Br. J. Pharmac. (1976), **58**, 89–100

THE RELEASE OF A CORONARY VASODILATOR METABOLITE FROM THE GUINEA-PIG ISOLATED PERFUSED HEART STIMULATED BY CATECHOLAMINES, HISTAMINE AND ELECTRICAL PACING AND BY EXPOSURE TO ANOXIA

K.J. BROADLEY

Department of Applied Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, King Edward VII Avenue, Cardiff CF1 3NU

1 A procedure involving two guinea-pig isolated hearts perfused in series is described for detecting in the recipient heart the release of a possible coronary vasodilator metabolite from the donor heart.

2 Adrenaline and isoprenaline stimulated the rate and force of contraction and produced a multiphasic coronary vascular response, the predominant phase of which was vasodilatation. When the β-adrenoceptors of the recipient heart were blocked, stimulation of the donor heart by the catecholamines was associated with a coronary vasodilatation of the recipient heart.

3 Histamine stimulated rate and force of contraction and was predominantly coronary vasodilator. After blockade of histamine H_1- and H_2-receptors in the recipient heart, coronary vasodilatation followed increases in activity of the donor heart in response to histamine.

4 These vasodilator responses of the recipient heart were attributed to the release from the donor heart of a vasodilator metabolite by the increased activity. This is the proposed mechanism for the predominant coronary vasodilator response to catecholamines and histamine.

5 Periods of electrically-paced tachycardia and anoxia of the donor heart also led to the release of vasodilator activity.

6 The possible identity of the metabolite is discussed.

Introduction

The overall coronary vascular response to catecholamines is vasodilatation (Wégria, 1951; Charlier, 1961; Parratt, 1968; Dempsey & Cooper, 1972) which is comprised of several components arising from both direct and indirect effects. The direct effects on the coronary vasculature yield both vasoconstriction mediated via α-adrenoceptors (Saito, 1959; Kaverina, 1965; Parratt, 1967; Broadley, 1970) and vasodilatation mediated via β-adrenoceptors differing from those of the myocardium (Adam, Boyles & Scholfield, 1970; Ross & Jorgensen, 1970; Bayer, Mentz & Förster, 1972) and therefore assigned to the β_2-type (Broadley, 1970; Parratt & Wadsworth, 1970; Mark, Abboud, Schmid, Heistad & Mayer, 1972; Ross, 1974). Others however disagree and claim that these resemble the β_1-adrenoceptors of the myocardium (Lucchesi & Hodgeman, 1971; Drew & Levy, 1972). The indirect effects result from the simultaneous positive inotropic and chronotropic responses, both of which restrict coronary flow by extravascular compression of the coronary vessels (Melville & Lu, 1950; Douglas, Armengol & Talesnik, 1960). The second indirect effect may account for the predominant vasodilator phase of the coronary vascular response. This component only occurs when there are significant changes in the rate and force of contraction and is reduced when these myocardial responses are antagonized (Hashimoto, Shigei, Imai, Saito, Yago, Uei & Clark, 1960; Siegal, Gilmore & Sarnoff, 1961). It is also prevented by metabolic inhibitors such as cyanide (Garcia-Ramos, Alanis & Luco, 1950) and fluoracetate (Saito, 1959) and is therefore attributed to the increased metabolic activity of the heart (Green, Wégria & Boyer, 1942; Melville & Lu, 1950; Douglas *et al.*, 1960; Hashimoto *et al.*, 1960; Hardin, Scott & Haddy, 1961; Siegal *et al.*, 1961; Berne, 1964). It has been suggested that a vasodilator metabolite is released by the myocardium during the increased activity (Berne, 1964; Haddy & Scott, 1968). Attempts to demonstrate such a metabolite in the venous outflow of hearts stimulated by adrenaline have been few, possibly because of interference by the simultaneous appearance of the adrenaline. Only the nucleoside, adenosine, has been

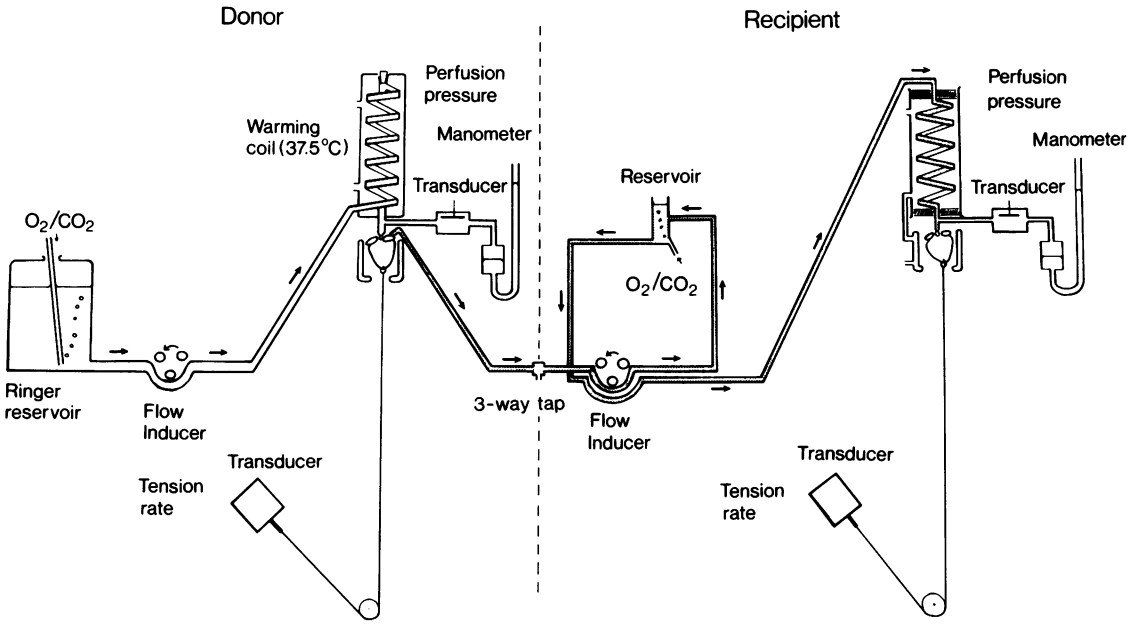

Figure 1 Schematic diagram of the apparatus used to perfuse two guinea-pig isolated hearts in series, with the recipient heart served either with fresh Krebs-bicarbonate solution or the perfusate from the donor heart. Perfusion pressure and rate and force of contractions were recorded in each heart.

successfully detected (Katori & Berne, 1966). Most work to substantiate the claim of a putative mediator has in fact been based upon the modification of the vasodilatation by substances known to affect the proposed substance. In the case of adenosine this evidence tended to disprove its role since dipyridamole, an inhibitor of its uptake, failed to potentiate the vasodilatation to catecholamines (Parratt & Wadsworth, 1972).

