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A comparison of the effects of a series of anti-anginal agents in a novel canine model of transient myocardial ischaemia

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1 An anaesthetized canine model of transient myocardial ischemia (TMI) has been developed in which reproducible and reversible electrocardiographic (ECG) and haemodynamic responses are exacerbated by electrical pacing.

2 The model could separate the ECG and haemodynamic effects of compounds with anti-ischaemic properties.

3 Compounds known to possess peripheral or coronary vasodilator properties did not necessarily alleviate the ECG consequences of TMI since glyceryl trinitrate was active whereas dipyridamole was not. The effects of verapamil were complicated by its adverse conduction effects while lidoflazine inhibited the ECG changes only during the ischaemic phase and the 'metabolic modulator' oxfenicine worsened the ECG response.

4 In a model considered to lack coronary reserve, improvements observed in the ischaemic ECG and global ventricular function were considered to result from a direct myocardial effect of the drugs examined rather than by a vascular influence. This was provided to the greatest degree by the Ca^{2+}-entry blockers nifedipine and nicardipine, with flunarizine adopting an intermediate position.

Introduction

The assessment of changes in S-T segment deflections obtained by epicardial electrocardiographic (ECG) mapping has been used experimentally as a means of dynamically representing the myocardial O_2 supply/demand ratio. Szekeres *et al.* (1976) described a canine model of electrical stress-pacing induced 'angina' employing a critical stenosis to reduce coronary blood flow (CBF) with epicardial recording of ECGs providing a gross electrophysiological index of oxygen debt. In addition it has been indicated that the ischaemic changes in this type of model were accompanied by increases in myocardial lactic acid and carbon dioxide production (Szekeres *et al.*, 1976; Szekeres & Udvary, 1983; Allely & Alps, 1987). Such models of transient myocardial ischaemia (TMI) have provided the basis of the version employed in the present study where temporary complete occlusion of the left anterior descending coronary artery (LAD) mirrors the ischaemic component of the disease state and superimposed electrical pacing provides a component of work-induced stress.

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The present study was carried out in order to: (a) evaluate the reproducibility and reversibility of the model and (b) assess a series of novel and clinically-employed 'anti-anginal' agents in terms of their electrophysiological and gross haemodynamic effects.

Methods

Experimental preparation

Adult beagles of either sex (10 to 20 kg) were deprived of food overnight and premedicated with acepromazine (0.2 mg kg^{-1} i.m.). General anaesthesia was induced with sodium pentobarbitone (Sagatal, 25 mg kg^{-1} i.v.) and maintained with this agent as required. The trachea was intubated and the dogs ventilated (respirator, Harvard) with room air at a rate of 10–12 min^{-1}, tidal volume 200–300 ml according to body weight. Body temperature was maintained at 38 ± 1°C by means of a rectal probe thermometer attached to a homeothermic blanket control unit (CFP 8185).

