effective concentration was about $0.74\,\mu M$, in the guinea-pig heart equivalent effects were obtained with approximately $0.38\,\mu M$. If the concentrations were raised several fold, as in a single bolus dose, then the phase of vasoconstriction was also obtained. The latter confirms results obtained by Shrör et al. (1978), in which guinea-pig hearts, perfused at a constant rate (10 ml/min), received $1-50\,\mu g$ of AA in single doses.

Although the formation of PGI_2 by the vascular wall has been extensively documented (Bunting et al., 1976; Needleman et al., 1977; McIntyre et al., 1978), its function in regulating coronary blood flow under physiological conditions remains questionable (Parratt & Marshall, 1978; Sunahara & Talesnik, 1979).

In summary, we postulate that the coronary vasoconstriction elicited by AA is due to its biotransformation into a thromboxane A_2-like substance by the vessel walls themselves. The TxA_2-like synthetase could be selectively inhibited by Dpy, Spyz or low concentrations of ASA. These data do not preclude the importance of platelet synthesized TxA_2 acting as a pro-aggregatory factor involved, for instance, in vascular injuries (Srivastava, 1978; Gorman, 1979; Bourgain, Andries & Finne, 1979). However, the vascular synthesis of TxA_2 may further aggravate the compromised coronary flow by inducing vasoconstriction.

We postulate that from a metabolic point of view, at least two types of coronary insufficiencies may originate: (1) in the myocardial cell compartment, in which increased levels of PGE_2 would block MCD by diminishing the adenylate cyclase activation, and subsequently reducing the levels of cyclic AMP that would trigger the actual mechanism inducing the MCD reaction (Sen et al., 1976; Sen, Sunahara, Talesnik & Endrenyi, 1977) and (2) in the coronary vascular compartment, in which an unopposed TxA_2-like substance, synthesized by the vascular wall, could induce a vasospastic condition. Thus, TxA_2

may compromise the coronary flow when its local synthesis is exaggerated.

References

AHUMADA, G.G., SOBEL, B.E. & NEEDLEMAN, P. (1980). Synthesis of prostaglandins by cultured rat heart myocytes and cardiac mesenchymal cells. *J. mol. cell. Cardiol.*, **12**, 685–700.

ALI, A.E., BARRETT, J.C. & ELING, T.E. (1980). Prostaglandin and thromboxane production by fibroblasts and vascular endothelial cells. *Prostaglandins*, **20**, 667–688.

ALI, M. & McDONALD, J.W.D. (1977). Effects of sulfinpyrazone on platelet prostaglandin synthesis and platelet release of serotonin. *J. lab. Clin. Med.*, **89**, 868–875.

AL-UBAIDI, F. & BAKHLE, Y.S. (1980). Differences in biological activation of arachidonic acid in perfused lungs from guinea-pig, rat and man. *Eur. J. Pharmac.*, **62**, 89–96.

ALLY, A.I., MANKU, M.S., HORROBIN, D.F., MORGAN, R.O., KARMAZYN, M. & KARMALI, R.A. (1977). Dipyridamole: A possible potent inhibitor of thromboxane A_2 synthetase in vascular smooth muscle. *Prostaglandins*, **14**, 607–609.

ANHUT, H., BERNAUER, W. & PESKAR, B.A. (1977). Radioimmunological determination of thromboxane release in cardiac anaphylaxis. *Eur. J. Pharmac.*, **44**, 85–88.

ANHUT, H., BERNAUER, W. & PESKAR, B.A. (1978). Pharmacological modification of thromboxane and prostaglandin release in cardiac anaphylaxis. *Prostaglandins*, **15**, 889–900.

ARMSTRONG, J.M., DUSTING, G.J., MONCADA, S. & VANE, J.R. (1978). Cardiovascular actions of prostacyclin (PGI_2), a metabolite of arachidonic acid which is synthesized by blood vessels. *Circulation Res.*, Suppl. I, **43**, I-112–I-119.