The present study was undertaken to develop a technique whereby the presence of a vasodilator metabolite following cardiac stimulation by catecholamines could be demonstrated pharmacologically and that it indeed had coronary vasodilator activity. The principle of the procedure was to take the perfusate from an isolated perfused heart to supply a second heart which would thus respond to the presence of any released substance(s). The choice of an alternative drug with which to stimulate the heart was virtually limited to histamine by the availability of an effective antagonist of the myocardial and coronary vascular responses of the drug in the recipient heart.

The myocardial effects of histamine may be abolished by H_2-receptor antagonists such as burimamide (Black, Duncan, Durant, Ganellin & Parsons, 1972; McNeill & Verma, 1974; Levi, Capurro & Lee, 1975). These have also been shown to antagonize the coronary vasodilatation (Ercan, Bökesoy & Türker, 1974) which presumably arises indirectly from the increased myocardial activity (Broadley, 1975a). However, histamine also exerts direct vasodilator and constrictor effects upon the coronary vasculature and these are mediated via H_1-receptors (Broadley, 1975a). This may account for the demonstration by others of antagonism of the overall coronary vasodilatation by mepyramine (Levi & Kuye, 1974). Therefore both antagonists were required to prevent the effects of histamine in the recipient heart.

To avoid the problems of contamination of the perfusate by the agonist, increased activity of the donor heart was also induced by electrically-paced tachycardia. Finally, in view of the possibility that the increased myocardial activity produces a relative anoxia which may serve as an intermediate to the release of a metabolically-linked vasoactive metabolite (Berne, 1958), experiments were performed in which the donor heart was exposed to periods of anoxia to determine whether this too would release a vasodilator metabolite. A preliminary account of the technique used has been presented to the British Pharmacological Society (Broadley, 1975b).

Methods

Guinea-pigs of either sex and weight range 275–675 g were killed by a blow on the head and the hearts and

lungs together were rapidly excised and transferred to a beaker containing Krebs-bicarbonate solution. Donor hearts were set up initially. A small incision was made in the aorta which was tied onto a glass cannula for retrograde perfusion of the coronary vessels by a modified (Broadley, 1970) Langendorff (1895) method (Figure 1). The venae cavae and pulmonary artery and vein were tied off and only then were the lungs trimmed free. The pulmonary artery was cut which thus provided the only route of escape for the perfusate. A constant rate (5–6 ml/min) of perfusion was achieved by means of a Watson-Marlow flow inducer (MHRE/30/T, 0.5 mm tube bore). The Krebs-bicarbonate solution, having the following composition in g/l distilled water; NaCl 6.92, KCl 0.345, $CaCl_2 2H_2O$ 0.28, $NaHCO_3$ 2.1, $MgSO_4 7H_2O$ 0.29, glucose 2.0, $NaH_2PO_4 2H_2O$ 0.16, was gassed with 5% CO_2 in O_2 before passing through the flow inducer to the warming coil at the base of which was the cannulated aorta. Alterations in perfusion pressure (resting level between 25 and 50 mmHg; 1 mmHg $\approx$ 133 Pa) arising from changes in coronary vascular resistance were recorded on a Devices M19 polygraph by means of a pressure transducer (Bell & Howell, Type 4-327-L221) situated between the flow inducer and warming coil. Also located at this point was a Condon manometer.

Isometric contractions were recorded with a transducer (Ether, Type UF1, 114 g sensitivity range) attached via a pulley to a clip on the apex of the ventricles. The transducer was adjusted to apply a resting diastolic tension of 1–2 g on the heart. Rate of contraction was recorded by a ratemeter (Devices, Type 2751) triggered by the signal from the tension trace.

Drugs were injected into the perfusion solution through the connecting rubber tubing immediately prior to entry into the warming coil. The dose volume was between 0.025 and 0.2 ml and this produced a small injection artefact which was well separated from the drug response.

The recipient heart was then set up, intially perfused with fresh Krebs-bicarbonate solution taken from a reservoir via a 3-way tap. The perfusion apparatus was essentially the same, but certain additional features were required to satisfy the criteria necessitated by the very small quantities of possible metabolites likely to be released. All the perfusate had to be collected from the donor heart and taken immediately to the second heart, the perfusion rates of both hearts had to match exactly and the perfusate required regassing and rewarming. A reservoir was provided for this purpose and also to serve as a trap for any air arriving from the donor heart and a point where fresh Ringer solution and the drugs could be added. The volume of this reservoir and all the tubing had to be minimal to avoid dispersion and dilution of the small amounts of any metabolite. The perfusion pressure and tension and rate of contraction of the recipient heart were recorded similarly to the donor heart and both hearts were perfused and jacketed at 37°C.

After both hearts had stabilized, the cut pulmonary artery of the donor heart was cannulated with a length of polythene tubing and by turning the 3-way tap, the perfusate was taken to the recipient heart.

Electrically-induced tachycardia of donor hearts was produced by placing bipolar electrodes on the surface of the ventricles and stimulating with square-wave pulses (5 Hz, 10 V, 5 ms) delivered by an SRI stimulator (Type 6053) for periods of 2 minutes.

In the experiments where the donor heart was exposed to anoxia, the Krebs-bicarbonate solution was replaced by Locke solution having the following composition in g/l distilled water: NaCl 9.0, KCl 0.42, $CaCl_2$, 0.24, $NaHCO_3$ 0.5 and glucose 1.0. This was gassed with O_2 so that the omission of the gas would deprive the heart of only O_2 and not both O_2 and CO_2 which would occur had Krebs-bicarbonate solution been used.

Drugs used were: (–)-adrenaline acid tartrate (BDH Ltd.), burimamide (SK & F Labs Ltd.), histamine acid phosphate (Sigma), (–)-isoprenaline bitartrate dihydrate (Ward Blenkinsop Ltd.), mepyramine maleate (Anthisan, May & Baker Ltd.), phentolamine mesylate (CIBA), propranolol (ICI Ltd.). All solutions were freshly prepared in 0.9% w/v NaCl solution (saline) and amounts referred to in the text are expressed as the base.

