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Effects of an increase in haemoglobin O_2 affinity produced by BW12C on myocardial function in the erythrocyte-perfused rabbit heart *in vitro* and myocardial infarct size in the dog

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1 The effects of BW12C on myocardial function in the erythrocyte-perfused rabbit heart and on myocardial infarct size in the anaesthetized dog have been evaluated.

2 Perfusion of rabbit hearts with erythrocytes pretreated with BW12C (10^{-3} M–4×10^{-3} M) produced concentration-dependent decreases in left ventricular pressure (LVP), LVP dP/dt and coronary perfusion pressure. A concomitant decrease in P_{O_2} and an increase in lactate production by the myocardium was also observed.

3 Perfusion of rabbit hearts with Krebs Henseleit buffer containing BW12C (10^{-5}–10^{-4} M) caused no change in measured variables. Although BW12C (10^{-3} M) caused a small decrease in LVP, coronary perfusion pressure and heart rate, these changes were not significant.

4 In anaesthetized dogs, an infusion of BW12C (total dose 50 mg kg^{-1}, i.v.) caused small, but significant, changes in haemodynamic status. The oxygen saturation curve was shifted to the left and relative % oxygenation (P_{20}) was shifted to the left throughout the course of the experiment. (P_{20}, control 16.3 ± 0.4 mmHg; after BW12C 7.9 ± 1.4 mmHg).

5 Pretreatment with BW12C (total dose 50 mg kg^{-1}) caused no change in area at risk but significantly increased the myocardial infarct size by 410%.

6 These studies with BW12C demonstrate that alteration in haemoglobin-oxygen affinity can induce adaptive physiological changes in tissue function and metabolism and can assume a critical role when oxygen supply may be impaired due to a flow-limiting stenosis.

Introduction

Under normal physiological conditions, changes in oxygen supply in response to a changing demand on the myocardium is adequately controlled by the coronary blood flow (Berne, 1964). During myocardial ischaemia as a result of a flow limiting coronary stenosis, tissue oxygenation and preservation becomes more dependent upon the coronary perfusion pressure and the formation of a collateral circulation (Kirk & Sonneblick, 1982). However, an often overlooked determinant of oxygen delivery to tissue is the relationship that describes the binding of oxygen to haemoglobin and its subsequent dissociation: the haemoglobin oxygen saturation curve (OSC) (Sladen, 1981). The relationship between haemoglobin oxygen affinity, oxygen delivery and myocardial function has not been adequately investigated due to the lack of specific pharmacological agents able to modify the OSC in a quantitative manner.

A pharmacological agent, BW12C (5-(2-formyl-3-hydroxyphenoxy pentanoic acid), has recently been found to bind preferentially to the oxy conformation of haemoglobin at a site between the amino terminal residues of the α sub units. BW12C increases the oxygen affinity of haemoglobin in a concentration-dependent fashion and thus can be used to produce graded leftwards shift of the OSC (Beddell *et al.*, 1984). We have used this agent to determine the importance of haemoglobin oxygen affinity on myocardial function using an erythrocyte-perfused rabbit heart preparation *in vitro*. In addition, the effects of an impaired oxygen delivery on the fate of ischaemic tissue in a canine occlusion/reperfusion model of experimental myocardial infarction has been studied.

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Methods

Erythrocyte-perfused rabbit heart studies in vitro

The erythrocyte-perfused rabbit preparation used for these studies was as described by Bergmann *et al.* (1979). Briefly, New Zealand white rabbits of either sex, weighing 2.2–2.5 kg were stunned by a blow to the head. The hearts were rapidly excised and perfused retrogradely at a constant flow rate via the aorta with non-recirculating oxygenated (95% O_2:5% CO_2) Krebs-Henseleit buffer at 37°C containing bovine serum albumin 0.3 mM, dextrose 10 mM, insulin 70 mu l^{-1} and the following ions in mmol l^{-1}: Na^+ 143, Cl^- 128, K^+ 6.1, Ca^{2+} 2.5, Mg^{2+} 1.2, SO_4^{2-} 1.2, HCO_3^- 24.9 and $H_2PO_4^-$ 1.4.

