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CORONARY REACTIONS TO CARDIAC HYPERACTIVITY AND TO HYPOXIA IN ISOLATED PERFUSED HEART OF RAT

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1 Continuous recording of cardiac force of contraction, heart rate and coronary flow from isolated perfused hearts of rats was used to study coronary reactions: (a) to cardiostimulation with noradrenaline, $CaCl_2$, or electrically induced tachycardia; (b) to short duration stoppage of coronary inflow (hypoxia).

2 The heart rate was controlled by electrical pacing. Coronary vasodilatation resulted from cardiostimulation or hypoxia. This coronary response was greater at higher heart rates.

3 In parallel experiments administration of noradrenaline to hearts paced at different frequencies resulted in a rate-dependent elevation of adenosine-3',5'-cyclic monophosphate (cyclic AMP).

4 Duration of hypoxia leading to different degrees of reactive hyperaemia did not change the cardiac cyclic AMP levels.

5 Coronary vasodilatation due to increased cardiac metabolism produced by noradrenaline, Ca^{2+} or tachycardia was enhanced by the phosphodiesterase inhibitors diazoxide and papaverine while it was inhibited during the administration of prostaglandin E_2.

6 Reactive hyperaemia was unaffected by diazoxide, papaverine or prostaglandin E_2.

7 Catecholamine depletion by reserpine did not influence metabolic coronary dilatation nor the reactive hyperaemic responses.

8 We postulate that there are at least two types of coronary reactions: one in response to hypoxia, 'reactive hyperaemia', and another resulting from cardiac hyperactivity, 'metabolic coronary dilatation'. The latter, blocked by prostaglandin E_2 and enhanced by diazoxide or papaverine, would be triggered by cyclic AMP while reactive hyperaemia would result from other mechanisms.

Introduction

Substantial evidence has accumulated to show that increased cardiac activity leads to enhanced coronary flow (CF). The latter results from a prolonged vasodilatation due to the action on the vasculature of certain metabolite(s) produced during the cardiac hyperactivity (Gregg & Fisher, 1963; Berne, 1969; Broadley, 1976). The intracardiac metabolic processes relating the cardiac hyperactivity to the enhancement of CF have been attributed to the increased level of cardiac adenosine-3',5'-cyclic monophosphate (cyclic AMP) (Sen, Sunahara & Talesnik, 1976). It has also been shown that metabolically-induced coronary dilatation (MCD) is inhibited by administration of prostaglandin E_1 (Sunahara & Talesnik, 1974) or E_2, and exaggerated in the presence of the phosphodiesterase inhibitor, diazoxide; corresponding attenuation or elevation in cyclic AMP levels were also observed after these treatments (Sen *et al.*, 1976). We also investigated the possibility that the endogenously synthesized prostaglandin could be a modulator of the adenylate cyclase activation, thus explaining the augmented MCD responses obtained in isolated perfused hearts by giving aspirin-like substances (Talesnik & Sunahara, 1973).

We advanced the hypothesis that the activation of the adenylate cyclase could be triggered by the increased cardiac contractile process itself. In the present paper, we tested this hypothesis further by examining the influence of different initial left ventricle distentions and of heart rate on the MCD response, as well as the level of cyclic AMP produced by cardiostimulation at different heart rates. It has been shown that the increase in heart rate results in an augmentation of CF (Pitt & Gregg, 1968; Cobb, Bache, Curry, Ebert, McHale & Greenfield, 1973; Broadley, 1976), but that changes in heart rate have

relatively little influence on coronary extravascular support (Raff, Kosche & Lochner, 1972). A distinction is also made between MCD (as a response to increase in cardiac metabolism) and 'reactive hyperaemia' (RH) that results from myocardial hypoxia. A preliminary account of these results has already been presented (Sunahara, Talesnik, Sen & Hrycyshyn, 1974).

Methods

Male Wistar or Sprague-Dawley rats, mean body weight 273 ± 13 g (mean $\pm$ s.e. mean) were obtained from Canadian Biobreeding Laboratories. The animals were anaesthetized with ether and injected with heparin (1.5 mg/kg i.v.). The hearts were quickly excised and chilled in Krebs-Henseleit-bicarbonate (K-H) solution at 4–5°C. Aortic retrograde perfusion by the Langendorff method was carried out at 50 mmHg perfusion pressure with K-H medium modified to contain half the recommended Ca^{2+} (Zachariah, 1961), and containing insulin 2 units/l (Bleehen & Fisher, 1954; Weissler, Atschuld, Gibb, Pollack & Kruger, 1973). The perfusate was gassed with 95% O_2 and 5% CO_2. Continuous measurement of CF was obtained from a differential pressure recording as described elsewhere (Sen *et al.*, 1976). Calibrations of flow were performed after each experiment. In several instances a magnetic flowmeter (Biotronex L1) was used in parallel to check the differential pressure method. Since the two recordings were identical and the low flow obtained from the rat heart was easier to measure with the differential pressure system, we adopted the latter technique for our experiments. The heart activity was recorded with a force-displacement transducer (Grass FT.03); the force transducer was attached to the apex of the heart and 2 g initial tension was applied. The signals from the force transducer were recorded on an oscillograph and the data expressed as 'force of contraction'. The force of contraction of the heart was also recorded in isovolumic conditions by implanting a water-filled vinyl balloon into the left ventricle according to the method described by Fallen, Elliott & Gorlin (1967); in this preparation the balloon was connected to a pressure transducer (Statham P23 Db).

Care was taken to set the initial volume so that zero pressure was obtained during diastole. The product of systolic pressure and heart rate is expressed as 'total pressure developed'. A tuberculin syringe manipulated by a micrometer driver permitted the changing of the volume of fluid in the system to produce different degrees of left ventricular distention. Spontaneous rhythm was suppressed by clamping the interventricular septum. Electrical pacing was regularly used. For this purpose, dipolar electrodes were placed at the base of the right ventricle, and square wave pulses 1 ms duration, current approximately 20% above threshold were applied at frequencies shown in the Results section. Drugs were administered in single bolus (0.1 ml) with a special injector (Sen *et al.*, 1976). The changes in CF were assessed by planimetry of the area under the flowmeter tracing as already described (Sen *et al.*, 1976). Similar methods for estimating changes in CF by planimetric integration over a time period have been described by other authors (Raberger, Weissel & Kraupp, 1971). At the end of each experiment the heart was removed, blotted and weighed. The changes in CF are calculated in ml/min per g of tissue (ml min^{-1} g^{-1}) and the data are expressed as mean percentage $\pm$ standard error of the mean (s.e. mean) over basal levels.