Results

With the two hearts satisfactorily connected in series and no loss of perfusate the effects of adrenaline were examined (Figure 2). Adrenaline (0.25 μg) added to the recipient heart reservoir increased the tension and rate of contraction and produced a characteristic coronary vascular response consisting of an initial rise in perfusion pressure, indicative of vasoconstriction, followed by a more prolonged vasodilatation. The same dose of adrenaline to the donor heart produced identical responses. This adrenaline was carried in the perfusate to the recipient heart where smaller responses were produced. These responses of the recipient heart to the adrenaline were sufficient to mask any effects of a metabolite arriving concurrently from the donor heart. The direct effects of adrenaline on the β-adrenoceptors of the recipient heart were therefore antagonized by the non-selective β-antagonist propranolol. This was used because it would block both the β_1-adrenoceptors responsible for the myocardial stimulation and the vascular β_2-adrenoceptors responsible for direct vasodilatation. Propranolol (15 μg) added to the recipient heart reservoir satisfactorily antagonized the positive

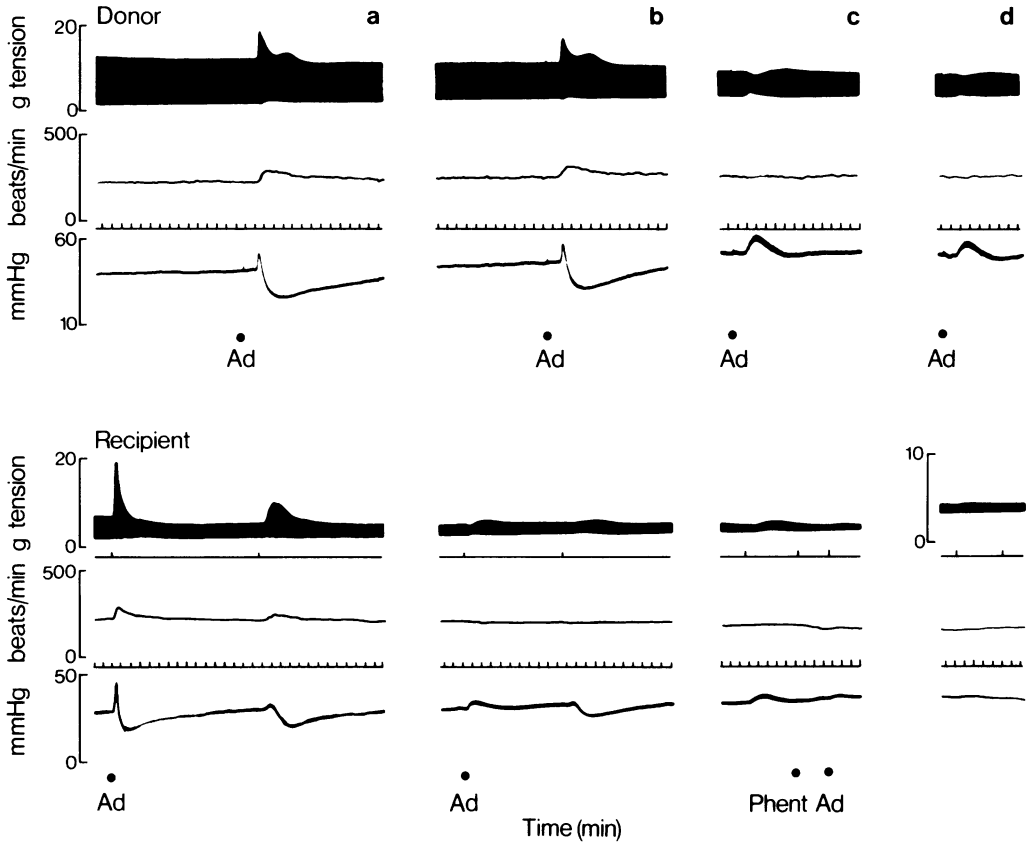

Figure 2 Donor and recipient isolated perfused (5 ml/min) hearts from guinea-pigs (518 and 458 g respectively). The effects of adrenaline (Ad, 0.25 μg) on the contractile force (upper records), heart rate (middle records) and coronary perfusion pressure (lower records) of each heart are shown. Propranolol was added to the recipient heart reservoir (15 μg) between (a) and (b) and to the donor heart reservoir (10 μg) between (b) and (c). Phentolamine (Phent, 10 μg) was added to the recipient heart in (c).

inotropic and chronotropic responses to adrenaline together with the vasodilatation. Only a small vasoconstriction remained (Figure 2b). The repeated dose of adrenaline in the donor heart yielded unchanged responses, however after a short delay (1 min) the perfusate reaching the recipient heart produced a vasodilatation unaccompanied by rate or tension changes. The positive inotropic and chronotropic responses to adrenaline and the coronary vasodilatation in the donor heart were antagonized by introduction of propranolol (10 μg) to the perfusion solution; only a vasoconstriction remained (Figure 2c). The anticipated vasodilatation that had previously followed adrenaline administration to the donor heart was also abolished, although a small vasoconstriction occurred. Finally, the α-adrenoceptor antagonist, phentolamine (10 μg), was added to the reservoir and this antagonized the residual vasoconstriction to adrenaline, whether added to the donor or recipient heart (Figure 2c and d).

Since this vasoconstriction tended to oppose the vasodilator activity carried over from the donor heart, the experiments were repeated using isoprenaline which has negligible constrictor activity. Isoprenaline (10 ng) was added to the recipient and donor hearts to produce positive inotropic and chronotropic responses together with a predominant coronary vasodilatation (Figure 3a). The myocardial and coronary vascular β-adrenergic effects in the recipient heart were antagonized with propranolol (10 μg) and the responses to a repeated dose of isoprenaline in the donor heart were unchanged. On arrival at the recipient heart, where no direct β-adrenoceptor effects were possible, vasodilatation occurred (Figure 3b). This was reproducible with a second dose of isoprenaline. Antagonism of the tension and rate

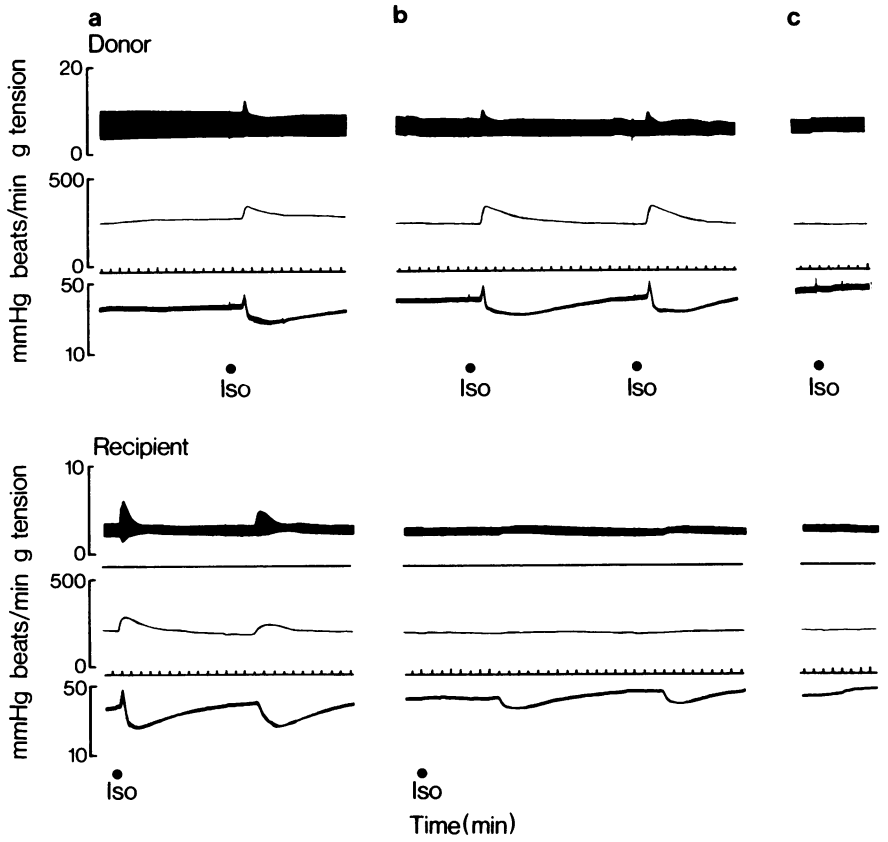

Figure 3 Donor and recipient isolated perfused (5 ml/min) hearts from guinea-pigs (485 and 630 g respectively). The effects of isoprenaline (Iso, 10 ng) on the contractile force (upper records), heart rate (middle records) and coronary perfusion pressure (lower records) of each heart are shown. Propranolol was added to the recipient heart reservoir (10 μg) between (a) and (b) and to the donor heart reservoir between (b) and (c).

