Minimal role of nitric oxide in basal coronary flow regulation and cardiac energetics of blood-perfused isolated canine heart

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1. The role of nitric oxide (NO) in the regulation of basal coronary perfusion and ventricular chamber energetics was studied in isovolumetrically contracting isolated blood-perfused canine hearts. Hearts were cross-perfused by a donor animal prior to isolation, and chamber volume controlled by a servo-pump. Coronary sinus flow and arterial–coronary sinus oxygen difference were measured to determine energetic efficiency.

2. NO synthase (NOS) was competitively inhibited by N^G-monomethyl-L-arginine (L-NMMA; 0·5 mg kg^{-1}, intracoronary), resulting in a reduction of acetylcholine (50 μg min^{-1})-induced flow augmentation from 143 to 62% ($P < 0·001$).

3. NOS inhibition had no significant effect on basal coronary flow. Coronary pressure–flow relationships were determined at a constant cardiac workload by varying mean perfusion pressure between 20 and 150 mmHg. Neither the shape of the relationship, nor the low-pressure value at which flow regulation was substantially diminished were altered by NOS inhibition.

4. Myocardial efficiency was assessed by the relationship between myocardial oxygen consumption and total pressure–volume area (PVA), with cavity volume altered to generate varying PVAs. This relative load-independent measure of energetic efficiency was minimally altered by NOS inhibition.

5. These results contrast with isolated crystalloid-perfused heart experiments and suggest that in hearts with highly controlled ventricular loading and whole-blood perfusion, effects of basal NO production on coronary perfusion and left ventricular energetics are minimal.

Nitric oxide (NO) released by the coronary arterial endothelium may play an important role in basal myocardial flow regulation, contractility and energetics. In isolated crystalloid-perfused rat or guinea-pig hearts (Amrani *et al.* 1992; Ueeda, Silvia & Olsson, 1992; Brown, Thompson & Belloni, 1993) and isolated blood-perfused rat hearts (Bouma, Ferdinandy, Sipkema, Allaart & Westerhof, 1992), NO synthase (NOS) inhibition lowers flow by 30–75%. In addition, NOS inhibition can depress left ventricular (LV) function (Amrani *et al.* 1992) while increasing lactate extraction. However, basal coronary tone is markedly reduced in these preparations, with resting myocardial flows 4–5 times greater than *in vivo* hearts (Smith & Canty, 1993). In addition, both coronary flow and LV function vary substantially with altered perfusion pressures (Kitakaze & Marban, 1988; Ueeda *et al.* 1992) revealing the lack of normal coronary flow autoregulation (Canty, 1988; Feigl, Neat & Huang, 1989). This loss of basal vascular tone might enhance the cardiac sensitivity to NOS inhibition.

Observations made *in vivo* and from whole-blood-perfused isolated hearts support this interpretation. In this setting, NOS inhibition generally has insignificant effects on basal coronary flow (Parent, Pare & Lavallée, 1992; Smith & Canty, 1993; Lefroy, Crake, Uren, Davies & Maseri, 1993; Duncker & Bache, 1994) and neither steady-state flow nor LV function are substantially influenced by variations in perfusion pressure over a broad physiological range (Sunagawa, Maughan, Friesinger, Guzuman, Chang & Sagawa, 1982; Canty, 1988; Smith & Canty, 1993). Basal contractile function also appears minimally changed by NOS inhibition in intact preparations (Chu *et al.* 1991; Paulus, Vantrimpont & Shah, 1994). One potentially important effect of NOS blockade on *in vivo* coronary flow regulation is an increase in the critical pressure (near

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50 mmHg) below which flow autoregulation is lost (Smith & Canty, 1993; Duncker & Bache, 1994). A major factor of these data, however, was that NOS inhibition also increased systemic vascular and LV workloads, which themselves can alter the coronary pressure–flow relationship (Mosher, Ross, McFate & Shaw, 1963; Feigl *et al.* 1989).

Because of the ambiguities raised by abnormal basal flow and flow regulation in crystalloid-perfused hearts, and systemic vascular loading changes with NOS inhibition *in vivo*, the role of NO in the basal regulation of coronary flow, cardiac contractility and energetic efficiency remains uncertain. The present study sought to address these issues employing an isolated whole-blood-perfused canine preparation in which coronary flow regulation was much closer to that observed *in vivo*, yet chamber workload could be precisely controlled. This model also enabled precise assessment of the relationship between total cardiac work and perfusion pressure, as well as work and myocardial oxygen consumption (i.e. efficiency). Thus, we tested the effect of NOS inhibition on the sensitivity of myocardial flow and LV function to varied coronary artery perfusion pressures, and on cardiac energy efficiency.