BELO, S. & TALESNIK, J. (1980). Coronary spasm induced by arachidonic acid metabolites formed in isolated perfused hearts. *Fedn. Proc.*, **39**, Part II: 1003.

BLEEHAN, N.M. & FISHER, R.B. (1954). The action of insulin in the isolated rat heart. *J. Physiol.*, **123**, 260–276.

BOURGAIN, R.H., ANDRIES, R. & FINNE, E. (1979). The role of prostaglandins in platelet-vessel wall interaction. *Archs int. Pharmacodyn.*, **239**, 161–163.

BRAUNWALD, E. (1978). Coronary spasm and acute myocardial infarction – New possibility for treatment and prevention. *New Engl. J. Med.*, **299**, 1301–1303.

BUNTING, S., GRYGLEWSKI, R., MONCADA, S. & VANE, J.R. (1976). Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins*, **12**, 897–913.

BURCH, J.W., STANFORD, N. & MAJERUS, P.W. (1978). Inhibition of platelet prostaglandin synthetase by oral aspirin. *J. clin. Invest.*, **61**, 314–319.

CARLSON, T.H., WENTLAND, S.H., LEONARD, B.D., RUDER, M.A. & REEVE, E.B. (1978). Effects of prostaglandins E_2, analogs, fatty acids, and indomethacin on fibrinogen level. *Am. J. Physiol.*, **235**, H223–H230.

DE DECKERE, E.A.M. (1979). Effects of prostaglandins on coronary flow rate and left ventricular work in isolated rat heart. *Eur. J. Pharmac.*, **58**, 211–213.

DE DECKERE, E.A.M., NUGTEREN, D.H. & TEN HOOR, F. (1977). Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart. *Nature*, **268**, 160–163.

DE KOCK, A., LOCHNER, A., KOTZÉ, J.C.N. & GEVERS, W. (1978). The hypoxic, low-flow perfused rat heart: Characterization as a model of global ischaemia. *Basic Res. Cardiol.*, **73**, 506–522.

DRAKE, A.J., HAINES, J.R. & NOBLE, M.I.M. (1980). Preferential uptake of lactate by normal myocardium in dogs. *Cardiovasc. Res.*, **14**, 65–72.

DUSTING, G.J., MONCADA, S. & VANE, J.R. (1977). Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. *Prostaglandins*, **13**, 3–15.

DUSTING, G.J., CHAPPLE, D.J., HUGHES, R., MONCADA, S. & VANE, J.R. (1978). Prostacyclin (PGI_2) induces coronary vasodilatation in anaesthetized dogs. *Cardiovasc. Res.*, **12**, 720–730.

DUSTING, G.J. & VANE, J.R. (1980). Some cardiovascular properties of prostacyclin (PGI_2) which are not shared by PGE_2. *Circulation Res.*, **46**: Suppl. I, I-183–I-187.

ELLIS, E.F., OELZ, O., ROBERTS, II, L.J., PAYNE, N.A., SWEETMAN, B.J., NIES, A.S. & OATES, J.A. (1976). Coronary artery smooth muscle contraction by a substance released from platelets: evidence that it is thromboxane A_2. *Science*, **193**, 1135–1137.

FALLEN, E.L., ELLIOTT, W.C. & GORLIN, R. (1967). Apparatus for study of ventricular function and metabolism in the isolated perfused rat heart. *J. appl. Physiol.*, **22**, 836–839.

FLOWER, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. *Pharmac. Rev.*, **26**, 33–67.

GERBER, J.G. & NIES, A.S. (1979). The hemodynamic effects of prostaglandins in the rat. Evidence for important species variation in renovascular responses. *Circulation Res.*, **44**, 406–410.

GORDON, J.L. & PEARSON, J.D. (1978). Effects of sulphinpyrazone and aspirin on prostaglandin I_2 (prostacyclin) synthesis by endothelial cells. *Br. J. Pharmac.*, **64**, 481–483.

GORMAN, R.R. (1979). Modulation of human platelet function by prostacyclin and thromboxane A_2. *Fedn. Proc.*, **38**, 83–88.