Within minutes of excision of the heart, the pulmonary artery was cannulated and the caval and pulmonary veins ligated, thus 90% of flow egressed via the pulmonary artery. Isovolumically beating preparations were prepared by passing a fluid-filled latex balloon into the left ventricle via the left atrium. The balloon was connected to a Gould P23 Gb pressure transducer via a short length of polyethylene tubing and left ventricular pressure (LVP) and its first derivative LVP dP/dt, were recorded continuously. Left ventricular end-diastolic pressure (LVEDP) was adjusted by changing the volume of the balloon. Coronary perfusion pressure (CPP) was monitored continuously with the use of a side arm cannula placed above the aortic valve and connected to a pressure transducer. Pacing electrodes were sutured to the right atrial appendage and hearts were paced at 180 beats min^{-1} throughout the experimental period.

After the preparation was complete, perfusion was either initiated with medium enriched with erythrocytes or maintained with erythrocyte-free Krebs-Henseleit albumin solution. Erythrocyte-enriched buffer was maintained at 0–4°C (to prevent the production of lactate) and warmed to 38°C before perfusion through the heart via a heating coil. Oxygenation of the erythrocyte suspension was achieved by equilibration with room air. Horse erythrocytes were used for the experimental preparation because of their availability and furthermore, a sufficient quantity of erythrocytes could be obtained from one animal for two isolated heart preparations. Horse blood (2l) was collected on the day prior to use in glass sterilized bottles containing an appropriate quantity of citrate, phosphate, dextrose buffer as an anticoagulant. On the day of use, the blood was centrifuged (15 min at 2000 *g* at 4°C) and the plasma and buffy coat aspirated. Erythrocytes were washed twice in physiological saline (at 4°C) and then resuspended in Krebs-Henseleit-albumin solution to yield a final haematocrit of 36–40%.

During the perfusion of the isolated heart with the erythrocyte-enriched buffer, the effluent from the pulmonary artery was taken at 15 min intervals and used for measurement of blood gas tensions, pH, haemoglobin concentration. (Radiometer, ABL3) and lactate concentration. The OSC was obtained for the erythrocyte enriched buffer before its perfusion through the isolated heart and measured with a Haemoscan (Aminco). From these the degree of left-shift was assessed and the saturation of haemoglobin with oxygen at the appropriate P_{O_2} measured. The oxygen content of the arterial (pre-heart) and the venous (pulmonary artery effluent) circulations and the amount of oxygen extracted by the myocardium were calculated from the product of the measured haemoglobin content, measured percent haemoglobin saturations and 1.39 (the amount of O_2 bound g^{-1} of saturated haemoglobin + $0.003 \times P_{O_2}$ mmHg). (The amount of dissolved O_2 as described by Bergmann *et al.*, 1979).

Five groups of experimental preparations were used. In group 1 ($n = 5$), rabbit hearts were perfused with an erythrocyte containing buffer for 90 min and the stability of myocardial function throughout the time period was assessed. In group 2 ($n = 5$), rabbit hearts were initially perfused for 30 min with the erythrocyte containing buffer and then with the buffer pretreated with BW12C 10^{-3} M for a further 30 min. Recovery of the preparation from drug action was monitored by returning to a perfusion with untreated erythrocytes. In groups 3 and 4 ($n = 5$), an identical protocol was used, but erythrocytes were pretreated with 2×10^{-3} M and 4×10^{-3} M BW12C, respectively.