In some parallel experiments, the cardiac level of cyclic AMP was determined. The hearts were frozen with Wollenberger tongs, chilled in liquid nitrogen and the cyclic AMP levels determined by the method of Gilman (1970) modified as indicated previously (Sen *et al.*, 1976).

Except in the cases specifically indicated, Student's *t* tests for paired and unpaired data were used to compare responses obtained in the different experimental conditions.

Drugs

The following drugs were used: noradrenaline bitartrate monohydrate (Levophed, Wintrop), reserpine (Serpasil, Ciba), prostaglandin E_2 (Upjohn), diazoxide (Hyperstat, Schering), papaverine hydrochloride (Merck), heparin and insulin (Connaught Laboratories) and [^{3}H]-adenosine 3′-5′-cyclic phosphate, specific activity 16.2 Ci/mmol (Schwartz-Bioresearch). All drug solutions were freshly prepared. The stock solution of prostaglandin E_2 was prepared by dissolving 2 mg of crystalline prostaglandin E_2 per ml of 90% ethanol.

Results

Influence of heart rate on metabolic coronary dilator response to cardiostimulation with noradrenaline or calcium (Ca^{2+})

Sudden increases in heart rate result in augmented CF. If the increased heart rate is maintained for a prolonged period of time (at least 30 min at each frequency) the CF returns towards the initial value. The challenging doses of noradrenaline (NA) or Ca^{2+} were given only after CF was stabilized at control levels as shown in the legend of Figure 2. A typical experiment is shown in Figure 1, in which a bolus dose of NA or Ca^{2+} was administered to a heart beating at different rates. When cardiac hyperactivity almost

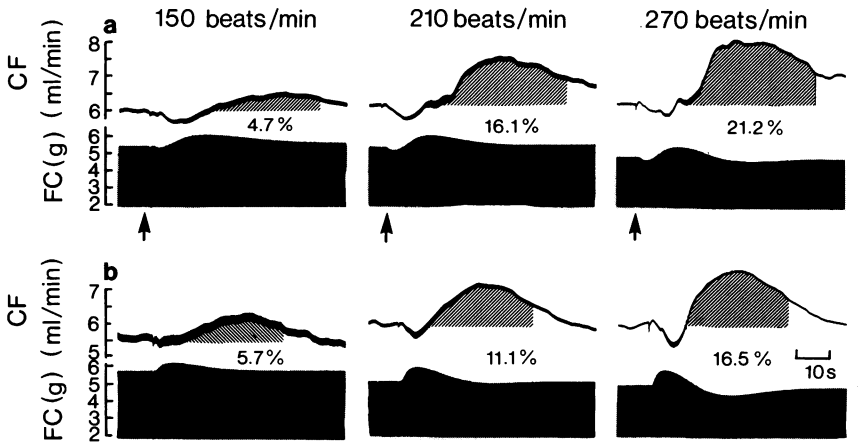

Figure 1 Influence of heart rate on metabolic coronary dilator responses. Perfused Wistar rat hearts paced at frequencies indicated at the top in beats/minute. FC (g) = force of contraction. CF (ml/min) = coronary flow. Numerals under the flow curve represent % change in CF calculated by planimetry of the hatched area. (a) ↑ = noradrenaline 0.01 μg; (b) ↑ = $CaCl_2$ 0.2 mg.

reached its peak, a slowly developing and long lasting increase in CF (MCD) was observed.

The data collected in this type of experiment show unequivocally that the cardiostimulation produced by NA or Ca^{2+} resulted in significantly greater MCD reactions as the heart rates were increased (Figure 2).

Effect of single doses of noradrenaline on cardiac cyclic AMP levels at different heart rates.

As in previous experiments, hearts of Wistar rats were paced at the indicated frequencies for about 30 min; a challenging dose of NA (0.02 μg) was then administered and the hearts were subsequently clamped with Wollenberger tongs for cyclic AMP determinations. The dose of NA adopted was based on previous experience (Sen *et al.*, 1976). After 30 min the basic coronary flow was, for 150 beats/min, 5.6 ml/min ($n=43$), for 200 beats/min, 6.7 ml/min ($n=36$) and for 250 beats/min, 6.4 ml/min ($n=36$). These mean CF values were not normalized for tissue weight. It can be seen in Figure 3 that the rate of rise of cardiac cyclic AMP was about the same for all three heart rates, and at 10 s after the NA administration they all increased to the same level; however, the 150 and 200 beats/min values started to decline towards control levels at 15 seconds. The hearts beating at 150 beats/min reached the basal cyclic AMP level at about 20 s while those beating at 200 beats/min remained significantly above normal even at 30 seconds. The cardiac cyclic AMP at a heart rate of 250 beats/min continued to rise after 10 s, reaching peak level at 15 s, and remained at a significantly higher level for over 30 seconds. These data indicate that the degree of cardiac hyperactivity plays an important role in the activation of the adenylate cyclase system.

Influence of acute changes of heart rate on coronary flow and on cardiac cyclic AMP levels

Changes affecting the CF when the pacing frequency was increased are shown in Figure 4. It can be seen that there was an initial brief period of diminished CF which was quickly overcome by a marked diminution in coronary resistance, giving rise to increased CF whose magnitude depended on the length and frequency of stimulation (Figures 4 and 5). The initial decrease of CF is not affected by the administration of α-adrenoceptor blocking agents (Talesnik & Sunahara, 1973).

To study the influence of the heart rate on the cardiac cyclic AMP content, parallel experiments were carried out in hearts paced at 150 beats/min, on which a rate of 350 beats/min was imposed for 30 seconds. It can be seen in Figure 6 that the cyclic AMP level rose during tachycardia and reached peak value about 20 s after initiation of the high rate of cardiostimulation. It is interesting to note that at the end of the 30 s period of tachycardia the cyclic AMP levels were already on the decline, although the increment of CF was still in the ascending phase (Figure 4). When tachycardia of 200, 250 or 300 beats/min was induced, the results were less conclusive. Because of large variations the values for the frequency of 350 shown in Figure 6 were not significantly different.