responses to isoprenaline in the donor heart by propranolol (10 μg) also abolished this vasodilatation in the recipient heart that had previously followed the isoprenaline response in the donor heart. Therefore both the catecholamines adrenaline and isoprenaline, in stimulating the donor heart, caused the appearance of vasodilator activity in the recipient heart where their direct effects were antagonized.

The study was next extended to determine whether cardiac stimulation by histamine would also lead to this vasodilator activity carried over to the second heart. Histamine (1 μg) was administered consecutively to the donor and recipient hearts (Figure 4a). Each heart exhibited positive inotropic and chronotropic responses and triphasic coronary vascular responses consisting of an initial fall in perfusion pressure followed by a rise in pressure and finally a more prolonged vasodilatation. The histamine added to the donor heart was carried over to the recipient heart which yielded smaller identical responses, and these, as with the catecholamines, masked any response to a possible metabolite. These effects of histamine on the second heart were antagonized by the combined addition to the reservoir of the H_2-receptor antagonist burimamide (2 mg) and the H_1-receptor antagonist mepyramine (5 μg). These antagonize the myocardial and vascular effects respectively and histamine repeated immediately produced no response. Because of the short-lived action of burimamide (Broadley, 1975a), this combined antagonist administration had to be repeated immediately before the subsequent histamine administration to the donor heart. This produced unchanged responses in the donor heart followed shortly by a vasodilatation in the recipient. Antagonism of the histamine responses in the donor

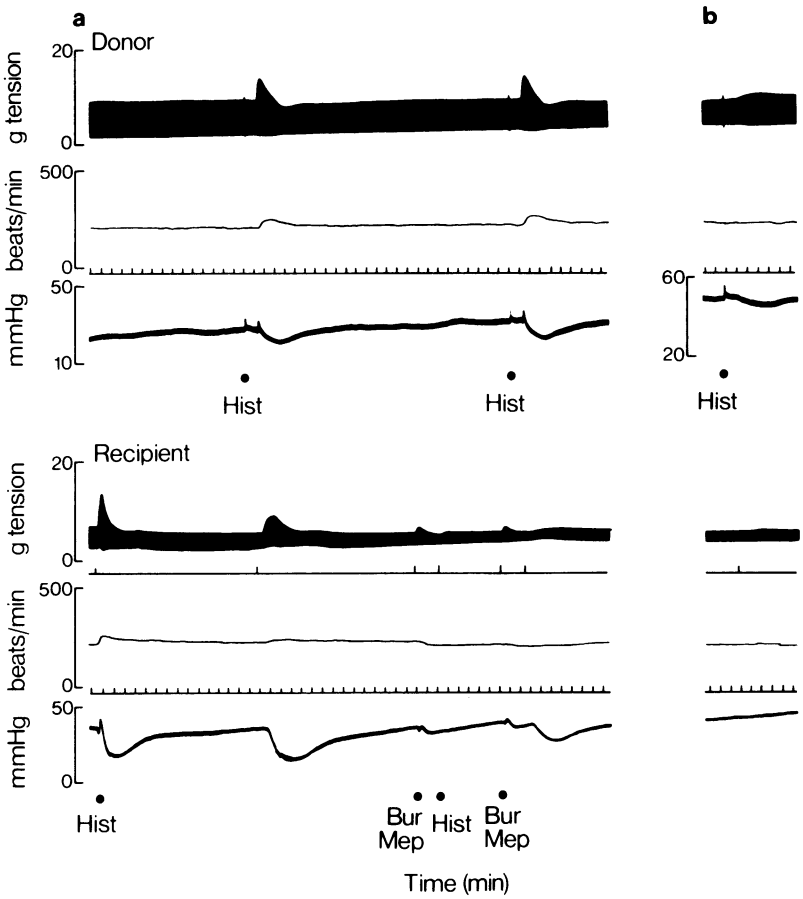

Figure 4 Donor and recipient isolated perfused (6 ml/min) hearts from guinea-pigs (663 and 575 g respectively). The effects of histamine (Hist, 1 μg) on the contractile force (upper records), heart rate (middle records) and coronary perfusion pressure (lower records) of each heart are shown. Burimamide (Bur, 2 mg) and mepyramine (Mep, 5 μg) were added simultaneously to the recipient heart reservoir immediately before the following dose of histamine added to either the donor or recipient hearts in (a). The donor heart was perfused with burimamide (20 μg/ml) and mepyramine (1 μg/ml) during (b).

heart by perfusing burimamide (20 μg/ml) and mepyramine (1 μg/ml) also abolished this vasodilatation in the recipient heart (Figure 4b).

At this stage the burimamide in the perfusate to the donor heart was omitted and immediately the myocardial responses of both hearts to histamine started to return (Figure 5) and were completely restored within 16 minutes. By this time the vasodilatation had also returned although the continued presence of mepyramine prevented any direct vascular effects. Repeating the antagonism of myocardial effects in the recipient heart with burimamide (2 mg) again revealed the carriage over of vasodilator activity from the donor heart when stimulated by histamine (Figure 5). This demonstrated both the reproducibility and the persistence of the releasing capabilities of the donor heart over a prolonged period of time.