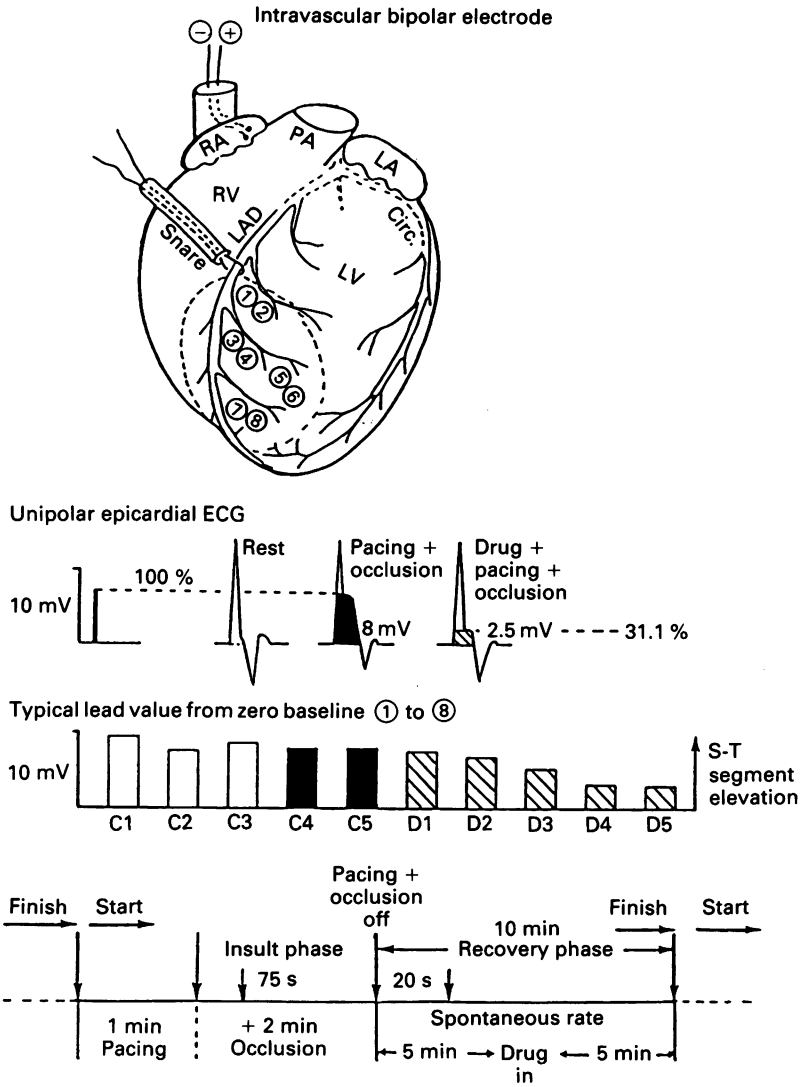

Figure 1 Arrangement of bipolar epicardial recording electrodes and experimental protocol to determine the electrocardiographic changes produced by transient myocardial ischaemia in the pentobarbitone-anaesthetized dog.

Haemodynamics

The left femoral vein was catheterized for drug administration. Ringer-lactate solution (250 ml) was infused before surgery as fluid replacement therapy. Phasic aortic blood pressure was recorded by means of a catheter inserted into the left femoral artery attached to a pressure transducer (Siemens, 746). Left ventricular systolic (LVSP) and end diastolic (LVEDP) pressures were measured by means of a catheter introduced into the left ventricular (LV) cavity via the left carotid artery and attached to a pressure transducer (Siemens, 746). The expression $dP\,dt^{-1}\,P^{-1}$ max was derived (Siemens 868 calculator) from LVSP to give a value for LV contractile force (CF). All haemodynamic data were recorded on a Mingograf (Siemens 804 inkspray oscillograph).

Coronary occlusion and S-T segment recording

Left lateral thoracotomy was performed via the 5th intercostal space. The lungs were retracted and the pericardial sac drawn up to 'cradle' the heart. A

length of the LAD was dissected free and a silk ligature loosely applied and drawn up a nylon tube for application of transient occlusion. Four close pairs of unipolar epicardial recording electrodes were sewn onto the potential ischaemic zone of the left ventricle (see Figure 1) for epicardial ECG recordings made via a Siemens 850 ECG amplifier and recorded on a Mingograf (Siemens, 804 inkspray oscillograph) made at a sensitivity of 1 mm = 1 mV. Pacing of the heart was performed by a pacing catheter inserted into the right atrium via the right jugular vein at 5 V, 1 ms duration (Grass S88 stimulator).

Experimental protocol

A schematic representation of the experimental protocol is shown in Figure 1.

The preparation was left to stabilize for 30 min. Pacing of the heart at a rate of 50–80 beat min^{-1} above resting heart rate (HR) was performed for 1 min before application of the LAD occlusion. Pacing plus occlusion was then carried out for a further 2 min. The next pacing event was after 10 min rest. Episodes of pacing and occlusion were repeated at least four times or until two consecutive challenges produced the same degree of S-T segment elevation with a return to baseline between challenges.

S-T segment elevations in response to each challenge were calculated for two distinct phases: (a) during the rising peak insult from ECG recordings made at 45, 60, 75, 90 and 105 s in the LAD occlusion period (5 time values). (b) Early during the recovery phase at 2 s intervals for the first 20 s (10 time values) following switching off electrical pacing and releasing LAD occlusion.