To assess the direct pharmacological effects of BW12C, as distinct from its actions on haemoglobin oxygen affinity, in group 5 ($n = 5$), the effect of increasing concentrations of BW12C on cardiac function in the Krebs-Henseleit perfused, erythrocyte-free, rabbit heart was evaluated by use of the same protocol as described for the previous 4 groups with the exception that these hearts were not paced. Since BW12C binds with a high affinity and specificity to the haemoglobin molecule the concentration appearing free in the erythrocyte buffer is very small (10^{-6} M); therefore the dose range evaluated in these preparations (10^{-5}–10^{-3} M) was in great excess of those concentrations which would appear free to exert a direct pharmacological action. Data were analysed by Student's paired *t* test.

Experimental myocardial infarction studies

Beagle dogs of either sex, weighing between 10.0 and 15.0 kg were used. Anaesthesia was induced by intravenous administration of thiopentone sodium (30 mg kg^{-1}) and supplemented as required with a pentobarbitone sodium and α-chloralose mixture (12 mg kg^{-1} pentobarbitone and 40 mg kg^{-1} α-

chloralose). The jugular and femoral veins were cannulated for administration of drugs and a femoral artery was cannulated for measurement of blood pressure and, at intervals throughout the experiment, withdrawal of blood samples for analysis of blood gases, OSC and pH. A cannula was inserted into the left ventricle via the left carotid artery for measurement of LVP and LVP *dP/dt*. A tracheal cannula was inserted for mechanical ventilation with room air from a Palmer, large animal, respiration pump (approx 15 ml kg^{-1} and 19 strokes min^{-1}) adjusted if necessary to maintain blood gases under control conditions within normal limits. The electrocardiogram was recorded from a standard limb lead II. Body temperature was maintained at 40°C by infrared heating lamps. All physiological recordings were made on a Beckman R611 Polygraph.

A left thoracotomy was performed in the fifth intercostal space, the lungs gently retracted and the heart supported in a pericardial cradle. The left anterior descending coronary artery (LAD) was cleared distal to the first major diagonal branch and an occlusive snare was placed around the vessel. The LAD was occluded for 1 h and then reperfused for 2 h. Following the period of reperfusion, a polyethylene cannula was inserted into the LAD at the site of occlusion and 0.9% NaCl was infused via the LAD cannula at a pressure equivalent to mean systemic blood pressure. A concentrated solution of Evans blue dye (approximately 30 ml) was administered systemically via the femoral vein, followed immediately by a saturated solution of potassium chloride to induce cardiac arrest. By using this procedure, all but the LAD coronary bed perfused with saline was stained blue, hence effectively demarcating the area at risk (Allan *et al.*, 1985).

The heart was rapidly removed, washed in saline and sectioned by transverse slicing (0.5–1 cm in thickness) from apex to base. The right ventricle was separated and discarded. Each slice was then photographed with a Nikon Medical camera to record the area at risk delineated by the Evans blue.

The transmural distribution of the infarct was measured by a macroscopic staining technique. After recording the area at risk, tissue slices were incubated at 37°C for 30 min in a phosphate-buffered solution of triphenyltetrazolium chloride (TTC) to differentiate normal and infarcted myocardium. Each transverse slice was photographed again to record the area of infarction delineated by the TTC.

Following photographic development, colour photographs of serial sections were independently viewed, magnified 5 times and traced onto clear acetate sheets. Areas of risk and infarction were then measured by computer assisted planimetry using an Apple Graphic system. The measurement procedure was carried out independently by an individual unaware of the experimental protocol.

Infarct size as delineated by TTC staining measured from tracings of the transverse slices of myocardium was calculated as a percentage of the total area at risk and expressed as a median of the group.

Experimental animals were assigned to two groups. One group served as a control group ($n = 8$) and received no treatment whereas the other group ($n = 5$) was pretreated with BW12C (50 mg kg^{-1}) administered by intravenous infusion 1 h before occlusion of the LAD. Differences in infarct size between groups was statistically evaluated by a Mann Whitney U test.