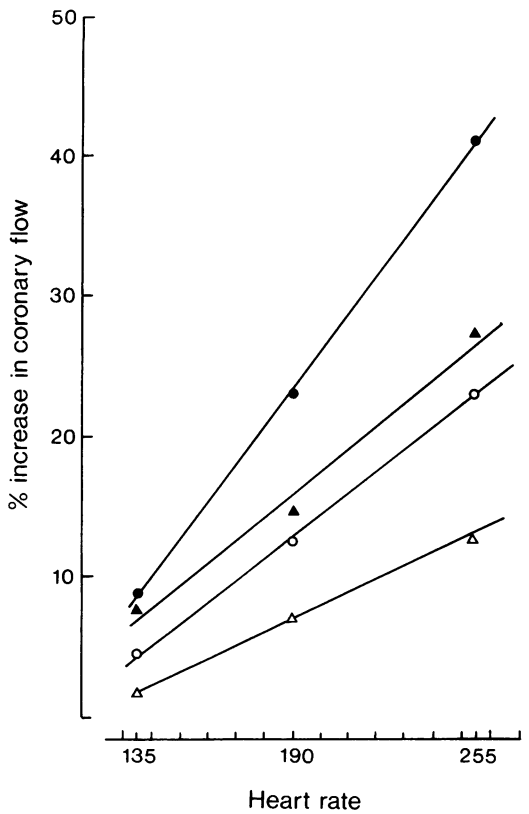

Figure 2 Influence of heart rate on metabolic coronary dilator (MCD) responses to noradrenaline (NA) and Ca^{2+}. Data obtained from Wistar rat hearts. Each point represents the mean of 7–9 experiments. The MCD responses are significantly greater as the cardiostimulation is produced at increased heart rates. (Addendum 1). (●) NA 10 ng; (○) NA 5 ng; (▲) $CaCl_2$ 0.2 mg; (△) $CaCl_2$ 0.1 mg.

	Heart rate (beats/min)		
	135	190	255
Mean basic CF ± s.e. mean (ml g^{-1} min^{-1}) after 30 min of imposed rhythm	6.3 ± 0.3	6.2 ± 0.3	6.2 ± 0.25

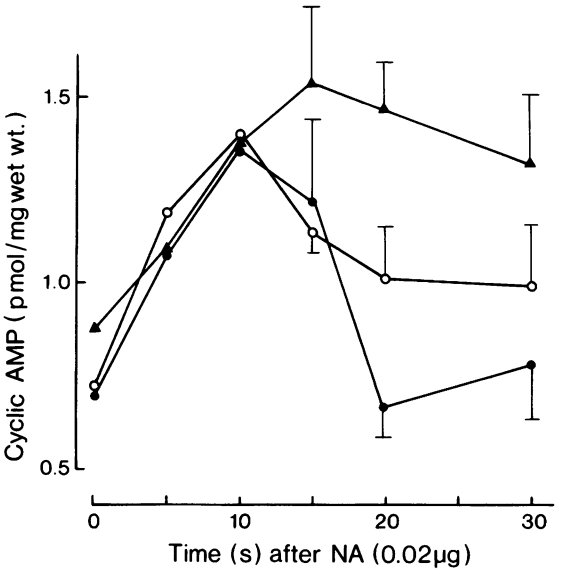

Figure 3 Influence of heart rate and noradrenaline (NA) on the cardiac cyclic AMP levels. Hearts of Wistar rats were electrically paced. Cyclic AMP measured at 0 time obtained after 20–30 min pacing at the indicated rates. A bolus dose of NA was injected after time 0. Each point represents the mean of at least 6 experiments. Vertical lines show s.e. mean. (●) 150 beats/min; (○) 200 beats/min; (▲) 250 beats/min.

Influence of diazoxide, papaverine and prostaglandin E_2 on the changes in coronary flow induced by tachycardia

The increase in CF produced by tachycardia was studied in different groups of animals in which the phosphodiesterase inhibitors, diazoxide or papaverine were added, in different concentrations, to the perfusate. The CF values were similar before, and during the administration of diazoxide or papaverine (Table 1) Diazoxide or papaverine concentrations above those shown in this Table could not be used because they themselves induced sustained coronary dilatation, thus preventing the study of MCD. After administration of diazoxide or papaverine, a substantial increase in CF reactions to tachycardia was observed (Figures 4 and 5). As a standard procedure, the effect of diazoxide or papaverine on the MCD due to tachycardia was compared with the effect on the responses to NA or Ca^{2+}. In all cases, in the presence of diazoxide or papaverine, there was a significant increase in MCD (Figure 5).

In another series of experiments, effects of tachycardia were studied before and during the continuous administration of prostaglandin E_2. When prostaglandin E_2 was given there was an initial vasodilatation that subsided to control levels after about 20 minutes. Some experiments in which CF remained above the initial values were discarded. The control CF values before and during prostaglandin E_2 administration are shown in Table 1. During prostaglandin E_2 administration the CF reaction to tachycardia was markedly inhibited (Figures 4 and 7). To ascertain whether this inhibition resembled that described in a previous paper (Sen *et al.*, 1976), NA and/or Ca^{2+} were also tested before, and during prostaglandin E_2 administration. In diazoxide-treated hearts, prostaglandin E_2 also inhibited the coronary

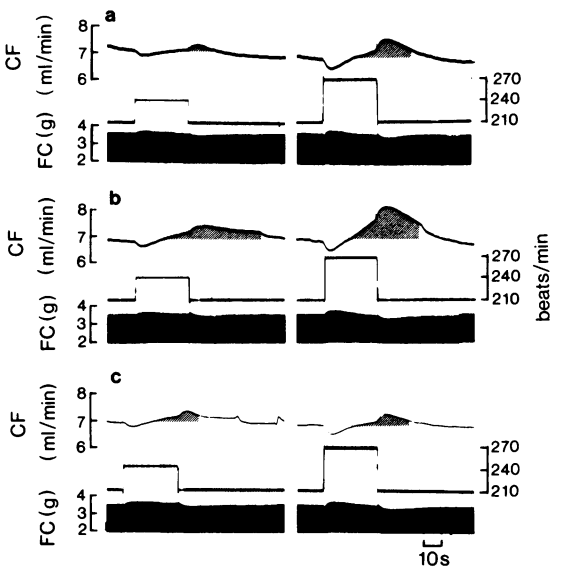

Figure 4 Influence of changes in heart rate on the coronary flow. Hearts of Sprague-Dawley rats were paced at 210 beats/min; sudden increases in heart rate (cardiotachometer calibration in beats/min on the right) for 30 s produced a metabolic coronary dilator (MCD) reaction that was enhanced by diazoxide and blocked by prostaglandin E_2. The hatched areas under the coronary flow curve were used to calculate % change of MCD. (FC=force of contraction). (a) Control; (b) during diazoxide 300 μg/l; (c) during diazoxide 300 μg/l + prostaglandin E_2 10 μg/l.

reaction to tachycardia, NA or Ca^{2+} (Figures 4 and 7). The administration of diazoxide, papaverine or prostaglandin E_2 did not alter either the basal or the stimulated force of contraction induced by NA or Ca^{2+} (Figure 7 and Table 1). These results confirm our previous observations (Sen *et al.*, 1976).