For each electrode the ischaemic effect was determined from a summation of the S-T segment voltages for a single heartbeat at the above time points. The two conditioning pre-drug S-T segment elevations were averaged and this value taken as 100%. Each test event thereafter was expressed as a percentage of this baseline value and a mean (±s.e.) percentage calculated. In 4 animals no drug was administered and without interrupting the timing cycle a further 8 challenges were performed to provide control responses. In treated animals a drug dose was administered 5 min into the reperfusion phase and the protocol repeated.

Drugs

Dipyridamole HCl (Boehringer Ingelheim); flunarizine HCl (Janssen Pharmaceuticals); glyceryl trinitrate (Wellcome); lidoflazine HCl (Janssen Pharmaceuticals); nicardipine HCl (Syntex); nifedipine (Bayer); oxfenicine HCl (Pfizer); verapamil HCl (Sigma).

Statistical analysis

Statistical significance of results was assessed by Student's two-tailed t test (Robson, 1973).

Results

Electrocardiographic parameters

Effects of repeat ischaemic episodes The initial 1 min pacing period produced only minimal effects on the S-T segment of the ECG. Pacing plus LAD occlusion produced a time-related rise in the S-T segment which gradually returned to its pre-insult level on reperfusion. Successive repeated ischaemic episodes subsequent to the conditioning challenges did not alter the electrocardiographic response to the challenge in the control dog group ($n = 4$) and those values are included for comparison in each figure.

Drug effects The effects of nicardipine ($n = 7$) and nifedipine ($n = 5$) on S-T segment elevations during the ischaemic and reperfusion phases are depicted in Figure 2a. Nifedipine had a greater effect than nicardipine during the ischaemic phase but both drugs produced almost identical effects during reperfusion.

Figure 2b shows the effects of dipyridamole (DPPP; $n = 5$) and glyceryl trinitrate (GTN; $n = 5$). Apart from the 5 $\mu g\,kg^{-1}$ dose during the ischaemic phase, GTN exerted a significant effect during both phases. DPPP reduced S-T segment elevation only during the ischaemic phase.

The effects of flunarizine ($n = 5$) and verapamil ($n = 5$) are shown in Figure 2c. Both drugs reduced S-T segment elevations to about the same extent at 200 $\mu g\,kg^{-1}$ during both phases, the effects being more pronounced during ischaemia. At 400 $\mu g\,kg^{-1}$ verapamil produced a 2:1 atrioventricular block in 2 dogs on electrical pacing. The S-T segment value is therefore artificially low at this point since the degree of ischaemia was not as great in these dogs and this data point has been omitted from the figure. Figure 2d depicts the effects of lidoflazine ($n = 5$) and oxfenicine ($n = 3$). Lidoflazine produced a significant effect during the ischaemic phase but on reperfusion it was ineffective at doses up to 1 $mg\,kg^{-1}$. Oxfenicine was also active in the ischaemic phase at doses of 200 $\mu g\,kg^{-1}$ and above but at low doses it actually exacerbated the S-T segment elevations. A small reduction was noted at 1 $mg\,kg^{-1}$ but this had disappeared by cumulative addition of 10 $mg\,kg^{-1}$.

General haemodynamics

Effects of repeat ischaemic episodes In the absence of drug treatment, the overall effect of the ischaemic challenge in any group was to reduce mean arterial