Tachycardia was induced into the donor hearts of another series of experiments. These hearts were paced with square wave pulses (10 V, 5 ms) at 5 Hz compared with their normal spontaneous rate of 210 beats/minute. This was the fastest possible rate that the hearts would follow. During the 2 min pacing, the force of contraction was diminished and there was a small vasoconstriction followed by a small vasodilatation (Figure 6a). Following this the recipient heart also produced a vasodilatation. However, this vasodilatation was small compared with those achieved with the catecholamines and histamine-induced tachycardia. Although several stimulation

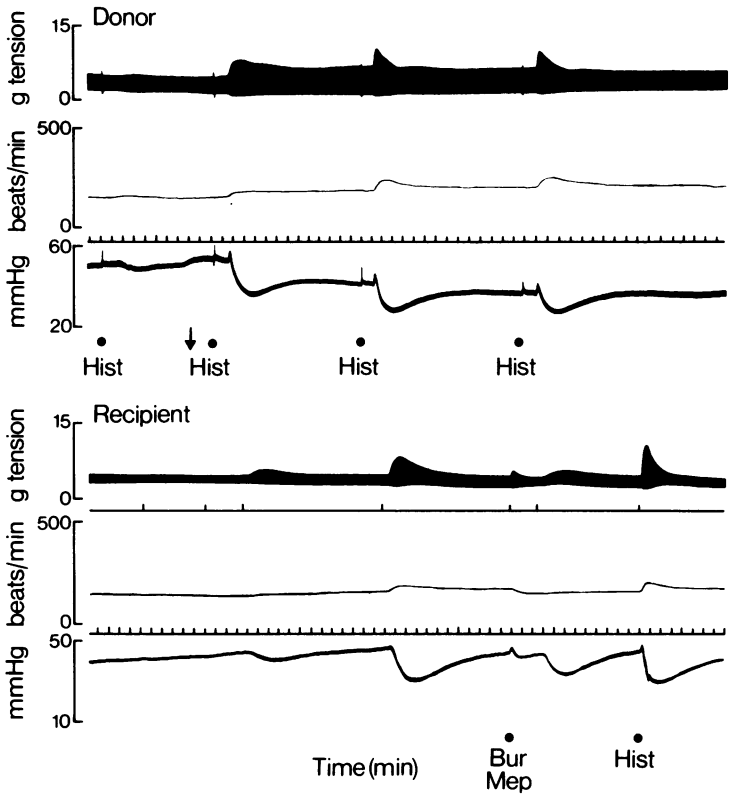

Figure 5 Continuation of the experiment using the donor and recipient guinea-pig isolated perfused hearts shown in Figure 4. The effects of histamine (Hist, 1 μg) on the contractile force (upper records), heart rate (middle records) and coronary perfusion pressure (lower records) of each heart, initially in the presence of burimamide (20 μg/ml) and mepyramine (1 μg/ml) perfusing from the donor heart reservoir are shown. At ↓ perfusion with a drug-free Krebs-bicarbonate solution was started. After recovery of the responses to histamine, the burimamide (Bur, 2 mg) and mepyramine (Mep, 5 μg) were returned to the recipient heart immediately before an injection of histamine to the donor heart.

parameters were attempted the vasodilatation of either heart could not be improved above that obtained with the quoted values.

Figure 6 also demonstrates the responses of the recipient heart that occur when the 3-way tap was turned to change from fresh Krebs-bicarbonate solution to the perfusate leaving the donor heart. The perfusion pressure fell and the tension usually declined. The effect of topping-up the reservoir with fresh Ringer was to produce a transient increase in pressure, and at the end of the experiment on returning completely to fresh Ringer the vasoconstriction was maintained (Figure 6c).

Finally, experiments were performed in hearts perfused with oxygenated Locke Ringer solution (Figure 7). The donor heart was exposed to a period of anoxia by perfusing with ungassed Ringer solution. There was a reduction in the force of contraction with arrhythmias and a large coronary vasodilatation. All three parameters were restored to normal on returning to oxygenated Locke solution. One minute after the onset of coronary vasodilatation in the donor heart there was a corresponding fall in perfusion pressure in the recipient heart but without the tension and rate changes. This returned to normal in parallel with the pressure changes in the donor heart.

Discussion

The multi-component coronary vascular response to adrenaline has been demonstrated in guinea-pig isolated perfused hearts. The overall effect of adrenaline is recognized as vasodilatation and an increased coronary blood flow (Wégria, 1951; Charlier, 1961; Parratt, 1968) and this was illustrated

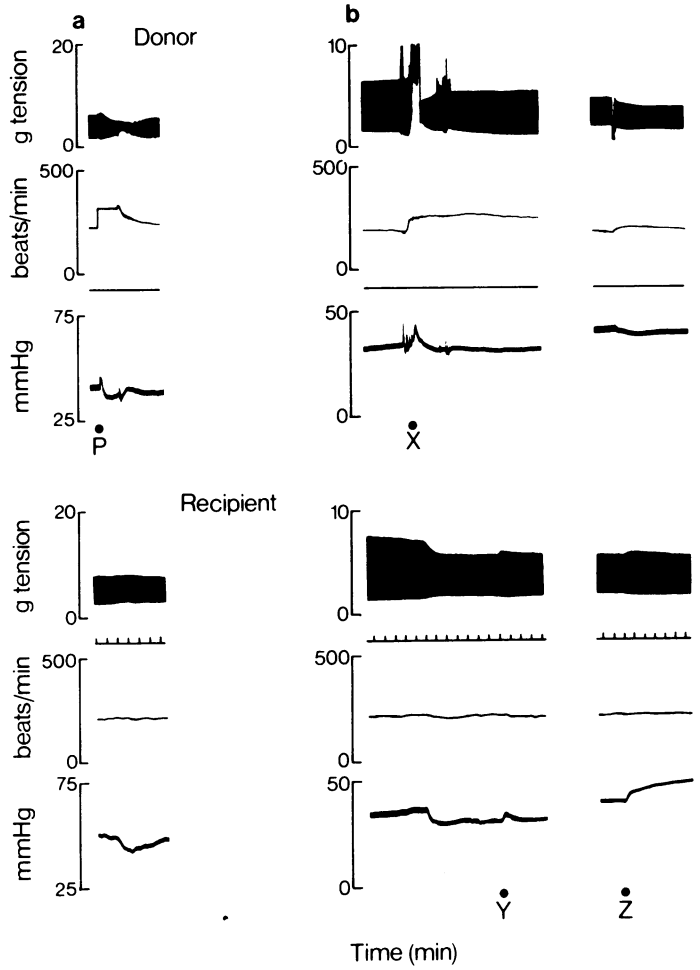

Figure 6 (a) Donor and recipient isolated perfused (5 ml/min) hearts from guinea-pigs (374 and 328 g respectively). The effects of electrically-paced ventricular tachycardia (P, 5 Hz, 10 V, 5 ms for 2 min) of the donor heart on the contractile force (upper records), heart rate (middle records) and coronary perfusion pressure (lower records) of each heart. (b) Donor and recipient isolated perfused (5 ml/min) hearts from guinea-pigs (431 and 278 g respectively). The effects are shown of connecting the two hearts at the beginning of an experiment and changing from normal Krebs-bicarbonate solution to the perfusate leaving the donor heart (X). At Y the effects of topping-up the recipient heart reservoir with fresh Krebs-bicarbonate solution are shown and at the end of the experiment (Z) the return to fresh Krebs-bicarbonate solution and disconnecting the hearts.