GREENWALD, J.E., WONG, L.K., RAO, M., BIANCHINE, J.R. & PANGANAMALA, R.V. (1978). A study of three vasodilating agents as selective inhibitors of thromboxane A_2 biosynthesis. *Biochem. biophys. Res. Commun.*, **84**, 1112–1118.

GRYGLEWSKI, R.J. & VANE, J.R. (1972). The release of prostaglandins and rabbit aorta contracting substance (RCS) from rabbit spleen and its antagonism by anti-inflammatory drugs. *Br. J. Pharmac.*, **45**, 37–47.

GRYGLEWSKI, R.J., BUNTING, S., MONCADA, S., FLOWER, R.J. & VANE, J.R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins*, **12**, 685–713.

HAMBERG, M., SVENSSON, J. & SAMUELSSON, B. (1975). Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. natn. Acad. Sci., U.S.A.*, **72**, 2994–2998.

HAMBERG, M., SVENSSON, J., HEDQVIST, P., STRANDBERG, K. & SAMUELSSON, B. (1976). Involvement of endoperoxides and thromboxanes in anaphylactic reactions. In *Advances in Prostaglandins and Thromboxane Research*, Vol. 1. ed. Samuelsson, B. & Paoletti, R. pp. 495–501. New York: Raven Press.

HARLAN, D.M., ROOKE, T.W., BELLONI, F.L. & SPARKS, H.V. (1978). Effect of indomethacin on coronary vascular response to increased myocardial oxygen consumption. *Am. J. Physiol.*, **235**, H372–H378.

HIGGS, G.A., CARDINAL, D.C., MONCADA, S. & VANE, J.R. (1979). Microcirculatory effects of prostacyclin (PGI_2) in the hamster cheek pouch. *Microvasc. Res.*, **18**, 245–254.

HINTZE, T.H. & KALEY, G. (1977). Prostaglandins and the control of blood flow in the canine myocardium. *Circulation Res.*, **40**, 313–320.

HORNSTRA, G., HADDEMAN, E. & DON, J.A. (1979). Blood platelets do not provide endoperoxides for vascular prostacyclin production. *Nature*, **279**, 66–68.

HSUEH, W. & NEEDLEMAN, P. (1978). Sites of lipase activation and prostaglandin synthesis in isolated, perfused hearts and hydronephrotic kidneys. *Prostaglandins*, **16**, 661–681.

HYMAN, A.L., KADOWITZ, P.J., LANDS, W.E.M., CRAWFORD, C.G., FRIED, J. & BARTON, J. (1978). Coronary vasodilatory activity of 13,14-dehydroprostacyclin methylester: comparison with prostacyclin and other prostanoids. *Proc. natn. Acad. Sci., U.S.A.*, **75**, 3522–3526.

HYMAN, A.L., SPANNHAKE, E.W. & KADOWITZ, P.J. (1980). Divergent responses to arachidonic acid in the feline pulmonary vascular bed. *Am. J. Physiol.*, **239**, H40–H46.

INGERMAN, C.M., AHARONY, D., SILVER, M.J., SMITH, J.B., NISSENBAUM, M., SEDAR, A.W. & MACARAK, E. (1980). Prostaglandin I_2 and thromboxane A_2 can be produced by endothelial cells in situ and in culture. *Fedn. Proc.*, **39**, Part I: 391.

ISAKSON, P.C., RAZ, A., DENNY, S.E., PURÉ, E. & NEEDLEMAN, P. (1977). A novel prostaglandin is the major product of arachidonic acid metabolism in rabbit heart. *Proc. natn. Acad. Sci., U.S.A.*, **74**, 101–105.

JOHNSON, R.A., MORTON, D.R., KINNER, J.H., GORMAN, R.R., McGUIRE, J.C., SUN, F.F., WHITTAKER, N., BUNTING, S., SALMON, J., MONCADA, S. & VANE, J.R. (1976). The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins*, **12**, 915–928.