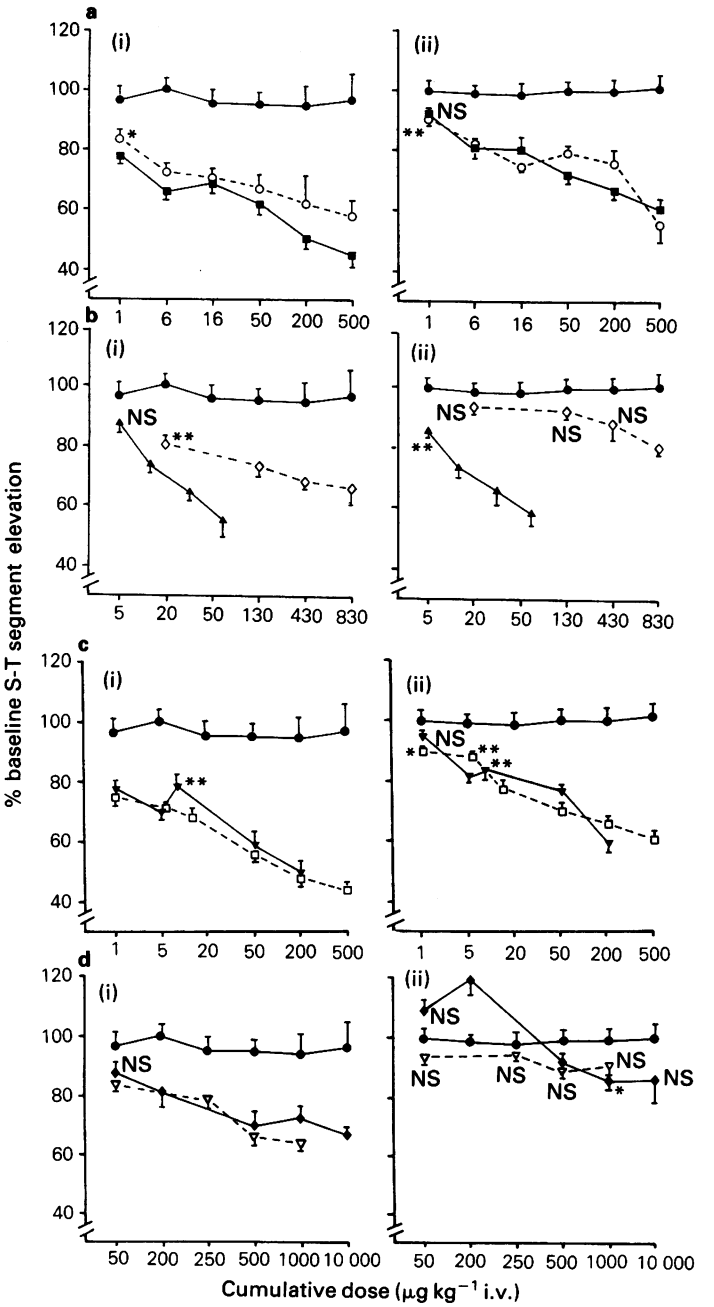

Figure 2 The effects of (a) nicardipine (○; $n = 7$) and nifedipine (■; n = 5); (b) glyceryl trinitrate (▲; $n = 5$) and dipyridamole (◇; $n = 5$); (c) flunarizine (□; $n = 5$) and verapamil (▼; $n = 5$); (d) lidoflazine (▽; $n = 5$) and oxfenicine (◆; $n = 5$), on epicardial S-T segment elevations during the ischaemic (i) and reperfusion (ii) phases of transient myocardial ischaemia in the pentobarbitone-anaesthetized dog. Values represent mean and vertical lines s.e.mean. All points are significantly lower than values in control dogs (●; $n = 4$) at $P < 0.001$ except as indicated NS, $*P < 0.05$ and $**P < 0.01$, Student's two-tailed t test.

blood pressure (MABP) and increase LVEDP while CF remained essentially unchanged. Changes in systolic and diastolic BP followed similar directional changes and the impact of ischaemia on the pump was predominantly reflected through the systolic component. For convenience, the data presented relates to MABP. MABP appeared to recover well between challenges on reperfusion but this was not always the case with LVEDP in some dogs where it remained elevated. Variability in haemodynamic responses to the challenge between animals in any given group appeared to depend upon how much of the myocardium was involved in each animal. A consideration of this variability and the possible impact of drug intervention is made later (Limitations of study section).

Drug effects Drug effects on haemodynamic parameters (excluding HR) are shown in Table 1. The values at the dose producing the optimal reduction in S-T segment elevation (or the highest dose examined in the absence of a reduction) are also given.

Nicardipine: Nicardipine ($16\,\mu g\,kg^{-1}$) had no effects on HR but it decreased resting MABP. The falls in MABP associated with ischaemia and reperfusion were not altered by nicardipine. Nicardipine reduced resting LVEDP and reduced the LVEDP elevated during the challenge. Resting CF was increased and this increase persisted during the challenge.