METHODS

Isolated heart preparation

Ten isolated canine hearts were studied. The surgical procedure employed for the isolated heart preparation has been previously described in detail (Sagawa, Suga, Maughan & Sunagawa, 1988; Harasawa, de Tombe, Sheriff & Hunter, 1992). Briefly, two mongrel dogs (20–30 kg) were used for each experiment. The support dog was premedicated with hydrocortisone (500 mg, I.M.), diphenhydramine (50 mg, I.V.), indomethacin (25 mg, per rectum), and heparin (10 000 i.u.) to minimize cardiopulmonary instability during cross-circulation and anaesthetized with pentobarbitone (35 mg kg^{-1} bolus, followed by 3 mg kg^{-1} h^{-1} infusion). Both femoral arteries and veins were cannulated to provide oxygenated blood perfusion to a heart isolated from a second (donor) dog. The donor dog was also anaesthetized with 35 mg kg^{-1} pentobarbitone (no paralytic agent) and the chest opened via a median sternotomy. The subclavian artery and right atrium were cannulated and connected to the perfusion system, so that the heart could be isolated while maintaining normal blood perfusion. The azygous vein and both vena cavae, the descending aorta and both pulmonary vascular hilae were tied off and ligated and the heart then removed from the chest. The left ventricle was vented to air and the left atrium opened and all chordae tendineae cut. A metal ring adaptor was sewn into the mitral annulus to which an intracavitary latex balloon was secured. The balloon was filled by a volume servo-pump and pressures were measured by micromanometer (PC-380, Millar, Houston, TX, USA).

Mean coronary perfusion pressure (CPP) as measured in the proximal aortic root was controlled by two peristaltic pumps (Harasawa *et al.* 1992). The first pump withdrew arterial blood from the support dog at a constant rate, while the second servo-controlled pump diverted a portion of this blood to the isolated heart. This maintained a constant haemodynamic load on the support dog independent of coronary flow to the isolated heart. The arterial blood O_2 and CO_2 concentrations and pH of the support dog were maintained in a normal range by adjusting ventilation and administering sodium bicarbonate or oxygen as required. Coronary blood flow (CBF) was measured by draining all blood from the right heart past an in-line ultrasonic flow probe (Transonics, Ithaca, NY, USA). The difference in arterial and coronary venous blood oxygen (ΔAV_{O_2}) was continuously measured by absorption spectrophotometry (AVOX Systems, TX, USA) calibrated to an oxygen analyser (Oxy-Con, TX, USA).

Protocol

Experiments were performed with the left ventricle contracting isovolumically. The mean weight of LV (free wall and septum) was 118·5 ± 18·7 g and that of the right ventricle (RV) was 42·7 ± 10·9 g. Data were measured before and after blockade of NOS by N^G-monomethyl-L-arginine (L-NMMA; 0·5 mg kg^{-1}, 40 μM) directly infused into the coronary perfusion line over a 10 min period. The efficacy of NOS blockade was established in each heart by testing for flow augmentation to continuous acetylcholine (ACh, 50–75 μg min^{-1}) infused into the perfusion line.

To test the effects of NOS blockade on the dependence of coronary flow or LV systolic function on mean coronary perfusion pressure, the CPP was gradually decreased from 150 mmHg to approximately 20 mmHg at a constant LV volume. Two to three minutes were provided after each change in CPP to achieve steady state prior to data collection.

To test effects of NOS blockade on LV efficiency, we assessed the linear relationship between total ventricular workload (pressure–volume area, or PVA) and myocardial oxygen consumption ($M\dot{V}_{O_2}$). The inverse slope of this relationship provides a measure of chemomechanical efficiency, while the offset assesses the O_2 costs of basal metabolism and excitation–contraction coupling (Suga, Hisano, Goto, Yamada & Igarashi, 1983; Suga, 1990). To measure $M\dot{V}_{O_2}$–PVA relationships, LV volume was changed at five to ten different steady-state values at a constant contractile state. Two to three minutes were provided after each volume change prior to data recording.