KADOWITZ, P.J., SPANNHAKE, E.W., GREENBERG, S., FEIGEN, L.P. & HYMAN, A.L. (1977). Comparative effects of arachidonic acid, bisenoic prostaglandins, and an endoperoxide analog on the canine pulmonary vascular bed. *Can. J. Physiol. Pharmac.*, **55**, 1369–1377.

KALSNER, S. (1975). Endogenous prostaglandin release contributes directly to coronary artery tone. *Can. J. Physiol. Pharmac.*, **53**, 560–565.

KULKARNI, P.S., ROBERTS, R. & NEEDLEMAN, P. (1976). Paradoxical endogenous synthesis of a coronary dilating substance from arachidonate. *Prostaglandins*, **12**, 337–353.

LANDS, W.E.M. (1979). The biosynthesis and metabolism of prostaglandins. *A. Rev. Physiol.*, **41**, 633–652.

LEWY, R.I., WIENER, L., WALINSKY, P., LEFER, A.M., SILVER, M.J. & SMITH, J.B. (1980). Thromboxane release during pacing-induced angina pectoris: possible vasoconstrictor influence on the coronary vasculature. *Circulation*, **61**, 1165–1171.

LIMAS, C.J. & COHN, J.N. (1973). Isolation and properties of myocardial prostaglandin synthetase. *Cardiovasc. Res.*, **7**, 623–628.

MACINTYRE, D.E., PEARSON, J.D. & GORDON, J.L. (1978). Localization and stimulation of prostacyclin production in vascular cells. *Nature*, **271**, 549–551.

MASERI, A., L'ABBATE, A., CHIERCHIA, S., PARODI, O., SEVERI, S., BIAGINI, A., DISTANTE, A., MARZILLI, M. & BALLESTRA, A.M. (1979). Significance of spasm in the pathogenesis of ischemic heart disease. *Am. J. Cardiol.*, **44**, 788–792.

MENTZ, P., BLASS, K.-E. & FÖRSTER, W. (1976). Effects of prostaglandin precursors and other unsaturated fatty acids on coronary flow and cardiac action in vivo and in vitro. *Acta biol. med. germ.*, **35**, 1159–1160.

MENTZ, P. & FÖRSTER, W. (1977). The influence of unsaturated fatty acids on prostaglandin release in isolated perfused guinea-pig hearts. *Prostaglandins*, **14**, 173–179.

MESSINA, E.J., RODENBURG, J., SLOMIANY, B.L., ROBERTS, A.M., HINTZE, T.H. & KALEY, G. (1980). Microcirculatory effects of arachidonic acid and a prostaglandin endoperoxide (PGH_2). *Microvasc. Res.*, **19**, 288–296.

MINKES, M.S., DOUGLAS, Jr., J.R. & NEEDLEMAN, P. (1973). Prostaglandin release by the isolated perfused rabbit heart. *Prostaglandins*, **3**, 439–445.

MONCADA, S., GRYGLEWSKI, R., BUNTING, S. & VANE, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*, **263**, 663–665.

MONCADA, S. & VANE, J.R. (1978). Prostacyclin (PGI_2), the vascular wall and vasodilatation. In: *Mechanisms of Vasodilatation*. Satellite Symp. *27th Int. Congr. Physiol. Sci.* (Wilrijk 1977), pp. 107–121. Basel: Karger.

MONCADA, S. & VANE, J.R. (1979). Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A$_2$, and prostacyclin. *Pharmac. Rev.*, **30**, 293–331.

NEEDLEMAN, P. (1976). The synthesis and function of prostaglandins in the heart. *Fedn. Proc.*, **35**, 2376–2381.

NEEDLEMAN, P., RAZ, A., KULKARNI, P.S., PURÉ, E., WYCHE, A., DENNY, S.E. & ISAKSON, P.C. (1977). Biological and chemical characterization of a unique endogenous vasodilator prostaglandin produced in isolated coronary artery and in intact perfused heart. In *Biochemical Aspects of Prostaglandins and Thromboxanes.* ed. Kharasch, N. & Fried, J. pp. 199–214. New York: Academic Press.