Nifedipine: Nifedipine ($16\,\mu g\,kg^{-1}$) produced a small fall in resting HR (about 6%). MABP was unaltered at rest or during ischaemia but the reduction associated with reperfusion was abolished. Resting LVEDP was reduced and the increase produced by the challenge was also decreased. No effects were noted on baseline CF but the depression associated with the challenge was abolished.

Table 1 The effects of nicardipine (Nic), nifedipine (Nif), glyceryl trinitrate (GTN), dipyridamole (DPPP), flunarizine (Flu), verapamil (Ver), lidoflazine (Lid) and oxfenicine (Oxf) on mean arterial blood pressure (MABP, mmHg): left ventricular end diastolic pressure (LVEDP, mmHg) and left ventricular contractility (CF, s) in a pentobarbitone-anaesthetized canine model of transient myocardial ischaemia

				Challenge			
		Baseline		*Ischaemia*		*Reperfusion*	
Drug	*Parameter*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Nic	MABP	104 ± 4	88 ± 6	92 ± 6	85 ± 6	97 ± 4	96 ± 4
$n = 7$	LVEDP	5.1 ± 0.7	4.1 ± 1.1	7.3 ± 0.9	6.6 ± 1.4	7.2 ± 0.8	6.1 ± 1.3
(16)	CF	129 ± 7	140 ± 14	135 ± 9	149 ± 13	138 ± 10	152 ± 11
Nif	MABP	106 ± 3	102 ± 7	68 ± 9	69 ± 6	85 ± 9	92 ± 8
$n = 5$	LVEDP	4.4 ± 0.5	3.8 ± 0.9	6.7 ± 0.4	5.6 ± 0.6	7.5 ± 0.4	6.0 ± 0.5
(16)	CF	132 ± 8	132 ± 10	120 ± 9	145 ± 22	124 ± 5	132 ± 6
GTN	MABP	91 ± 8	91 ± 8	78 ± 5	76 ± 9	79 ± 6	75 ± 8
$n = 5$	LVEDP	6.4 ± 0.5	5.8 ± 1.1	9.5 ± 0.7	7.8 ± 1.5	9.8 ± 0.6	8.1 ± 0.7
(35)	CF	125 ± 12	123 ± 14	131 ± 10	158 ± 7	115 ± 12	116 ± 8
DPPP	MABP	124 ± 8	97 ± 13	87 ± 7	88 ± 11	83 ± 10	80 ± 12
$n = 5$	LVEDP	6.7 ± 0.6	7.8 ± 0.6	7.2 ± 0.6	7.5 ± 0.6	9.1 ± 1.1	10.4 ± 1.5
(830)	CF	127 ± 20	130 ± 9	136 ± 8	129 ± 40	109 ± 7	110 ± 13
Flu	MABP	111 ± 7	109 ± 7	72 ± 12	68 ± 7	101 ± 14	93 ± 3
$n = 5$	LVEDP	4.2 ± 0.3	4.4 ± 1.2	7.3 ± 0.7	8.2 ± 1.6	7.6 ± 0.5	7.2 ± 1.1
(16)	CF	142 ± 10	154 ± 27	140 ± 20	116 ± 21	143 ± 12	130 ± 23
Ver	MABP	104 ± 9	99 ± 10	96 ± 9	101 ± 13	99 ± 9	100 ± 12
$n = 5$	LVEDP	3.1 ± 0.6	3.2 ± 1.1	4.1 ± 0.7	3.8 ± 0.8	4.8 ± 0.7	4.5 ± 1.1
(50)	CF	119 ± 4	110 ± 9	137 ± 3	136 ± 6	127 ± 6	120 ± 9
Lid	MABP	109 ± 12	97 ± 11	85 ± 9	94 ± 11	92 ± 7	97 ± 11
$n = 5$	LVEDP	4.9 ± 0.8	4.1 ± 1.0	6.7 ± 0.9	6.9 ± 1.8	7.2 ± 0.2	6.5 ± 1.7
(1000)	CF	127 ± 12	138 ± 10	128 ± 4	128 ± 7	139 ± 8	140 ± 7
Oxf	MABP	79 ± 12	81 ± 3	65 ± 12	62 ± 6	77 ± 11	92 ± 4
$n = 3$	LVEDP	6.6 ± 0.8	6.7 ± 1.4	7.0 ± 0.8	6.8 ± 1.3	9.0 ± 1.6	6.2 ± 1.9
(10,000)	CF	127 ± 12	140 ± 20	122 ± 18	113 ± 33	121 ± 16	103 ± 17