In still another set of experiments, hearts were paced at 150 beats/min and an indwelling balloon (filled with 0.03 ml of fluid) was used for recording cardiac activity. Tachycardia (200 and 250 beats/min) for periods of 30 s was applied before and during the administration of diazoxide (1.29×10^{-6}M). The basic CF (6.5 ± 0.26 ml min^{-1} g^{-1}) was unaffected by diazoxide administration (6.3 ± 0.12 ml min^{-1} g^{-1}) but the MCD induced by tachycardia was significantly increased (Figure 8). After the MCD enhancement by diazoxide was clearly produced, prostaglandin E_2 was added to the perfusate so that one group received it at a concentration of 1 μg/l (2.8×10^{-9}M), while the other two groups received 3 μg/l (8.5×10^{-9}M) and 5 μg/l (1.4×10^{-8}M), respectively. The addition of prostaglandin $E+^2$ did not alter the basic CF, whose values remained about the same as in the controls (6.2 ± 0.32; 6.0 ± 0.09 and 6.1 ± 0.78 ml min^{-1} g^{-1}, respectively). As can be seen in Figure 8, the addition of prostaglandin E_2 produced a concentration-dependent inhibition of MCD responses to tachycardia.

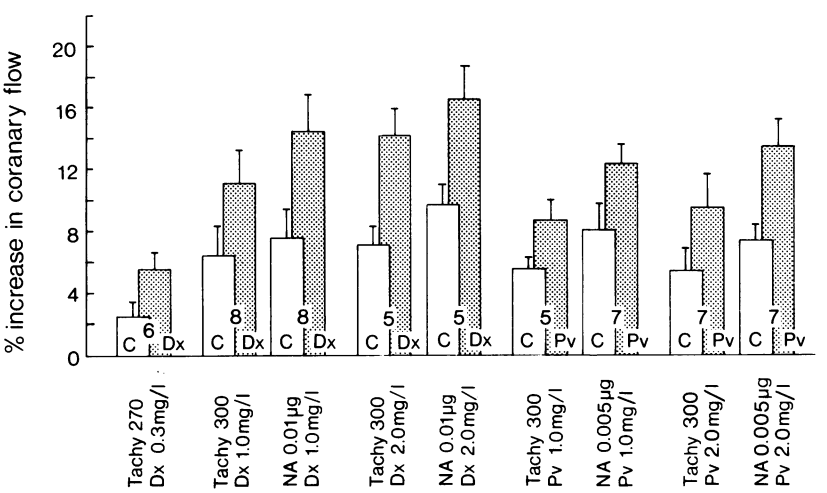

Figure 5 Influence of diazoxide (Dx) and papaverine (Pv) on the metabolic coronary dilator (MCD) responses to tachycardia (Tachy). Hearts of Sprague-Dawley rats paced at a basal rate of 210 beats/min were submitted to tachycardia for 30 seconds. For brevity only tachycardia at 270 or 300 are shown. C=control. Noradrenaline (NA) was used to assess the effectiveness of diazoxide or papaverine. Shown in the graph are the mean % increase in coronary flow (CF); vertical lines shown s.e. mean; paired analysis (number of experiments shown in columns) indicate that in every case diazoxide or papaverine produced a significant difference in MCD response ($P < 0.025$).

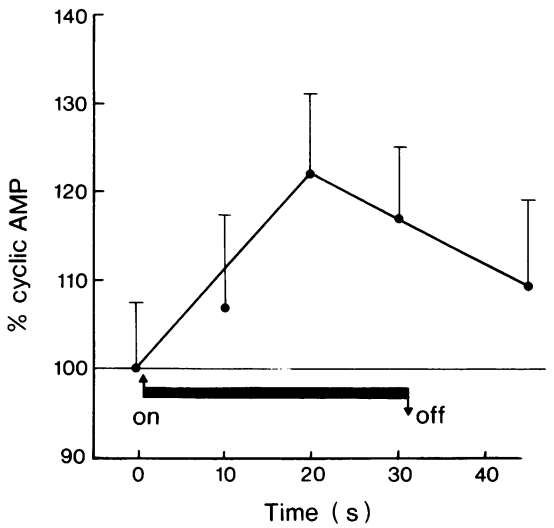

Figure 6 Influence of tachycardia on the cardiac cyclic AMP levels. Hearts of Sprague-Dawley rats were paced at 150 beats/minute. After–30 min, values at time 0 were obtained (0.719 pmol/mg wet wt. = 100%). Tachycardia at 350 beats/min was imposed for 30 s and hearts clamped at indicated intervals. Each point represents the mean % of at least 6 experiments. Vertical lines show s.e. mean.

Influence of heart rate, phosphodiesterase inhibition and prostaglandin E_2 on coronary reactive hyperaemia

Brief stoppage of the coronary inflow results in reactive hyperaemia (RH) (Figure 9). It is clear that the magnitude of RH is dependent both on the heart rate and on the duration of the coronary flow arrest.

Experiments were carried out in hearts paced at 210 beats/min and RH induced for 5, 10, 15 and 20 seconds. The RH produced at 10 and 20 s is shown in Figure 7; results obtained before and during the administration of diazoxide and papaverine are also plotted. It can also be seen that RH was unaffected by diazoxide or papaverine while MCD reaction to cardiostimulation (NA, Ca^{2+} or tachycardia) was markedly enhanced. Furthermore, prostaglandin E_2 had no effect on RH, contrary to its inhibitory effect on MCD. Thus, a clear difference between these two types of increases in CF was established. Once more, the administration of diazoxide, papaverine or prostaglandin E_2 did not alter the effects of NA and Ca^{2+} on the force of contraction (Figure 7).

Influence of reserpine on the coronary reaction to cardiostimulation and to hypoxia

Release of catecholamines from rat hearts by different agents is well known. Therefore, we decided to

Table 1 Influence of diazoxide (Dx), papaverine (P_v) and prostaglandin E_2 (PGE_2) on coronary flow and myocardial force of contraction

Condition	n	Coronary flow (ml min^{-1} g^{-1}) Before	Coronary flow (ml min^{-1} g^{-1}) During	Force of contraction (g) Before	Force of contraction (g) During
Dx (1.29×10^{-6} M)	20	7.4 ± 0.19	6.9 ± 0.23	2.7 ± 0.22	2.9 ± 0.25
Dx (4.3×10^{-6} M)	10	7.6 ± 0.24	7.2 ± 0.15	2.7 ± 0.10	2.5 ± 0.12
Dx (8.6×10^{-6} M)	10	7.8 ± 0.34	7.5 ± 0.42	2.9 ± 0.27	2.9 ± 0.18
Pv (2.66×10^{-6} M)	14	7.3 ± 0.26	7.1 ± 0.31	2.9 ± 0.26	2.8 ± 0.19
Pv (5.32×10^{-6} M)	22	7.6 ± 0.36	7.4 ± 0.40	2.5 ± 0.20	2.6 ± 0.32
PGE_2 (1.4 to 2.8×10^{-8} M)	20	7.4 ± 0.20	6.9 ± 0.18	3.1 ± 0.27	3.1 ± 0.18

The mean ± s.e. mean of basic coronary flow and force of contraction remained unaltered by the administration of diazoxide, papaverine or prostaglandin E_2. Higher concentrations of diazoxide or papaverine produced sustained coronary dilatation.