Results

Effects of BW12C on myocardial function in the rabbit isolated heart

In the series of control experiments (Group 1) the rabbit isolated heart preparation perfused with horse-

Table 1 Myocardial function in atrial-paced, rabbit isolated hearts ($n = 5$) perfused at a coronary flow of 1.49 ± 0.03 ml g^{-1} min^{-1} with horse erythrocyte-enriched Krebs-Henseleit buffer†

Time (min)	*LVP* (mmHg)	*LVP* dP/dt (mmHg s^{-1})	*LVEDP* (mmHg)	*CPP* (mmHg)	*O_2 extraction* $\frac{Ao_2 - Vo_2}{Ao_2}$	*Venous* Po_2 (mmHg)	*Venous* Pco_2 (mmHg)	*Lactate extraction* $\frac{A_{Lact} - V_{Lact}}{A_{Lact}}$
0	68 ± 6	1650 ± 540	1.8 ± 0.7	58 ± 8	0.198 ± 0.041	40.9 ± 4.0	34.4 ± 3.0	0.009 ± 0.039
+15	72 ± 5	1860 ± 200	1.2 ± 0.4	71 ± 6	0.192 ± 0.032	41.4 ± 3.3	38.1 ± 3.0	0.068 ± 0.040
+30	72 ± 6	1800 ± 225	1.0 ± 0.5	65 ± 4	0.175 ± 0.035	43.1 ± 4.0	38.2 ± 3.0	0.093 ± 0.060
+60	72 ± 7	1750 ± 250	0.8 ± 0.4	69 ± 4	0.172 ± 0.036	44.3 ± 3.5	38.8 ± 3.0	0.091 ± 0.014
+90	72 ± 7	1775 ± 250	1.0 ± 0.5	74* ± 2	0.176 ± 0.028	43.3 ± 3.0	36.6 ± 3.0	0.069 ± 0.032

*$P < 0.05$ from reading at time 0.
Values are mean ± s.e.mean.
†The arterial perfusate contained 27.6 ± 3 vol % O_2, Po_2 166 ± 9 mmHg, Pco_2 29 ± 5 mmHg lactate 193 ± 27 nM, haemoglobin 18.4 ± 1.4 g % and arterial pH: 7.434 ± 0.020.

erythrocyte-enriched Krebs Henseleit buffer was found to be stable for 90 min with no significant variation in myocardial performance observed throughout this time period (Table 1).

Perfusion of the rabbit isolated heart with erythrocytes pretreated with BW12C 10^{-3} M for 30 min (Group 2) did not elicit any significant change in LVP, LVP *dP/dt* or CPP when compared to control, although, there were significant decreases in the Po_2 of the venous effluent and an increased myocardial lactate production during perfusion (Figure 1). On returning to perfusion with untreated, erythrocyte-enriched buffer the changes in venous Po_2 and lactate production returned to pre-drug control levels.

Perfusion of the isolated heart with erythrocytes pretreated with BW12C 2×10^{-3} M (Group 3) and 4×10^{-3} M (Group 4) for 30 min resulted in significant, concentration-dependent decreases in LVP, LVP *dP/dt* and CPP concomitant with decreases in venous Po_2 and a further increase in lactate production by the myocardium (Figure 1). On returning to the perfusion with untreated erythrocytes, myocardial function, venous Po_2 and myocardial lactate production returned towards pre-drug control levels. When perfusion was maintained with erythrocytes treated with 4×10^{-3} M BW12C for longer periods (>30 min) a progressive decline in left ventricular function with cardiac failure occurred. The magnitude of the changes in cardiac function following perfusion of erythrocytes pretreated with BW12C was associated with the degree of leftward shift of the OSC of the erythrocyte-enriched buffer. Treatment with BW12C resulted in a progressive decrease in P_{50} from 25 ± 1.2 mmHg (control) to 18 ± 1 mmHg (Group 2), 12.0 ± 0.5 mmHg (Group 3) and 7.0 ± 0.5 mmHg (Group 4) (Figure 2). BW12C did not affect the arterial Po_2, Pco_2, pH or oxygen carrying capacity of the perfusate.