Analysis

Data were displayed and digitized at 200 Hz using custom-designed acquisition software. Coronary pressure–flow relationships demonstrated a biphasic behaviour, with the flatter portion at perfusion pressures between 60 and 120 mmHg and a steeper declining portion at pressures below 55 mmHg. To quantify the lower pressure break-point in the coronary pressure–flow (CPP–CBF) curves, each portion was fitted by linear regression and the intersection point calculated. One regression line was applied to the CPP–CBF data at CPP between 80 and 100 mmHg and a second line to data between CPP of 20 and 50 mmHg.

$M\dot{V}_{O_2}$ for the whole heart was calculated as the product of CBF and ΔAV_{O_2}. Since the right ventricle was vented by continuous blood drainage it performed negligible work. Therefore, RV $M\dot{V}_{O_2}$ was considered equal to the ratio of RV to total heart mass multiplied by the $M\dot{V}_{O_2}$ of the combined unloaded RV and LV. This RV contribution was subtracted from total $M\dot{V}_{O_2}$ to yield the fraction related to the LV. The energetics of the LV were assessed by the relationship between $M\dot{V}_{O_2}$ and PVA, as described by Suga *et al.* (1983) and Suga (1990). For an isovolumic beat, PVA is defined as the triangular area bounded by the developed pressure, the end-systolic pressure–volume relationship (ESPVR) extended to zero

pressure, and the diastolic pressure–volume relationship. PVA was normalized per 100 grams of LV, with dimensions of (mmHg ml) beat^{-1} (100 g LV)$^{-1}$.

Since contractile state can vary the relationship between $M\dot{V}_{O_2}$ and PVA (Suga *et al.* 1983; Suga, 1990), we also assessed contractility from the same set of pressure–volume data used to evaluate chamber energetics in each heart employing the ESPVR (Sagawa *et al.* 1988). The linear slope (E_{es}) and volume intercept (V_0) of the ESPVR was determined from these data before and after L-NMMA. The linear slope (E_{es}) was also normalized per 100 grams of LV. Contractile function was also measured just prior to and 20 min after L-NMMA by isovolumic dP/dt_{max}.

Preparation of free reduced oxyhaemoglobin

To study the potential influence of free plasma haemoglobin (Hgb) due to haemolysis from the pump-perfusion system on ACh-induced flow reserve, free reduced haemoglobin (Sigma) was injected I.C. in four additional intact hearts. For this purpose, four dogs were anaesthetized with pentobarbitone (35 mg kg^{-1}), paralysed with pancuronium bromide (0·1 mg kg^{-1}) and the heart exposed by left lateral thoracotomy and placed in a pericardial cradle. Adequacy of anaesthesia was confirmed by the stability of heart rate and arterial pressure. The left anterior descending artery was dissected below the first diagonal and cannulated by a 2 mm o.d. tube with an in-line ultrasound flow probe (Transonics No. 2 N), perfused with blood diverted from the left carotid artery.

Free reduced oxyhaemoglobin was prepared by standard technique (Myers, Banitt, Guerra & Harrison, 1989). Haemoglobin, 6·45 g, was dissolved in 100 ml distilled water containing 0·174 g sodium dithionite. This solution was dialysed against distilled water (100 × vol) for 4 h, with one change of water. Fresh haemoglobin was prepared before each experiment and used within 18 h. The oxygen saturation was confirmed as < 20% by co-oximetry. Reduced Hgb was infused into the coronary cannula to achieve steady-state concentrations between 10 and 20 μM. Coronary flow responses to 50 μM min^{-1} ACh were tested before and after Hgb infusion.

Free plasma Hgb was determined by polychromatic spectrophotometry (Noe, Weedn & Bell, 1984). Plasma was centrifuged at 10^2 *g* for 10 min, diluted 1 : 10 in Tris buffer and spectrophotometric absorbance (A) was determined at three wavelengths (380, 415 and 470 nm, in this order), with free plasma Hgb = $1{\cdot}65 \times A_{415} - 0{\cdot}93 \times A_{380} - 0{\cdot}73 \times A_{470}$.