here as a predominant fall in perfusion pressure following an initial vasoconstriction. This vasodilatation is attributed to both a direct stimulation of β-adrenoceptors in the coronary vasculature (Adam *et al.*, 1970; Parratt & Wadsworth, 1970; Ross & Jorgensen, 1970) and an indirect effect arising from the concomitant increased myocardial activity (Berne, 1964). In the present study stimulation of the donor heart by adrenaline and the accompanying vasodilatation were followed by similar responses in the recipient heart due to the presence of the relatively large dose of adrenaline in the perfusate. When the direct and indirect β-adrenergic effects of adrenaline were antagonized in the recipient heart by propranolol, stimulation of the donor heart by adrenaline was followed only by the appearance of vasodilator activity in the recipient heart. The time lapse was consistent with the time taken for the perfusate to reach the second heart and the course of the dilatation of both hearts ran approximately in

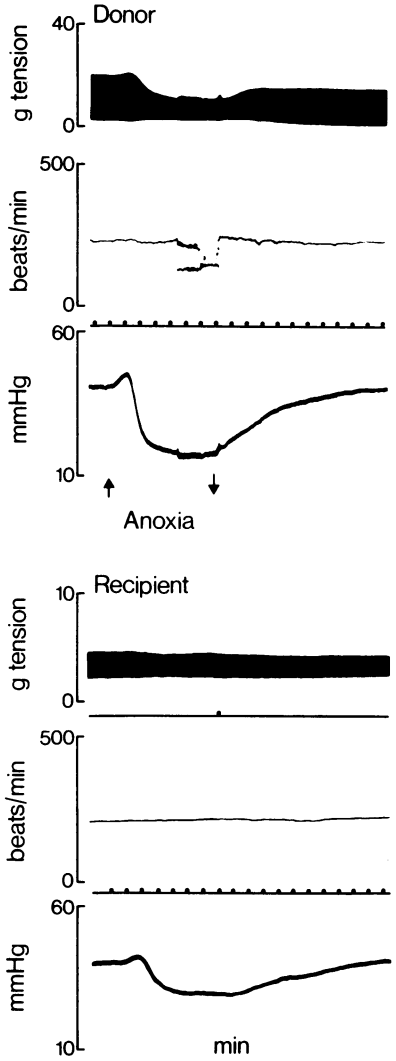

Figure 7 Donor and recipient isolated hearts from guinea-pigs (518 and 458 g respectively) perfused with Locke Ringer solution (gassed with oxygen) at 5 ml/minute. The effects of a period of perfusion of the donor heart with ungassed Ringer solution (anoxia) between the arrows upon the contractile force (upper records), heart rate (middle records) and coronary perfusion pressure (lower records) of each heart are shown. The perfusate was gassed normally before entry into the recipient heart throughout.

parallel. When the increases in activity of the donor heart were prevented by propranolol then the anticipated vasodilatation no longer occurred. In the case of adrenaline only a rise in pressure remained in both hearts which was attributed to residual direct vasoconstriction of the coronary vessels mediated via α-adrenoceptors (Saito, 1959; Kaverina, 1965; Parratt, 1967; Broadley, 1970).

The experiments were repeated with isoprenaline whose direct vascular effects are solely vasodilator. However, the response of the donor heart was biphasic consisting of an initial constriction due to extravascular compression arising from the myocardial effects (Melville & Lu, 1950; Douglas *et al.*, 1960), followed by the characteristic vasodilatation. When the β-adrenoceptors in the recipient heart were blocked by propranolol this vasodilatation was followed by a vasodilatation in the recipient heart. It is therefore concluded that both adrenaline and isoprenaline, by increasing the rate and force of contraction of the donor heart, cause the release of a vasodilator metabolite into the perfusate.

Increases in myocardial activity were next induced by histamine which, like the catecholamines, produced a multiple coronary vascular response the predominant phase of which was a fall in perfusion pressure. Histamine receptors in the recipient heart were antagonized to prevent their stimulation by histamine carried over in the perfusate. The myocardial effects are mediated via H_2-receptors (Black *et al.*, 1972; McNeill & Verma, 1974; Levi *et al.*, 1975) although recent findings suggest that this might only apply to the positive chronotropic responses (Reinhardt, Wagner & Schümann, 1974; Steinberg & Holland, 1975). Nevertheless, both the positive inotropic and chronotropic responses of the recipient heart were abolished here by the H_2-antagonist burimamide. The direct constriction and dilatation of coronary vessels mediated via H_1-receptors (Broadley, 1975a) were antagonized by mepyramine. Histamine-induced stimulation and vasodilatation of the donor heart were then followed by the appearance of vasodilator activity in the recipient heart.

Thus an increased activity in the donor heart to catecholamines and histamine not only produced a predominant coronary vasodilatation of that heart but has clearly been shown to yield vasodilator activity in the recipient heart on the arrival of the perfusate. It would appear to be due to the presence of a substance in the perfusate released by the increased activity and not by the drugs themselves since abolition of the myocardial response by the appropriate antagonist also prevented the vasodilatation in both hearts. If the release were drug-induced then it would be mediated by identical receptors for the myocardial effects. To eliminate this possibility, experiments were performed in which increases in activity were produced without drugs, namely by electrical pacing. This procedure only increased the rate of contraction, unlike the catecholamines and histamine which increased both rate and tension. In fact the tension usually declined. Although a range of stimulation parameters was attempted, poor vasodilator responses of the donor heart were

generally encountered. This was presumably due to an increased resistance from extravascular compression, known to accompany ventricular tachycardia (Anrep & Häusler, 1929; Corday, Gold, DeVera, Williams & Fields, 1959), which opposed the vasodilatation due to the increased activity of the heart. However, the tachycardia of the donor heart was followed by a more prominent vasodilatation in the recipient heart. Electrically-induced tachycardia was therefore also associated with the release of a vasodilator metabolite, although histamine and the catecholamines appeared to be superior in stimulation of the heart and therefore in producing the vasodilator activity. Generally the degree of vasodilatation of both hearts was related to the size of the positive inotropic and chronotropic responses of the donor heart.

The question now arises as to the identity of the metabolite released by the hyperactive heart and whether it is common for all forms of myocardial stimulation. The metabolic link between an increased activity and vasodilatation has been made by several authors (Berne, 1964; Haddy & Scott, 1968). However the presence of a coronary vasodilator metabolite has not previously been pharmacologically demonstrated. Early suggestions as to the nature of the substance included potassium ions (Driscol & Berne, 1957), lactic acid (Mohme-Lundholm, 1957) and bradykinin (Lochner & Parratt, 1966). More recently Berne and his colleagues have proposed (Berne, 1964) that adenosine, which has long been known to possess vasodilator activity (Drury & Szent-Gyorgyi, 1929), is released by the hyperactivity due to catecholamines. They have indeed measured an increased output of this nucleoside from the heart by adrenaline (Katori & Berne, 1966).