Administration of BW12C (10^{-5}–10^{-4} M) to the isolated Krebs-Henseleit perfused heart (Group 5) did not modify resting heart rate, LVP or CPP. Administration of BW12C at a concentration of 10^{-3} M elicited small decreases in LVP, CPP and heart rate although none of these changes was significant ($P > 0.05$) (Figure 3).

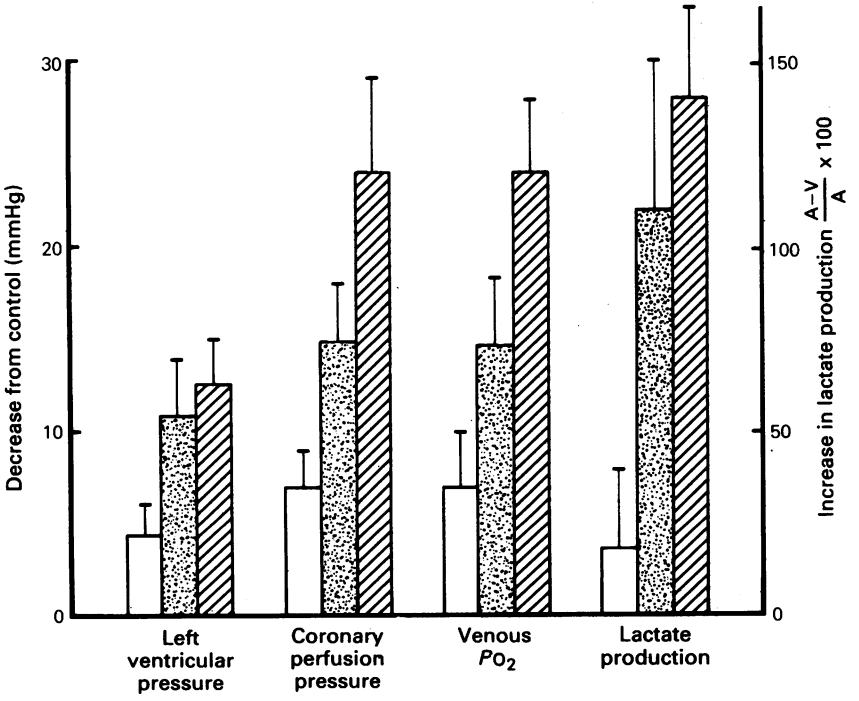

Figure 1 The effects of increased haemoglobin oxygen affinity, induced by BW12C, on myocardial function, venous Po_2 and lactate production in rabbit isolated perfused hearts. $n = 5$ for all groups. Open columns: 10^{-3} M BW12C; stippled columns: 2×10^{-3} M BW12C; hatched columns: 4×10^{-3} M BW12C. Significant changes in venous Po_2 and lactate production were induced by all concentrations of BW12C whereas significant changes in left ventricular pressure and coronary perfusion pressure were induced by 2×10^{-3} M and 4×10^{-3} M BW12C. (Data analysed by Student's *t* test for paired data).

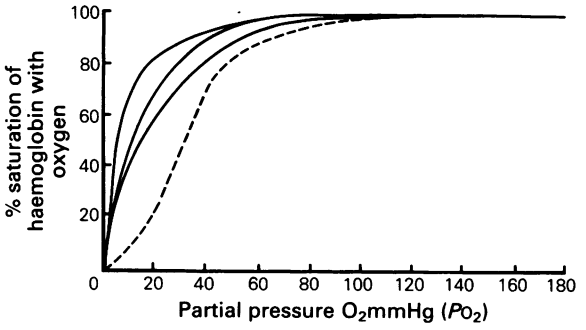

Figure 2 A representative haemoglobin oxygen saturation curve of erythrocyte-enriched buffer pretreated with increasing concentrations of BW12C (1, 2 and 4×10^{-3} M: solid lines) and of normal untreated erythrocytes in buffer (broken line). A progressive leftwards shift of the curve is produced by the increasing concentration of BW12C.