Each of the 'pre-values' for baseline and peak challenge represent the mean ± s.e.mean values for the pre-drug test cycles with each animal contributing its average value of the final two conditioning events at each point. The 'post-values' indicate the new level for each parameter associated with the cumulative dose of the drug concerned producing the optimal reduction in S-T segment elevation, or the highest dose examined in the absence of a reduction. The group n values are given at the doses ($\mu g\,kg^{-1}$ i.v.) shown in parentheses

Glyceryl trinitrate: HR was essentially unaltered by GTN ($35\,\mu g\,kg^{-1}$) while MABP was only slightly reduced on reperfusion. Resting LVEDP was reduced by GTN and the increase noted during the challenge also decreased. CF was increased only during reperfusion.

Dipyridamole: DPPP at $830\,\mu g\,kg^{-1}$ (a dose which only just began to affect S-T segment elevations on reperfusion) had no effects on MABP. Resting LVEDP was increased by DPPP and the increases produced by the challenge were not lessened. CF was not altered.

Flunarizine: Flunarizine ($16\,\mu g\,kg^{-1}$) did not affect resting MABP or HR and hardly affected the depressor effects of the challenge. There was no alteration of LVEDP during the resting or reperfusion phases but it increased during ischaemia. CF was increased at rest but decreased during the challenge.

Verapamil: Verapamil exerted little effect on MABP or HR at $50\,\mu g\,kg^{-1}$. Resting LVEDP was unaltered by verapamil and the elevated LVEDP noted during the challenge was very slightly reduced. CF was reduced slightly at rest and on reperfusion.

Lidoflazine: Lidoflazine ($1\,mg\,kg^{-1}$) had no effects on resting HR or MABP, but a slight lessening of the depressor effect of the challenge was noted. Resting and reperfusion LVEDP were decreased. Resting CF was increased with no alteration during the challenge.

Oxfenicine: Oxfenicine did not affect resting or ischaemic MABP but the depressor effect of reperfusion was reversed to a pressor effect. Resting and ischaemic LVEDP values were unaltered while the rise on reperfusion was abolished. Resting CF was slightly increased but CF was decreased during the challenge.

Discussion

The two-component model (ischaemia and reperfusion) of TMI described in the present study and the method of data analysis has provided a reproducible and reversible epicardial ECG response to the ischaemic conditioning of the canine myocardium to an on/off stress-pacing protocol.

The compounds studied appeared to exert a range of effects on the ischaemic myocardium. The onset of useful haemodynamic and ECG effects of a drug did not always coincide. It was evident that not all drugs demonstrated ECG activity in both phases of the challenge, although agents which have aroused clinical interest were clearly active in both phases. This applied to nicardipine, nifedipine, verapamil, GTN and flunarizine. Where LVEDP was reduced and CF enhanced, ventricular performance was considered improved and this was a feature seen with several drugs during the ischaemic phase. In the event that this feature persisted into the reperfusion phase, as with nicardipine and nifedipine, metabolic recovery must have been facilitated, with a resultant global increase in ventricular compliance. Rousseau *et al.* (1985, 1986) have shown that nicardipine improved biochemical function of the human ischaemic myocardium with only marginal effects on coronary blood flow (CBF). The haemodynamic and ECG properties of nicardipine appear to be separated: at doses below $16\,\mu g\,kg^{-1}$ it lowered S-T segment rises in the absence of haemodynamic effects.

Where CF was not enhanced on reperfusion as with lidoflazine, GTN and verapamil, LVEDP could still be lowered. Here it was evident that the ventricle could still operate effectively at a reduced muscle fibre length.