Table 2 Cardiac cyclic AMP levels during reactive hyperaemia

Time	0	5	10	20
Cyclic AMP (pmol/mg wet wt.)	0.998 ± 0.078	0.993 ± 0.0275	1.082 ± 0.076	1.082 ± 0.0669
Reactive hyperaemia (% increase in coronary flow)	0	6.4 ± 0.8	8.6 ± 1.2	11.3 ± 1.6

The levels of cyclic AMP measured in the same hearts in which reactive hyperaemia was induced, remained unchanged. Results are expressed as mean ± s.e. mean ($n = 6$ in each column).

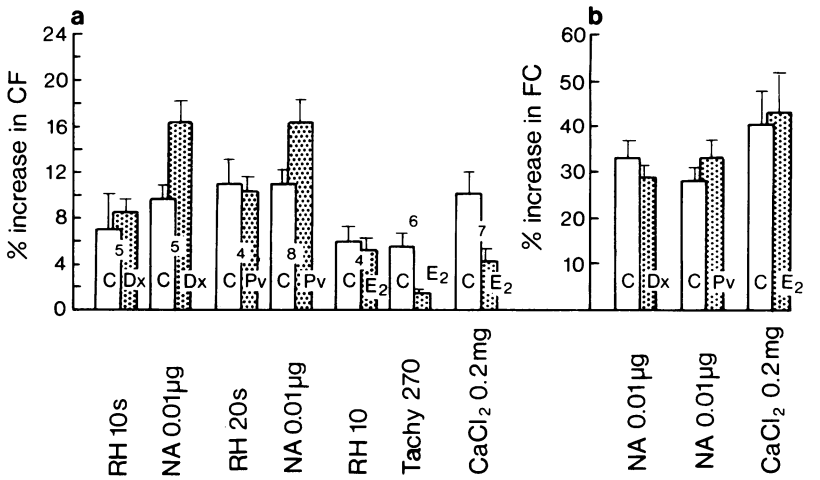

Figure 7 Influence of diazoxide, papaverine and prostaglandin E_2 on coronary reactive hyperaemia. Hearts of Sprague-Dawley rats paced at 210 beats/min were challenged by stoppage of the coronary inflow (RH) for 10 or 20 s, by noradrenaline (NA), tachycardia for 30 s (Tachy) and Ca^{2+}. (a) Mean % change of coronary flow (CF) is shown only for one dose of NA, Ca^{2+} or one period of tachycardia. Vertical lines show s.e. mean. C=control; Dx=diazoxide 2 mg/l; Pv=papaverine 2 mg/l; E_2=PGE_2 5–10 µg/l. In contrast to metabolic coronary dilatation induced by NA, Ca^{2+} or tachycardia, RH was unchanged by diazoxide, papaverine or prostaglandin E_2. (b) The force of contraction (FC) remains unchanged in response to NA or Ca^{2+} after diazoxide, papaverine or prostaglandin E_2.

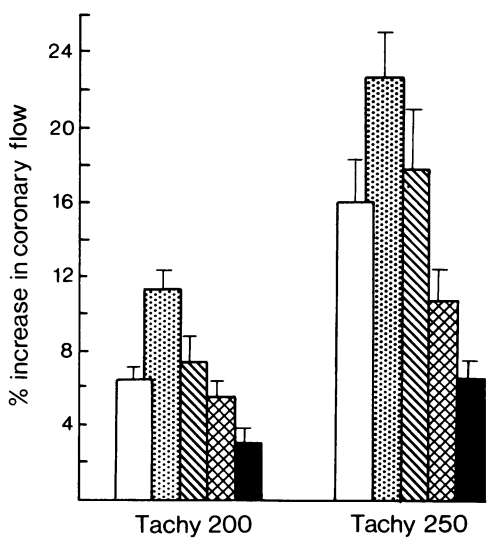

Figure 8 Influence of diazoxide and prostaglandin E_2 on the metabolic coronary dilator responses to tachycardia. Isovolumic hearts of 13 Wistar rats paced at 150 beats/min were challenged by tachycardia at 200 beats/min (Tachy 200) for 30 s and at 250 beats/min (Tachy 250) during control period (open columns) and during diazoxide 0.3 mg/l (stippled columns). This group of rats was subdivided to receive in addition prostaglandin E_2 1 µg/l, $n=4$ (hatched columns); 3 µg/l, $n=5$ (cross-hatched columns); and 5 µg/l, $n=4$ (solid columns).

investigate whether cardiac catecholamines are implicated in the coronary reactions to cardiostimulation or to hypoxia. CF reactions to NA, Ca^{2+}, tachycardia and RH in hearts from reserpine-treated rats are shown in Figure 10. These results indicate that endogenous catecholamines do not influence the MCD nor the RH reactions. The effectiveness of reserpine pretreatment was tested in Wistar rats in which heart catecholamine determinations were performed (5 normals and 5 reserpine-treated) by the modified method of Sellers, Flattery & Steiner, 1974 (by courtesy of Dr K.V. Flattery). Results indicate that with this procedure almost complete depletion was achieved.

Reactive hyperaemia and cardiac cyclic AMP levels

The hearts in which cyclic AMP was measured were paced at 210 beats/minute. In each case, the magnitude of RH induced by stopping the coronary inflow for 5, 10 or 20 s was recorded. About 20 min later the RH procedure was repeated, but the hearts were clamped at 5, 10 or 20 s for cyclic AMP determinations. Hypoxia up to 20 s did not change the cyclic AMP level of the rat myocardium (Table 2).