NEEDLEMAN, P. (1978). Experimental criteria for evaluating prostaglandin biosynthesis and intrinsic function. *Biochem. Pharmac.*, **27**, 1515–1518.

NUGTEREN, D.H., JOUVENAZ, G.H. & DUTILH, C.E. (1978). Determinations of prostaglandins and other products of arachidonate oxygenation in perfusates and during platelet aggregation. *Acta biol. med. germ.*, **27**, 701–706.

OGLETREE, M.L., SMITH, J.B. & LEFER, A.M. (1978). Actions of prostaglandins on isolated perfused cat coronary arteries. *Am. J. Physiol.*, **235**, H400–H406.

OPIE, L.H. (1965). Effect of extracellular pH on function and metabolism of isolated perfused rat heart. *Am. J. Physiol.*, **209**, 1075–1080.

OWEN, T.L., EHRHART, I.C., WEIDNER, W.J., SCOTT, J.B. & HADDY, F.J. (1975). Effects of indomethacin on local blood flow regulation in canine heart and kidney. *Proc. Soc. exp. Biol. Med.*, **149**, 871–876.

PARRATT, J.R. & MARSHALL, R.J. (1978). Are prostaglandins involved in the regulation of coronary blood flow? A review of the evidence. *Acta biol. med. germ.*, **37**, 747–760.

RANE, A., OELZ, O., FROLICH, J.C., SEYBERTH, H.W., SWEETMAN, B.J., WATSON, J.T., WILKINSON, G.R. & OATES, J.A. (1978). Relation between plasma concentration of indomethacin and its effect on prostaglandin synthesis and platelet aggregation in man. *Clin. Pharmac. Ther.*, **23**, 658–668.

RAZ, A., STERN, H. & KENIG-WAKSHAL, R. (1973). Indomethacin and aspirin inhibition of prostaglandin E$_2$ synthesis by sheep seminal vesicles microsome powder and seminal vesicle slices. *Prostaglandins*, **3**, 337–352.

SALZMAN, P.M., SALMON, J.A. & MONCADA, S. (1980). Prostacyclin and thromboxane A$_2$ synthesis by rabbit pulmonary artery. *J. Pharmac. exp. Ther.*, **215**, 240–247.

SAMUELSSON, B. & WENNMALM, Å. (1971). Increased nerve stimulation reduced release of noradrenaline from the rabbit heart after inhibition of prostaglandin synthesis. *Acta physiol. scand.*, **83**, 163–168.

SAMUELSSON, B. (1976). Prostaglandin endoperoxides and thromboxanes: Role in platelets and in vascular and respiratory smooth muscle. *Acta biol. med. germ.*, **35**, 1055–1063.

SCHAPER, W. & SCHAPER, J. (1977). The coronary microcirculation. *Am. J. Cardiol.*, **40**, 1008–1012.

SCHRÖR, K., MONCADA, S., UBATUBA, F.B. & VANE, J.R. (1978). Transformation of arachidonic acid and prostaglandin endoperoxides by the guinea pig heart. Formation of RCS and prostacyclin. *Eur. J. Pharmac.*, **47**, 103–114.

SCHRÖR, K., LINK, H.-B., RÖSEN, R., KLAUS, W. & RÖSEN, P. (1980). Prostacyclin induced coronary vasodilatation. Interactions with adenosine, cyclic AMP and energy charge in the rat heart in vitro. *Eur. J. Pharmac.*, **64**, 341–348.

SCHRÖR, K., SAUERLAND, S., KUHN, A. & RÖSEN, R. (1980). Different sensitivities of prostaglandin-cyclooxygenases in blood platelets and coronary arteries against non-steroidal anti-inflammatory drugs. *Naunyn-Schmiedebergs Arch. Pharmac.*, **313**, 69–76.