Nifedipine is a vasodilator with little direct myocardial effect *in vivo* and has also shown good activity in a dog TMI model of critical LAD stenosis (Alps *et al.*, 1985). In the present study its effect in reducing S-T segment elevations was very similar to that of nicardipine. Our observations for GTN were in keeping with the findings of Szekeres & Udvary (1983). It would appear that the ECG and haemodynamic effects of this drug are not directly linked. Although in the non-ischaemic canine ventricle there is no transmural flow gradient (Jennings & Reimer, 1979), there is the possibility of an association of the haemodynamic and ECG effects of GTN via its reported improvement of subendocardial blood flow to the ischaemic zone, by direct coronary conductance vessel dilatation which may not be of a sufficient magnitude to affect our overt haemodynamic recordings (Cohen & Kirk, 1973; Szekeres & Udvary, 1983). The cardioinhibitory activity of verapamil has been described by Himori *et al.* (1976) who found it to be a negative inotrope in the same dose range as that producing coronary vasodilatation. It is interesting to note that the beneficial electrocardiographic effect of verapamil at $1–200\,\mu g\,kg^{-1}$ overlapped the dose range $100–200\,\mu g\,kg^{-1}$ which according to Warltier *et al.* (1981) produced coronary vasodilatation. Our earlier studies with verapamil in the critical stenosis model also showed activity for this drug at low doses (Alps *et al.*, 1985). The doses producing S-T segment reduction and those producing detrimental myocardial conduction effects ($400\,\mu g\,kg^{-1}$ in the present study with a pacing frequency-dependent atrioventricular block at $100–400\,\mu g\,kg^{-1}$ in a previous study – Allely & Alps, 1988) do not appear to be very well separated.

Lew & Ban-Hayashi (1985) demonstrated that by elevating LVEDP above the ischaemia-induced

increase, the global mechanical disadvantage imposed by the ischaemic zone was diminished. The three drugs in this study demonstrating such an effect during ischaemia with corresponding ECG improvements were flunarizine, DPPP and lidoflazine. The ECG benefit of flunarizine was sustained throughout both phases of the challenge but it failed to improve ventricular performance; CF fell whilst LVEDP rose. The other two drugs failed to confer ECG improvement on reperfusion. In their model of critical stenosis Szekeres *et al.* (1976) also found DPPP to be inactive ($60\,\mu g\,kg^{-1}$). This is an interesting observation since DPPP is a coronary dilator exerting its effects primarily on coronary resistance vessels (Becker, 1976) thus increasing subendocardial flow in the ischaemic zone and, as with GTN, this may be responsible for the onset of ECG improvement at high doses. DPPP certainly did not improve ventricular performance. Like DPPP, lidoflazine is a long-acting coronary artery dilator (Schaper *et al.* 1966; Gobel, 1980). It has been shown to be of benefit in the long-term treatment of effort angina (Bernstein & Peretz, 1972), a property attributed to its calcium entry blocking activity (Van Neuten & Wellens, 1979), yet it has negligible effects on the myocardium (Vanhoutte & Van Neuten, 1980).

Oxfenicine was included in the present study since it has been shown to increase cardiac glucose oxidation in ischaemia at the expense of utilizing free fatty acids (Higgins *et al.*, 1981), thus yielding more ATP than aerobic oxidative pathways alone (Drake-Holland & Passingham, 1983). The haemodynamic activity of oxfenicine could be interpreted cautiously as providing an overall improvement in left ventricular function and efficiency since the ventricle was able to contract to a low LVEDP. Since a fall in CF was accompanied by an increased blood pressure on reperfusion, a systemic pressor effect may be exerted during this phase which falls back to baseline at rest. However, these haemodynamic effects must also have occurred at the expense of improving segmental efficiency, since there was no decrease in S-T segment elevations. Indeed, at lower doses the increase in S-T segment elevations was exacerbated, suggesting that a global preload improvement on reperfusion accompanied a local worsening of ventricular performance.