Influence of ventricular distention on metabolic coronary dilator response to noradrenaline, Ca^{2+} and tachycardia

The same cardiac stimuli imposed on a heart whose

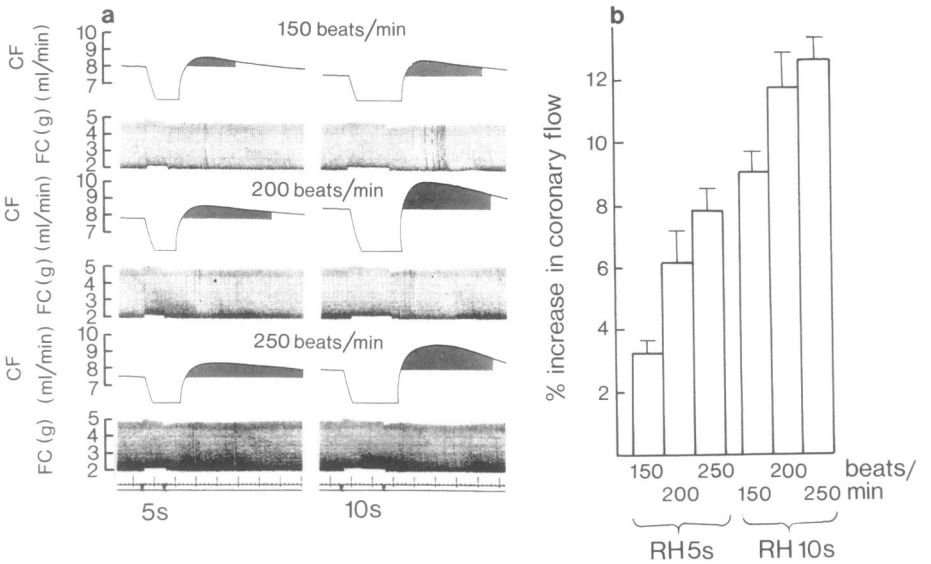

Figure 9 Influence of heart rate on coronary reactive hyperaemia (RH). RH induced in hearts paced at different rates indicated at the top of panels in (a) CF=coronary flow; FC=force of contraction. (b) The columns (mean %) indicate that the magnitude of RH depends on the heart rate and duration of the occlusion. Vertical lines show s.e. mean.

activity was recorded by an intraventricular balloon, produced greater increases in CF than in hearts in which a force transducer was used for measuring cardiac activity (Figure 11), even at initial tensions up to 5 grams. We therefore decided to investigate the MCD responses elicited by various agents at different initial distentions of the left ventricle. NA-elicited dose-dependent increases in CF can be demonstrated by both methods, but in the preparation with the ventricular balloon the CF response was about 2-fold higher than with force transducer (Figure 11). Hearts paced at 210 beats/min with an initial balloon volume set at 0.04 ± 0.0005 ml developed a systolic ventricular pressure of 59.3 ± 3.9 mmHg. When the balloon volume was increased to 0.09 ± 0.018 ml the systolic pressure increased to 76.8 ± 7.1 mmHg, i.e. about a 30% increase. Challenging doses of NA, Ca^{2+} or tachycardia, applied at the two ventricular volumes, resulted in an increase in total pressure developed and in a concomitant increase in CF. Thus, an increase in the intraventricular pressure enhanced the MCD reactions to cardiac hyperactivity even further.

Discussion

In the present experiments, the perfusion fluid supplied enough O_2 to ensure a substantial coronary reserve. Therefore, increases in CF could still occur as a result of diminished coronary vascular resistance. The data presented in this paper confirm that increases in CF are directly related to cardiac hyperactivity. This compensatory increase in CF, which appears so closely related to the increase in cardiac metabolism, was designated MCD (Sunahara & Talesnik, 1974). The concept of 'metabolic coronary dilatation' (MCD) is well recognized and included in many updated discussions on cardiovascular control (Vatner & Braunwald, 1975) but the question as to the mechanisms involved in this process is still open (Broadley, 1976). According to the adenosine hypothesis (Rubio & Berne, 1969; Rubio, Wiedmeier & Berne, 1974) the reduction in myocardial O_2 tension, or increased cardiac metabolic activity, would lead to breakdown of adenine nucleotides to adenosine or to its synthesis in the extramyocardial cell space (Nakatsu & Drummond, 1972). This would then induce dilatation of the coronary resistance vessels. Nevertheless, doubts concerning this hypothesis have been raised by several authors (Bittar & Pauly, 1971; Afonso, Ansfield, Berndt & Rowe, 1972) who showed that the administration of aminophylline left reactive hyperaemia responses unchanged but blocked the responses to adenosine. Moreover, it has been shown that adenosine 5′-triphosphate (ATP), adenosine 5′-pyrophosphate (ADP) and adenosine 5′-phosphate (AMP) are more potent coronary vasodilators than adenosine (Moir & Downs, 1972, 1973); ATP has been found in the perfusate of hypoxic guinea-pig heart (Paddle & Burnstock, 1974). Neither the

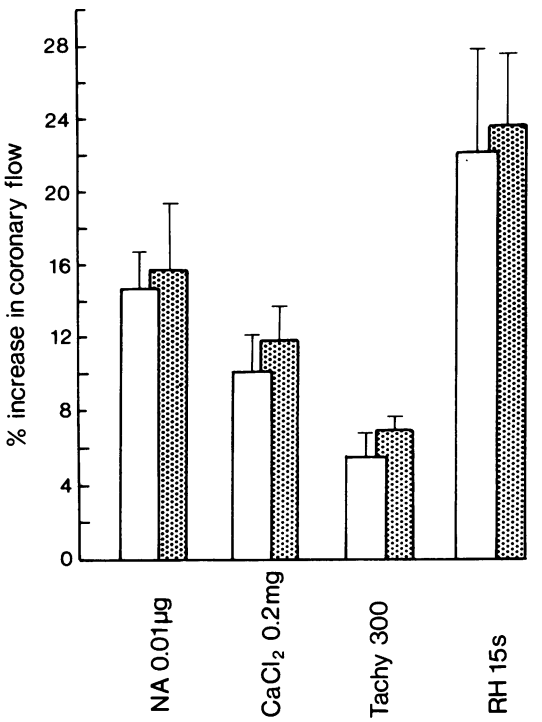

Figure 10 Coronary reactions in hearts of reserpine-treated rats. Similar metabolic coronary dilator and reactive hyperaemic responses were obtained in paced hearts of reserpine-treated and control Wistar rats; control (open columns) and reserpine-treated rats 5 mg/kg i.p. 24 h before the experiment (stippled columns).