Prostaglandins have also been implicated in the regulation of coronary blood flow. Coronary vasodilatation of isolated hearts following adrenaline or electrically-induced tachycardia has been potentiated by the prostaglandin synthetase inhibitor indomethacin (Talesnik & Sunahara, 1973), leading to the suggestion that the prostaglandins exert a suppressant effect upon the vasodilatation. In contrast, prostaglandin E_2 has been shown to have coronary vasodilator activity (Krebs & Schrör, 1975) and it has been proposed as the mediator of the coronary vasodilatation that accompanies anoxia (Kent, Alexander, Pisano, Keiser & Cooper, 1973; Wennmalm, Pham-Huu-Chanh & Junstad, 1974). There is a continuous release of prostaglandins by the spontaneously beating heart which is increased following anoxia (Block, Feinberg, Herbaczynska-Cedro & Vane, 1975). However, the visodilatation was not attributed to prostaglandin release since it was not modified by indomethacin, although the release was inhibited.

Anoxia has also been shown to release adenosine (Katori & Berne, 1966), which supports the suggestion that a local anoxia may serve as an intermediate in the predominant vasodilator response to catecholamines due to the increased myocardial activity (Berne, 1958, 1964). Pacing-induced tachycardia has also been shown to produce anoxia (Klarwein, Kako, Chrysohou & Bing, 1961). In the present study, anoxia of the donor heart produced a powerful vasodilatation in that heart which was followed by a similar vasodilatation as the perfusate reached the recipient heart. This could not be a direct effect of the anoxia since the perfusate was reoxygenated before entering the recipient heart. It is therefore concluded that anoxia releases a vasodilator metabolite into the perfusate. Whether this is the same as that released by the catecholamines, histamine and electrical pacing cannot be determined from the present study. However, it is worth speculating that this is a common metabolite whose release is triggered by a local relative anoxia occurring during the myocardial hyperactivity. Prostaglandins or adenosine would appear to be likely candidates and further experiments may clarify their role in the response(s). It has been pointed out however, that prostaglandin release requires adequate oxygen levels, and one would only expect their appearance in the perfusate *after* the period of anoxia (Block *et al.*, 1975). In the present work the vasodilatation in both the donor and recipient hearts occurred while the donor heart was still exposed to the hypoxic Ringer solution or increased activity and this might exclude prostaglandins as possible mediators.

The presence of a vasodilator metabolite has been clearly demonstrated pharmacologically in the perfusate leaving a heart which has undergone a period of increased activity. So far it has been assumed that the fall in perfusion pressure when the perfusate arrives at the recipient heart is due to the presence of a released substance. Alternatively, one could argue that the increased activity of the donor heart merely removes or utilizes one or more constituents of the Ringer solution and that its absence leads to a fall in perfusion pressure. However this possibility can confidently be discounted on the following grounds. It is unlikely that a local change in Ringer composition could occur to such an extent as to affect the donor heart for well after the activity has returned to normal and then to be maintained during transit from donor to recipient heart. Furthermore, in the case of anoxia there is no hyperactivity and this must be a release phenomenon.

It is clear that the substance or substances are probably released from the contracting heart continuously since vasodilatation in the recipient heart occurred immediately the perfusate was taken to this heart. This persisted throughout the experiment and on returning to fresh Ringer solution the perfusion pressure rose again. This resting level of metabolite output is therefore raised by an increased cardiac activity due to drugs or electrical pacing leading to further vasodilatation. The amounts released are

presumably very small, yet there is apparently no tachyphylaxis since the vasodilator responses were readily repeatable over experiments of long duration.

This proposed hyperactivity-anoxia-metabolite system for coronary vasodilatation may well be the basis of the autoregulation of the coronary flow that provides the circulatory need of the active myocardium. It now remains to adapt the procedure described here to aid the clarification of the nature of the metabolite and to determine whether a common substance is involved in the procedures used here to induce its release.

I am grateful to Dr R.W. Brimblecome of SK & F for the generous gift of burimamide, to Ward Blenkinsop for the (−)-isoprenaline and to ICI for the propranolol.

References

ADAM, K.R., BOYLES, S. & SCHOLFIELD, P.C. (1970). Cardio-selective β-adrenoceptor blockade and the coronary circulation. *Br. J. Pharmac.*, **40**, 534–536.

ANREP, G.V. & HÄUSLER, H. (1929). The coronary circulation. II. The effect of changes in temperature and of heart rate. *J. Physiol., Lond.*, **67**, 299–314.

BAYER, B.L., MENTZ, P. & FÖRSTER, W. (1972). Über spezifische Unterschiede zwischen den adrenergen β-Rezeptoren an Hertz und Koronarien. *Arch. int. Pharmacodyn*, **200**, 341–347.

BERNE, R.M. (1958). Effect of epinephrine and norepinephrine on coronary circulation. *Circulation Res.*, **6**, 644–655.

BERNE, R.M. (1964). Regulation of coronary blood flow. *Physiol. Rev.*, **44**, 1–29.

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition of antagonism of histamine H_2-receptors. *Nature, Lond.*, **236**, 385–390.

BLOCK, A.J., FEINBERG, H., HERBACZYNSKA-CEDRO, K. & VANE, J.R. (1975). Anoxia-induced release of prostaglandins in rabbit isolated hearts. *Circulation Res.*, **36**, 34–42.

BROADLEY, K.J. (1970). An analysis of the coronary vascular responses to catecholamines, using a modified Langendorff heart preparation. *Br. J. Pharmac.*, **40**, 617–629.

BROADLEY, K.J. (1975a). The role of H_1 and H_2-receptors in the coronary vascular response to histamine of isolated perfused hearts of guinea-pigs and rabbits. *Br. J. Pharmac.*, **54**, 511–521.

BROADLEY, K.J. (1975b). A procedure to demonstrate the release of a vasoactive metabolite by catecholamines from perfused guinea-pig hearts. *Br. J. Pharmac.*, **54**, 223–224P.

CHARLIER, R. (1961). *Coronary Vasodilators*. Oxford: Pergamon Press.

CORDAY, E., GOLD, H., DEVERA, L.B., WILLIAMS, J.H. & FIELDS, J. (1959) Effect of the cardiac arrythmias on the coronary circulation. *Ann. intern. Med.*, **50**, 535–553.

DEMPSEY, P.J. & COOPER, T. (1972). Pharmacology of the coronary circulation. *Ann. Rev. Pharmac.*, **12**, 99–110.

DOUGLAS, R.C., ARMENGOL, V. & TALESNIK, J. (1960). Influence of cardiac activity on coronary flow control. *Acta physiol. latinoam.*, **10**, 205–216.

DREW, G.M. & LEVY, G.P. (1972). Characterization of the coronary vascular β-adrenoceptors in the pig. *Br. J. Pharmac.*, **46**, 348–350.

DRISCOL, T.E. & BERNE, R.M. (1957). Role of potassium in regulation of coronary blood flow. *Proc. Soc. exp. Biol. Med.*, **96**, 505–508.

DRURY, A.N. & SZENT-GYORGYI, A. (1929). The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J. Physiol., Lond.*, **68**, 213–237.

ERCAN, Z.S., BÖKESOY, T.A. & TÜRKER, R.K. (1974). A study of the histamine H_2-receptors in heart muscle and coronary vessels. *Eur. J. Pharmac.*, **27**, 259–262.