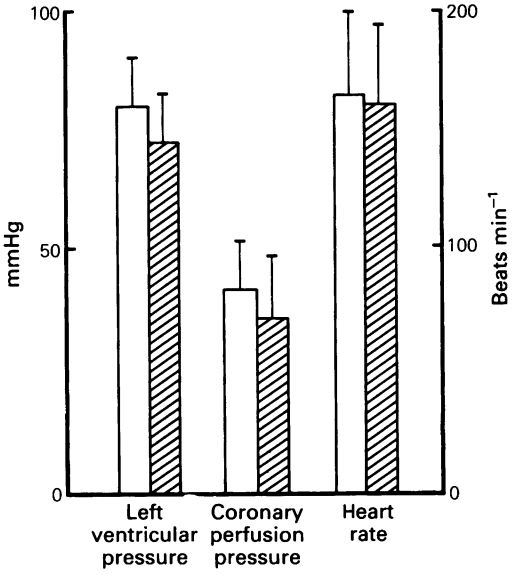

Figure 3 The effects of BW12C (10^{-3} M) (hatched columns) on myocardial function in rabbit isolated hearts perfused with Krebs-Henseleit, erythrocyte-free, buffer ($n = 5$). Left ventricular pressure, coronary perfusion pressure and heart rate were not significantly modified by this concentration of BW12C. Controls (without BW12C): open columns.

Effects of BW12C on myocardial infarct size

Occlusion of the LAD for 1 h, followed by coronary reperfusion, produced an area of injured myocardium predominantly located in the subendocardium. Transmural extension of the infarct was commonly seen in myocardium nearer the apex of the heart, whereas epicardial involvement from the mid-myocardium to the base of the heart was negligible.

The histological features of the myocardial infarction were essentially as previously described for experimentally induced infarcts in the dog (Allan *et al.*, 1985). Briefly, the infarcted myocardium stained more intensely with eosin than the surrounding normal tissue and frequently showed prominent myofibrillar contraction bands and tissue oedema.

In untreated dogs, occlusion of the LAD for 1 h followed by 2 h of coronary reperfusion, resulted in an area of risk (expressed as a percentage of the total left ventricle) of 28.2%, and a myocardial infarct size (expressed as a percentage of the area of risk) of 11.5% (Figure 4). The incidence of haemorrhage into the myocardium and the incidence of ventricular fibrillation on coronary reperfusion was low in untreated animals (haemorrhage, 2/8; ventricular fibrillation, 0/8).

Intravenous administration of BW12C (50 mg kg^{-1}) 1 h before coronary occlusion caused a significant ($P < 0.05$) leftward shift of the OSC at the P_{20} level (16 ± 0.4 to 8 ± 1 mmHg), the P_{50} level (29 ± 0.7 to 25 ± 1 mmHg) and at the P_{80} level (47 ± 1 mmHg to 44 ± 1 mmHg). The leftwards shift (P_{20}) was maintained throughout the duration of the experimental period. Pretreatment with BW12C did not significantly alter the haemodynamic status of the anaesthetized dog before coronary occlusion, although, small but significant increases in heart rate (from 170 ± 8.4 to 185 ± 6.3 beat min^{-1}, $P < 0.05$) and LVP (from 117 ± 5.6 to 124 ± 4.8 mmHg, $P < 0.05$) were observed.

In anaesthetized dogs pretreated with BW12C, the area of risk following coronary occlusion was not significantly altered (28.2% versus 25.7%, $P > 0.05$) whereas the myocardial infarct size increased significantly from 11.5% in untreated animals to 47.1% in treated animals, representing an increase of 410% above control ($P < 0.001$, Figure 4). Visual inspection of the infarcts demonstrated that the extension of the infarct was associated with a greater transmural involvement in all areas of the left ventricle. Neither myocardial haemorrhage nor ventricular fibrillation occurred in the treated animals.