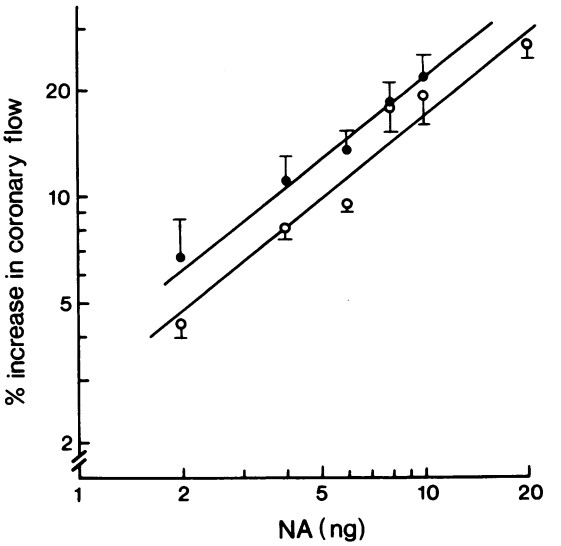

Figure 11 Comparative metabolic coronary dilator (MCD) reactions produced by cardiostimulation recorded with intraventricular balloon or with force-transducer. Hearts of Sprague-Dawley rats paced at 210 beats/min were challenged with noradrenaline (NA). The MCD response to cardiostimulation produced in isovolumic hearts (balloon) (●) was significantly higher than in non-working (force-transducer) hearts (○) (Addendum 2).

nucleotides nor adenosine may be claimed to be the sole mediators for the coronary responses elicited by myocardial hypoxia. Nevertheless, as Berne (1975) points out, since these studies and interpretations are quite controversial, we should await further investigations before the problem can be satisfactorily resolved.

An alternative model that could explain the MCD responses (Sen *et al.*, 1976) involves activation of the cardiac adenylate cyclase system. We have shown that this activation may be inhibited by prostaglandin E_1 or E_2 (Sunahara & Talesnik, 1974) and that the inhibition of cardiac synthesis of prostaglandin E_2 by aspirin-like substances leads to an enhancement of MCD (Talesnik & Sunahara, 1973). We postulated that the cardiac hyperactivity would trigger the formation of cyclic AMP. The present results support this hypothesis since the administration of cardiostimulating agents resulted in greater increases in cyclic AMP when the heart rates were higher. The MCD was also greater at higher heart rates. The direct correlation between cyclic AMP and MCD responses (Sen *et al.*, 1976) does not imply that the myocardial cyclic AMP produces the relaxation of the smooth muscle of the coronary resistance vessels. After a bolus dose of Ca^{2+} or NA, the peak values and full normalization of the cyclic AMP precede the increases in CF. Therefore, the cyclic AMP may only be considered a trigger metabolite for a more complex process in which adenosine or other substances may be involved.

It has been reported repeatedly that increases in heart rate are associated with increased myocardial oxygen consumption; this increased oxygen demand may be met by an increased coronary flow rate (Pitt & Gregg, 1968; Brink, Bester & Lochner, 1972; Cobb *et al.*, 1973). The characteristics of the enhancement of CF response to sudden increases in heart rate are similar to the MCD obtained with NA or Ca^{2+} inasmuch as they are enhanced by phosphodiesterase inhibitors (diazoxide or papaverine) and blocked by prostaglandin E_2. Moreover, since MCD can be elicited by Ca^{2+} or tachycardia, in hearts of reserpine-treated rats, catecholamine release need not necessarily be involved in the production of MCD. During the first period of induced tachycardia an

increase in cyclic AMP levels could be observed only at very high imposed heart rates; however, we have not yet studied this in hearts from reserpine-treated rats.

The most interesting finding, nevertheless, is that the challenge with the same dose of NA at higher heart rates, leading to larger cardiostimulation, produced greater elevations of MCD and cyclic AMP; the latter would be responsible for triggering the MCD responses. The observation obviously raises the question as to the mechanism by which cardiostimulation signals the adenylate cyclase for its modulated response. One might speculate that an activator would be involved which could be similar to the one described by Cheung, Bradham, Lynch, Lin & Tallant (1975), and which activates 3'5' nucleotide phosphodiesterase as well as the adenylate cyclase in the brain. The stimulation of adenylate cyclase requires Ca^{2+}, the effect being immediate and reversible. According to this, our original model (Sen *et al.*, 1976) could be modified by replacing the hypothetical signal I by the protein activator that would establish the link between cardiac hyperactivity and adenylate cyclase activation. In response to cardiac stimuli, the influx of Ca^{2+} or its release from its binding sites would activate the adenylate cyclase, resulting in an increase of intracellular cyclic AMP.

That the Ca^{2+} exchangeability may be increased in the isolated perfused heart muscle by different cardiostimulating agents is a well established fact (Nayler, McInnes, Chipperfield, Carson & Daile, 1970). Although the regulation of intramyocardial cell Ca^{2+} is an unsolved problem, there are indications that the Ca^{2+} taken up *in vitro* by the mitochondria, in absence of complexing anions, is easily released by a variety of treatments (Carafoli, 1975). It is relevant in this context to remember that the myocardium is dependent on the external calcium levels (Forester & Mainwood, 1974). Furthermore, the Ca^{2+} binding to the mitochondria is particularly important for the work performance, contractility and tension developed by the heart (Sulakhe & Dhalla, 1971). In addition, a source of activator calcium within the cardiac cell apparently exists in the sarcolemma, mitochondria and sarcoplasmic reticulum (Kirchberger, Tada, Repke & Katz, 1972; Endoh, Brodde & Schümann, 1975; Steer, Atlas & Levitzøki, 1975; Nayler, Dunnett & Burian, 1975; Lee, Balasubramanian & Dhalla, 1976).

In previous publications we showed that diazoxide produce an enhancement of the MCD response to NA and Ca^{2+}-induced cardiac hyperactivity. The interpretation offered was that this substance permitted the accumulation of cyclic AMP by inhibiting phosphodiesterase (Sen *et al.*, 1976). We do not have information about the actual degree of enzyme inhibition reached and therefore we decided to reinvestigate the problem by using larger concentrations of the same drug and also papaverine, whose effectiveness in inhibiting both Form I and Form II phosphodiesterases (Lugnier & Stoclet, 1974) is utilized as a standard reference for phosphodiesterase inhibition (Hanna, O'Dea & Goldberg, 1972; Amer & Kreighbaum, 1975). Both diazoxide and papaverine caused enhancement of MCD due to NA or Ca^{2+}; the increase of CF following tachycardia was also enhanced. Furthermore, the augmentation in MCD responses was greater with larger concentrations of diazoxide or papaverine, indicating that only a fraction of the phosphodiesterase was inhibited in our experiments. Unfortunately, these inhibitors are also smooth muscle relaxants, and the coronary dilatation elicited by higher concentrations of them interferes with the demonstration of MCD.