GARCIA-RAMOS, J., ALANIS, J. & LUCO, J. (1950). Estudios sobre la circulation coronaria. II. Las acciones del vago y del simpatico. *Archos Inst. Cardiol. Méx.*, **20**, 534–550.

GREEN, H.D., WÉGRIA, R. & BOYER, N.H. (1942). Effect of epinephrine and pitressin on the coronary artery inflow in anaesthetized dogs. *J. Pharmac. exp. Ther.*, **76**, 378–391.

HADDY, F.J. & SCOTT, J.B. (1968). Metabolically linked vasoactive chemicals in local regulation of blood flow. *Physiol. Rev.*, **48**, 688–707.

HARDIN, R.A., SCOTT, J.B. & HADDY, F.J. (1961). Effects of epinephrine and norepinephrine on coronary vascular resistance in dogs. *Am. J. Physiol.*, **201**, 276–280.

HASHIMOTO, K., SHIGEI, T., IMAI, S., SAITO, Y., YAGO, N., UEI, I. & CLARK, R.E. (1960). Oxygen consumption and coronary vascular tone in the isolated fibrillating dog heart. *Am. J. Physiol.*, **198**, 965–970.

KATORI, M. & BERNE, R.M. (1966). Release of adenosine from anoxic hearts. Relationship to coronary flow. *Circulation Res.*, **19**, 420–425.

KAVERINA, N.V. (1965). *Pharmacology of the Coronary Circulation*. Oxford: Pergamon Press.

KENT, K.M., ALEXANDER, R.W., PISANO, J.J., KEISER, H.R. & COOPER, T. (1973). Prostaglandin dependent coronary vasodilator responses. *Physiologist*, **16**, 361.

KLARWEIN, M., KAKO, K., CHRYSOHOU, A. & BING, R.J. (1961). Effect of atrial and ventricular fibrillation and ventricular tachycardia on carbohydrate metabolism of the heart. *Circulation Res.*, **9**, 819–825.

KREBS, R. & SCHRÖR, K. (1975). Actions of prostaglandin E_2 on myocardial mechanics, coronary vascular resistance and oxygen consumption in the guinea-pig isolated heart preparation. *Br. J. Pharmac.*, **55**, 403–408.

LANGENDORFF, O. (1895). Untersuchungen am überlebenden Säugetierherzen. *Pflügers Arch. ges. Physiol.*, **61**, 291–332.

LEVI, R., CAPURRO, N. & LEE, C.-H. (1975). Pharmacological characterization of cardiac histamine receptors: sensitivity to H_1- and H_2-receptor agonists and antagonists. *Eur. J. Pharmac.*, **30**, 328–335.

LEVI, R. & KUYE, J.O. (1974). Pharmacological characterization of cardiac histamine receptors: sensitivity to H_1-receptor antagonists. *Eur. J. Pharmac.*, **27**, 330–338.

LOCHNER, W. & PARRATT, J.R. (1966). A comparison of the effects of locally and systemically administered kinins on coronary blood flow and myocardial metabolism. *Br. J. Pharmac. Chemother.*, **26**, 17–26.

LUCHESSI, B.R. & HODGEMAN, R.J. (1971). Effect of 4-(2-hydroxy-3-isopropylaminopropoxy) acetanilide (AY 21,011) on the myocardial and coronary vascular responses to adrenergic stimulation. *J. Pharmac. exp. Ther.*, **176**, 200–211.

McNEILL, J.H. & VERMA, S.C. (1974). Blockade by burimamide of the effects of histamine and histamine analogs on cardiac contractility, phosphorylase activation and cyclic adenosine monophosphate. *J. Pharmac. exp. Ther.*, **188**, 180–188.

MARK, A.L., ABBOUD, F.M., SCHMID, P.G., HEISTAD, D.D. & MAYER, H.E. (1972). Differences in direct effects of adrenergic stimuli on coronary, cutaneous and muscular vessels. *J. clin. Invest.*, **51**, 279–287.

MELVILLE, K.I. & LU, F.C. (1950). Effects of epinephrine, aminophylline, nitroglycerine and papaverine on coronary inflow and on heart contractions as recorded concurrently. *J. Pharmac. exp. Ther.*, **99**, 286–303.

MOHME-LUNDHOLM, E. (1957). Mechanism of the relaxing effect of adrenaline on bovine coronary vessels. *Acta physiol. scand.*, **38**, 255–264.

PARRATT, J.R. (1967). Adrenergic receptors in the coronary circulation. *Am. Heart J.*, **73**, 137–140.

PARRATT, J.R. (1968). Pharmacological aspects of the coronary circulation. In *Progress in Medicinal Chemistry*, ed. Ellis, G.P. & West, G.B., vol. 6, pp. 11–66. London: Butterworth & Co. Ltd.

PARRATT, J.R. & WADSWORTH, R.M. (1970). The effect of "selective" β-adrenoceptor blocking drugs on the myocardial circulation. *Br. J. Pharmac.*, **39**, 296–308.

PARRATT, J.R. & WADSWORTH, R.M. (1972). The effects of dipyridamole on the myocardial vasodilator actions of noradrenaline, isoprenaline and adenosine. *Br. J. Pharmac.*, **46**, 585–593.

REINHARDT, D., WAGNER, J. & SCHÜMANN, H.J. (1974). Differentiation of H_1- and H_2-receptors mediating positive chrono- and inotropic responses to histamine on atrial preparations of the guinea-pig. *Agents and Actions*, **4**, 217–221.

ROSS, G. (1974). Myocardial and coronary vascular effects of salbutamol. *Cardiovasc. Res.*, **8**, 1–7.

ROSS, G. & JORGENSEN, C.R. (1970). Effects of a cardio-selective beta-adrenergic blocking agent on the heart and coronary circulation. *Cardiovasc. Res.*, **4**, 148–153.

SAITO, H. (1959). Effects of adrenaline and noradrenaline on the coronary outflow of the isolated rabbit heart. *Bull. Osaka med.* Sch., **5**, 15–33.

SIEGAL, J.H., GILMORE, J.P. & SARNOFF, J.J. (1961). Myocardial extraction and production of catecholamines. *Circulation Res.*, **9**, 1336–1350.

STEINBERG, M. & HOLLAND, D.R. (1975). Separate receptors mediating the positive inotropic and chronotropic effects of histamine in guinea-pig atria. *Eur. J. Pharmac.*, **34**, 95–104.

TALESNIK, J. & SUNAHARA, F.A. (1973). Enhancement of metabolic coronary dilatation by aspirin-like substances by suppression of prostaglandin feed-back control? *Nature, Lond.*, **224**, 351–353.

WÉGRIA, R. (1951). Pharmacology of the coronary circulation. *Pharmac. Rev.*, **3**, 197–246.

WENNMALM, Å., PHAM-HUU-CHANH & JUNSTAD, M. (1974). Hypoxia causes prostaglandin release from perfused rabbit hearts. *Acta physiol. scand.*, **91**, 133–135.

(Received January 30, 1976. Revised March 16, 1976.)