Discussion

Previous studies, designed to examine the effects of altered haemoglobin-oxygen affinity on tissue function both *in vivo* and *in vitro* have employed a variety of indirect methods to modulate haemoglobin oxygen

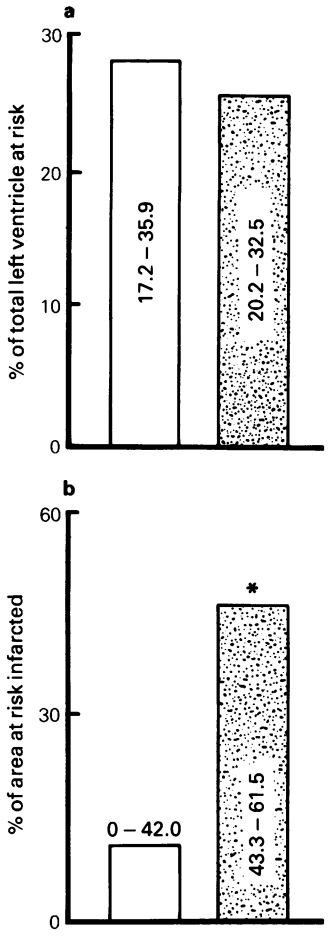

Figure 4 The effects of BW12C (50 mg kg^{-1}) on areas of left ventricle at risk and myocardial infarct size in anaesthetized beagle dogs with a 1 h occlusion of the LAD followed by 2 h of coronary reperfusion. Myocardial infarct size is expressed as a percentage of the area of risk infarcted (b) and the area at risk is expressed as a percentage of the total area of left ventricle (a). Area of risk was not modified by treatment with BW12C whereas myocardial infarct size was significantly increased by pretreatment with BW12C. Values are expressed as the median with the range inset, $n = 8$ for control (open columns) and 5 for BW12C group (stippled columns). *$P < 0.001$, Mann Whitney U test.

affinity. Increased haemoglobin-oxygen affinity, or a left shift of the OSC has been induced *in vivo* with transfusions of 2-3 diphosphoglycerate (2-3 DPG), depleted blood (Holsinger *et al.*, 1973), alkalotic blood (Woodson *et al.*, 1982), or carbamylated blood (Martin *et al.*, 1979; Woodson *et al.*, 1982). Decreased haemoglobin-oxygen affinity or a right shift of the OSC curve has been induced by infusion of high concentrations of glycolytic intermediates to elevate 2-3 DPG (Bristow *et al.*, 1977; Pantely *et al.*, 1981), or by pharmacological manipulation with ortho-iodosodium benzoate (Litwin *et al.*, 1976; Gross *et al.*, 1977; Rude *et al.*, 1983). Although the results of such studies have demonstrated the importance of haemoglobin-oxygen affinity as a determinant of tissue function, the methods by which the OSC were manipulated are questionable in terms of their specificity.

In our study, we have employed a novel pharmacological agent, BW12C, which exhibits a high binding affinity and specificity for the haemoglobin molecule (Beddell *et al.*, 1984). BW12C induces a concentration-dependent left shift of the OSC thereby permitting the evaluation of the effects of graded increases in haemoglobin oxygen affinity on myocardial function *in vitro* and *in vivo*.

Administration of BW12C-treated erythrocytes to the isolated perfused heart of the rabbit produced a concentration-dependent reduction in myocardial function and marked lactate production by the myocardium, the magnitude of which were related to the degree of left shift of the OSC elicited by this agent. BW12C had no direct pharmacological action on the isolated heart at concentrations higher than those which would appear unbound in the erythrocyte perfusate, thus confirming the specificity of this novel tool. Administration of BW12C-treated erythrocytes to the isolated heart also resulted in a decrease in the coronary perfusion pressure, presumably reflecting coronary autoregulation in response to hypoxia. Coronary vasodilatation in response to hypoxia is well documented (Berne, 1964; Seigl, 1983), although controversy exists as to the primary mediator responsible for this effect. Berne (1980) has proposed adenosine but other studies suggest a direct vascular effect of oxygen (Drake-Holland *et al.*, 1984). In the present study the decrease in coronary vascular resistance was related to the degree of hypoxia implying some association between oxygen supply and vascular tone, however, with the data we have obtained it is not possible to speculate on the contribution of adenosine to this response.