Statistical analysis

Data are presented as means ± standard deviation. Tests for effects of L-NMMA effect on ESPVR and $M\dot{V}_{O_2}$–PVA relationships were made in each heart using covariance analysis. For the combined data a multivariate regression model was used in which all raw data were combined into a single regression and dummy variables used to code for interanimal variation about the mean. Paired differences in flow response or contractility before and after L-NMMA were tested by Student's paired t test. Statistical significance is reported at $P < 0{\cdot}05$.

RESULTS

Response to acetylcholine

Figure 1 displays the percentage increase in CBF from ACh infusion before and after L-NMMA. CBF increased from 93·0 ± 37·3 to 210·6 ± 59·8 ml min^{-1} (+143%) at baseline and from 97·5 ± 39·5 to 150·7 ± 49·4 ml min^{-1} (+62·4%) after L-NMMA ($P < 0{\cdot}001$). This depression of flow reserve is consistent with prior data measured in conscious dogs with L-NMMA (Richard, Berdeaux, la Rochelle & Giudicelli, 1991 (0·5–1 mg kg^{-1}); Yamabe, Okumura, Ishizuka, Tsuchiya & Yasue, 1992 (2 μg min^{-1}); Duncker & Bache, 1994 (1·5 mg kg^{-1}, I.C.)) and very similar to results using L-NAME (Smith & Canty, 1993).

Effect of L-NMMA on LV function and basal coronary blood flow

Figure 2 displays results for changes in mean CBF and contractile function (dP/dt_{max}) for each heart before and after L-NMMA. NOS inhibition induced little change in either resting CBF (at identical workload) or contractility in each heart, with no significant difference between means.

Coronary flow regulation

Figure 3*A* and *B* displays an example from one heart of relationships between CBF and CPP and ventricular workload (PVA) and CPP. In each relationship, there was an upper range of CPP over which CBF and particularly PVA were less affected, as well as a corner point below which both variables declined steeply. The continuous and dotted regression lines delineate this corner point. Both the relationships and corner point were minimally altered by L-NMMA infusion.

Figure 3*C* and *D* displays group results for the same relationships. To combine individual data, CPP was normalized to 90 mmHg and flow (or PVA) normalized to its respective value when CPP was 90 mmHg. As in the individual example, NOS inhibition had negligible influence

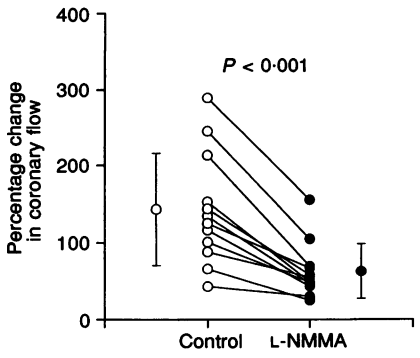

Figure 1. Influence of 40 μM intracoronary L-NMMA on flow augmentation induced by acetylcholine in twelve isolated canine hearts

Data shown are the percentage change in flow relative to baseline for control and post-L-NMMA conditions.

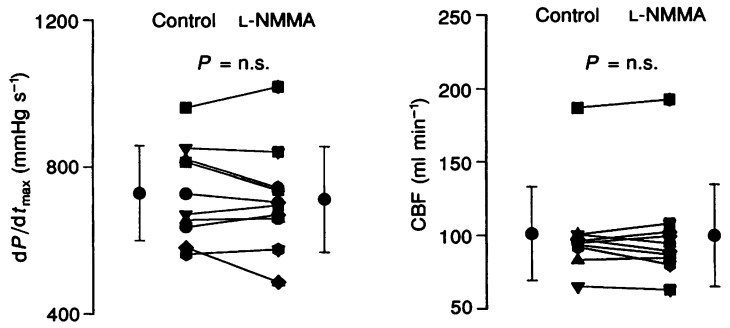

Figure 2. Influence of intracoronary L-NMMA on basal chamber contractile function and coronary blood flow

Function was indexed by isovolumic dP/dt_{max}. There was minimal change in both parameters as a consequence of L-NMMA infusion.

on either relationship, or the CPP at which flow and functional autoregulation were diminished.