The overall findings of the study indicate that the canine model of TMI developed here is a useful predictor of the anti-anginal efficacy of a compound. The differentiation of the onset of ECG and haemodynamic effects at different doses of a compound may indicate a separation of its mechanisms of action and these can be determined in the model. The two compounds shown to be inactive on the ECG effects during the reperfusion (lidoflazine and DPPP) are those which have aroused most clinical controversy in terms of whether or not they alleviate the symptoms of angina pectoris and the side-effects which they produce (Sbar & Schlant, 1967; Hanley & Hampton, 1983; Loeb *et al.*, 1983; Fazekas & Kiss, 1984). The compound which actually exacerbated the S-T segment elevations during the recovery phase at lower doses (oxfenicine) exerted a complex, seemingly contradictory, haemodynamic profile and a clinical application for such a metabolic modulator with no ECG benefit is uncertain. Nicardipine and nifedipine were not cardiodepressant at doses showing optimum ECG benefit during the challenge. They improved ventricular performance during the challenge while flunarizine displayed intermediate effects. In contrast, in the case of verapamil and GTN this feature was projected predominantly only during the ischaemic phase with little influence on ventricular function during reperfusion. Verapamil did not improve ventricular CF at doses associated with optimum beneficial ECG effects, and increasing the dose precluded any effective further benefit due to the A-V block incurred.

Limitations of the study

Inter-dog variability, with regard to the pattern and distribution of epicardial coronary arteries and their anastomoses, determined that the occluded vascular beds would be unequal in size and volume between hearts. This in turn dictated for any given animal whether TMI would have sufficient impact on the ventricular segment involved to embarrass mechanically the whole cardiac pump, even though the ECG evidence indicated serious local metabolic dysfunction.

We do know from other studies using our model that the reversible S-T segment changes are accompanied by reversible changes in lactic acid and CO_2 production, plasma K^+ levels and pH in local myocardial venous blood collected during ischaemia (Allely *et al.*, 1987), even though the hearts involved were not obviously compromised mechanically.

In an anaesthetized, thoracotomized animal the ischaemic heart cannot compensate by dilating to overcome the mechanical disadvantage imposed by the ischaemic segment. Rather, compensation features ballooning with an elevated LVEDP. In some animals this was obvious, and made worse by repeated ischaemic challenges, while in others no changes were evident. Thus, some hearts did not recover to their pre-ischaemic condition and featured a persistently elevated 'baseline' LVEDP. This condition did appear to be improved following the application of some drugs (e.g. nicardipine). Variation in haemodynamic responses to ischaemia made it impossible to apply statistical analysis methods to such changes and the effects of drugs on them in

small groups of animals. Nevertheless, when obvious haemodynamic responses did occur in any group the apparent effectiveness of a particular drug treatment was considered to be 'clinically' relevant to the known pharmacological profile of the agent.

Under the experimental conditions operated in the study, allowing 10 min between challenges, we were able to make only limited observations on the ability of drugs to favour the extent, as opposed to the rate which we measured, of recovery of the ischaemic myocardium. Nevertheless, as previously stated, the recovery period does allow time for reversal of local ECG and biochemical changes.

Whilst appreciating that the myocardium has been 'stunned' by repeated insults, and that not all hearts recovered haemodynamically between challenges, the time intervals followed have been evolved to standardize the acute experimental conditions. The assumption has been made that TMI is largely reversible, as in angina, otherwise the heart would infarct or fail, and thus the acute effects created in the model simulate some of the classical features of the human disease.

The technique is one used for *in vivo* 'screening' of drugs with unknown mechanism, it identifies different classes of drugs of known clinical interest and allows multiple doses to be examined in a reasonably short-duration study.

It is known from other studies in our laboratory (unpublished findings) employing the technique of radiolabelled microspheres, to measure regional and total CBF, that in this model CBF is only approximately 34% of normal during ischaemia. This residual flow must include contributions made from any pre-existing collateral circulation. Under these conditions doses of nicardipine which would cause marked coronary dilatation in normal hearts relieve S-T segment changes but do not increase ischaemic blood flow, other than to favour slightly the epicardial/endocardial gradient, and do not cause coronary steal.

Thus the model of TMI as operated here is one which has very limited coronary reserve and appears to identify pharmacological effects which are exerted predominantly on the myocardium and not via changes in blood supply. Certainly, when the critical stenosis technique is used there does appear to be coronary reserve and excessive dilatation compromises the benefit of direct myocardial action.

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