SEN, A.K., SUNAHARA, F.A. & TALESNIK, J. (1976). Prostaglandin E$_2$ and cyclic-AMP in the coronary vasodilatation due to cardiac hyperactivity. *Can. J. Physiol. Pharmac.*, **54**, 128–139.

SEN, A.K., SUNAHARA, F.A. & TALESNIK, J. (Addenda by ENDRENYI, L) (1977). Coronary reactions to cardiac hyperactivity and to hypoxia in isolated perfused heart of rat. *Br. J. Pharmac.*, **61**, 381–393.

SILBERBAUER, K., SINZINGER, H., WINTER, M., FEIGL, W. & RING, F. (1978). Prostacyclin-like activity of endothelium and subendothelium – important for atherosclerosis? *Experientia*, **34**, 1471–1472.

SIVAKOFF, M., PURÉ, E., HSUEH, W. & NEEDLEMAN, P. (1979). Prostaglandins and the heart. *Fedn. Proc.*, **38**, 78–82.

SMITH, W.L. & LANDS, W.E.M. (1971). Stimulation and blockade of prostaglandin biosynthesis. *J. biol. Chem.*, **246**, 6700–6704.

SRIVASTAVA, K.C. (1978). Arachidonic acid as a precursor in the production of substances necessary for platelet aggregation and its control. *Danish Med. Bull.*, **25**, 130–133.

SUNAHARA, F.A. & TALESNIK, J. (1979). Myocardial synthesis of prostaglandin-like substances and coronary reactions to cardiostimulation and to hypoxia. *Br. J. Pharmac.*, **65**, 71–85.

TERASHITA, Z.-I., FUKUI, H., NISHIKAWA, K., HIRATA, M. & KIKUCHI, S. (1978). Coronary vasospastic action of thromboxane A$_2$ in isolated, working guinea pig hearts. *Eur. J. Pharmac.*, **53**, 49–56.

TUVEMO, T., STRANDBERG, K.H., HAMBERG, M. & SAMUELSSON, B. (1976). Formation and action of prostaglandin endoperoxides in the isolated human umbilical artery. *Acta physiol. scand.*, **96**, 145–149.

VAPAATALO, H., PARANTAINEN, J., METSÄ-KETELÄ, T. & KANGASAHO, M. (1978). Prostaglandins and cyclic nucleotides in cardiac function. *Pol. J. Pharmac. Pharm.*, **30**, 195–213.

WEISSLER, A.M., ATSCHULD, R.A., GIBB, L.E., POLLACK, M.E. & KRUGER, F.A. (1973). Effect of insulin on the performance and metabolism of the anoxic isolated perfused rat heart. *Circulation Res.*, **32**, 108–116.

WEKSLER, B.B., LEY, C.W. & JAFFE, E.A. (1978). Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and ionophore A23187. *J. clin. Invest.*, **62**, 923–930.

WEKSLER, B.B., MARCUS, A.J. & JAFFE, E.A. (1977). Synthesis of prostaglandin I$_2$ (prostacyclin) by cultured human and bovine endothelial cells. *Proc. natn. Acad. Sci., U.S.A.*, **74**, 3922–3926.

WENNMALM, Å. (1979). Prostacyclin-dependent coronary vasodilatation in rabbit and guinea-pig hearts. *Acta physiol. scand.*, **106,** 47–52.

WICKS, T.C., ROSE, J.C., JOHNSON, M., RAMWELL, P.W. & KOT, P.A. (1976). Vascular responses to arachidonic acid in the perfused canine lung. *Circulation Res.*, **38,** 167–171.

WILLEMS, C.H., VAN AKEN, W.G., PEUSCHER-PRAKKE, E.M., VAN MOURIK, J.A., DUTILH, C. & TEN HOOR, F. (1978). Prostaglandin I_2 (prostacyclin) production by cultured human vascular endothelial cells in the absence of platelets. *J. mol. Med.*, **3,** 195–201.

ZACHARIAH, P. (1961). Contractility and sugar permeability in the perfused rat heart. *J. Physiol.*, **158,** 59–72.