The increase in cardiac cyclic AMP level correlates with the MCD responses, but it does not correlate with the inotropic or chronotropic actions of NA or Ca^{2+} (Sen *et al.*, 1976). In the present experiments, we confirmed that the larger concentrations of diazoxide and papaverine, that should have produced greater accumulations of cyclic AMP, did not alter the inotropic action of NA; furthermore, when MCD was inhibited by prostaglandin E_2, the inotropic action also remained unaffected. These results are incompatible with the concept that there is a dose-relationship between levels of cyclic AMP and inotropic actions (Entman, 1974; McNeill & Verma, 1974). We may well be dealing with a dissociation process like that described by Lefkowitz & Levey (1972) in which the adenylate cyclase may be totally activated by NA at a time when only a small portion of available binding sites are occupied. In our experiments, the adenylate cyclase could be activated by the cardiostimulation itself, perhaps through a system similar to that suggested by Cheung *et al.* (1975). For these reasons, it would be interesting to investigate whether prostaglandin E_2 affects the protein-Ca^{2+} complex that activates adenylate cyclase.

At present we do not have an explanation for the signal which triggers the coronary vasodilatation in response to cardiostimulation. Increasing the left ventricular volume leads to a corresponding increase in myocardial oxygen consumption (Simaan, 1974) and increase in ventricular work leads to an increase in coronary blood flow (Nayler *et al.*, 1970). Our experiments confirmed these observations, because NA, Ca^{2+} or tachycardia resulted in greater MCD responses at increased levels of ventricular distention.

RH is often used to investigate the mechanisms of the vasodilatation response to myocardial O_2 demands. For this reason we investigated the RH responses in the presence of prostaglandin E_2, diazoxide or papaverine. At increasing heart rates, the volume of RH was also increased; this confirms the reports of Pauly, Zarnstoff & Bittar (1973) and Bache,

Cobb & Greenfield (1973). In contrast to MCD, the magnitude of RH responses was unaffected by either diazoxide or papaverine; furthermore, the addition of prostaglandin E_2 at concentrations which markedly inhibited MCD, left RH unchanged. It becomes obvious, then, that the coronary dilatation (MCD) response to increase in oxygen demands is produced by a different mechanism from that related to RH. These differences are further emphasized by the failure of hypoxia to induce changes in cyclic AMP levels like those produced by NA, Ca^{2+} or tachycardia.

Smooth muscle relaxation during RH apparently is not due to inability of the subcellular organelles to bind and accumulate calcium during hypoxia of short duration, because the diminution of Ca^{2+} available for release from the intracellular stores is still found when the heart is kept hypoxic for 10 min, or even longer (Lee *et al.*, 1976). During hypoxia there is release of NA and the consequent glycolysis could lead to RH (Wollenberger, Krause & Shahab, 1967) or MCD. However, in catecholamine-depleted hearts we obtained similar RH or MCD responses, confirming, at least in part, the observations of Koyama & Nakagawa (1972) in reserpine-treated dogs. At present, we cannot rule out the production of adenosine by the ventricular muscle due to short-lasting ischaemia (Thomas, Rubio & Berne, 1975). Our data do not provide evidence against a role of adenosine in the coronary dilatation produced by increased oxygen demands. The use of adenosine-sparing drugs such as dipyridamole has been extensively studied and their effects are taken as support for the adenosine hypothesis (Miura, Tominaga & Hashimoto, 1967; Berne, 1969; Liu & Feinberg, 1973; Sano, 1974). However, dipyridamole, besides affecting adenosine metabolism, is also a potent phosphodiesterase inhibitor (Huang & Daly, 1974). Moreover, certain phosphodiesterase inhibitors such as carbochromen (Aporti, Leon & Toffano, 1975) affect the adenosine uptake system similarly to dipyridamole or lidoflazine (Jageneau, Schaper & van Gerven, 1969).

In summary, we postulate that there are at least two types of regulatory processes of the coronary circulation, one in response to hypoxia, 'reactive hyperaemia', and another resulting from cardiac hyperactivity, metabolic coronary dilatation'. Olsson (1975) pointed out that RH is a complex response with physical and chemical determinants. We feel that MCD is also a complex response and that there are many situations in which it is difficult to separate one from the other; furthermore, it may be that they often interact with each other. In the present experiments the separation of mechanisms is based upon the fact that while RH is unaffected by prostaglandin E_2, diazoxide or papaverine, MCD responses are blocked by prostaglandin E_2 and enhanced by diazoxide or papaverine.

Statistical Addenda

L. ENDRENYI

Statistical addendum No. 1

The statistical comparisons including the association between heart rate and the change in coronary flow (Figure 2) were based on analyses of variance which considered the low and high concentrations of either NA or Ca^{2+} at the three heart rates, and the observations at each of these treatment combinations.

In the presence of either concentration of NA or Ca^{2+} the percentage volume change increased linearly with the heart rate ($t=3.53$, $P<0.05$ and $t=6.45$, $P<0.001$, respectively, with d.f.$=6$ for the average linear components, and $t=0.88$ and 0.99, $P>0.10$, respectively, for the quadratic contributions; separate analyses at the two dose levels also yielded statistically highly significant linear components).

At the two doses of Ca^{2+}, the slopes relating the percentage volume changes to heart rates were different ($t=3.55$, d.f.$=12$, $P<0.001$). The difference between the slopes observed at the two NA doses was statistically not significant, even though this significance was only barely missed ($t=1.89$, d.f.$=12$, $0.05<P<0.10$). The average percentage changes in coronary flow measured at the two concentration levels of NA and Ca^{2+} were also different ($t=3.28$ and 3.13, respectively, d.f.$=6$, $P<0.05$). In all these contrasts the quadratic contributions were negligible.

Very similar conclusions were reached when the absolute rather than the percentage changes of the coronary flow were considered.

Statistical addendum No. 2

Statistical assessment of the volume changes (ΔV) induced by various NA doses (ε) was based on a double-logarithmic relationship (Figure 11):

$$\log \Delta V = A + B \log \varepsilon$$

The expression was mechanistically and statistically reasonable since: (1) dose-response curves could be described (Parker & Waud, 1971) by

$$\Delta V = A' + c^n/(B'^n + c^n)$$

where A', B' and n are constants; (2) according to preliminary analysis the concentration range of the experiments was restricted to $c \ll B'$; (3) the relative and not the absolute observational error was found to be homogeneous.

Analysis of covariance indicated parallelism of the lines for the force transducer and balloon treatments, regardless of whether the volume changes were analysed in absolute or in percentage terms (for testing lack of parallelism $t=0.23$ and 0.78, respectively, with d.f.$=56$, $P \gg 0.05$). With both measures, the response

lines characterizing the force transducer and balloon treatments were substantially separated (for testing lack of coincidence $t=5.73$, $P<0.001$ and $t=2.95$, $P<0.01$ with d.f. $=57$ for lines involving absolute and percentage volume changes, respectively). The distinct responses attributable to the two treatments were indicated also by the adjusted volume changes: $11.90 \pm 0.53\%$ for the force transducer and $15.55 \pm 1.15\%$ for the balloon treatment.

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