During perfusion with erythrocytes treated with BW12C, myocardial function was well maintained, despite the very marked reductions in O_2 delivery. Lactate production by the myocardium was significantly increased, indicating anaerobic respiration. However, no permanent damage from hypoxia ensued as all these parameters returned towards normal when hearts were returned to perfusion with normal erythrocytes. When perfusion was maintained with erythrocytes treated with BW12C (4×10^{-3} M) for longer periods, cardiac failure did occur indicating that

Table 2 Haemodynamic effects before and after an intravenous infusion of BW12C (total dose 50 mg kg^{-1}) to the anaesthetized dog

	Heart rate (beats min^{-1})	*Blood pressure* (mmHg)	*LVP* (mmHg)	*LVP* dP/dt (mmHg s^{-1})
Control	170 ± 8.4	128 ± 9.3/90 ± 6.3	117 ± 5.6	2780 ± 137
After BW12C	185 ± 6.3*	134 ± 6.5/93 ± 4.6	124 ± 4.8*	3040 ± 317

Mean values ± s.e.mean are given; $n = 8$ for control and 5 for BW12C group.
*Significantly different from control ($P < 0.05$, Student's t test for paired data).

prolonged hypoxia will induce severe myocardial dysfunction.

Pretreatment of anaesthetized dogs with BW12C did not induce any significant change in the resting haemodynamic status, other than small increases in LVP and heart rate. The changes in LVP and heart rate may reflect adaptive compensatory increases in myocardial function in response to the increased haemoglobin oxygen affinity although previous workers have demonstrated an acute increase in haemoglobin oxygen affinity is not accompanied by changes in cardiac output (Riggs *et al.*, 1973). A direct positive inotropic and chronotropic action of BW12C cannot be overruled, but such properties of BW12C were not apparent in the rabbit isolated heart studies.

In previous studies we have demonstrated that in anaesthetized dogs, coronary reperfusion following a 1 h period of occlusion results in salvage of ischaemic tissue with maximum infarct reduction occurring following 2 h of coronary reperfusion (Allan *et al.*, 1985). Using this experimental protocol we have now demonstrated that by increasing the haemoglobin-oxygen affinity with BW12C, and hence reducing oxygen delivery to the jeopardized myocardium, reperfusion salvage is compromised with the result that infarct size is dramatically increased. Recently, it has been suggested that the reoxygenation which occurs on coronary reperfusion may actually increase tissue damage and infarct size due to the production of oxygen-derived, free radicals (Shlafer *et al.*, 1982; Gardner *et al.*, 1983; Jolly *et al.*, 1984). However, our study highlights the critical role of oxygen delivery to the ischaemic myocardium during reperfusion as a determinant of tissue survival and increased myocardial salvage. Indeed, in a canine occlusion model where oxygen delivery was enhanced by decreasing haemoglobin-oxygen affinity via elevation of endogenous 2-3 DPG or pretreatment with ortho-iodosodium benzoate, a reduction in myocardial infarct size was attained (Pantely *et al.*, 1981; Rude *et al.*, 1983). These studies therefore further confirm the importance of oxygen and thus haemoglobin-oxygen affinity in governing oxygen delivery and hence tissue salvage during ischaemia.

In conclusion, our studies with BW12C demonstrate that alterations in haemoglobin-oxygen affinity can induce adaptive physiological changes in tissue function and metabolism and assume a critical role when oxygen supply may be impaired due to flow limiting stenosis. Pharmacological manipulations of haemoglobin-oxygen affinity with specific agents, like BW12C represent a novel approach for further elucidation of the role of oxygen on tissue function.

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