L-NMMA and chamber energetics

To test if basal LV metabolic efficiency was modified by NOS inhibition, $M\dot{V}_{O_2}$–PVA relationships were compared before and after L-NMMA. $M\dot{V}_{O_2}$–PVA relationships were highly linear under both conditions (mean $r^2 = 0{\cdot}983$ and 0·980, respectively). Six of nine hearts displayed a small downward displacement of $M\dot{V}_{O_2}$–PVA data (e.g. Fig. 4*A*), while the remaining three had no change (e.g. Fig. 4*B*). Mean results (Table 1) revealed no significant change in slope (chemomechanical efficiency) and only a small downward shift in the intercept ($P < 0{\cdot}01$, by multiple regression). However, as there was a commensurate equally small decline in simultaneously measured contractile

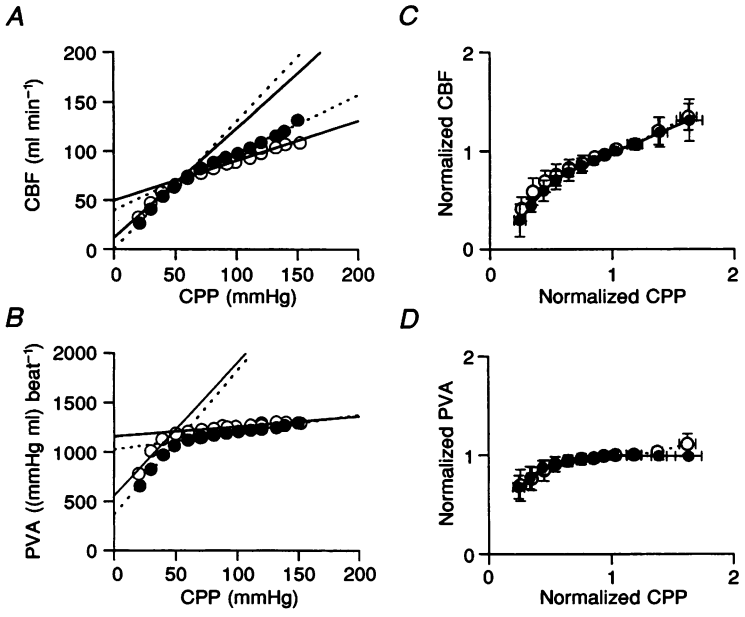

Figure 3

A, an example of the effect of L-NMMA on steady-state basal coronary pressure–flow relationships. These relationships displayed a biphasic pattern, with a shallower portion above coronary perfusion pressures (CPP) of 50–60 mmHg and a steeper portion at lower pressures. Linear regressions were used to define the corner points. The relationships and corner points were only slightly altered by L-NMMA infusion ○, before; ●, after L-NMMA. *B*, an example of the cardiac function–coronary pressure relationship from the same animal. A biphasic pattern was produced again with very little influence of CPP on LV function above the coronary point. L-NMMA also had minimal effect on this relationship. *C* and *D* show the group results for coronary pressure–flow and pressure–function relationships before and after I.C. L-NMMA. Data were normalized to values measured at a CPP of 90 mmHg prior to pooling results. As demonstrated in the previous example, the two sets of relationships were virtually superimposable.

Table 1. Influence of L-NMMA on cardiac chamber energetics and contractile function

	$M\dot{V}_{O_2}$–PVA		ESPVR	
	Slope ((ml O_2) $mmHg^{-1}$ ml^{-1} $\times 10^5$)	Intercept ((ml O_2) $beat^{-1}$ $(100\ g)^{-1}$ $\times 10^2$)	Slope (mmHg ml^{-1})	Intercept (ml)
Before L-NMMA	2·20 ± 0·061	2·80 ± 0·053	2·81 ± 0·043	4·73 ± 0·49
After L-NMMA	2·25 ± 0·049	2·63 ± 0·034	2·60 ± 0·054	6·50 ± 0·64
P	n.s.	< 0·001	< 0·005	n.s.

Mean slope and intercept of the relationship between myocardial oxygen consumption and total pressure–volume area ($M\dot{V}_{O_2}$–PVA) and end-systolic pressure–volume relationship (ESPVR) are provided. Data are means ± S.E.M. derived from a multiple regression analysis in the form:

$$y = B_0 + B_1 x + C_0 + C_1 x + \sum_{i=1}^{8} D_i + \sum_{i=1}^{8} D'_i\, x,$$

where B_0 and B_1 are the intercept and slope before L-NMMA, $(B_0 + C_0)$ and $(B_1 + C_1)$ the respective values after L-NMMA, and D_i and D'_i dummy variables coding for interanimal variability. n.s., not significant.

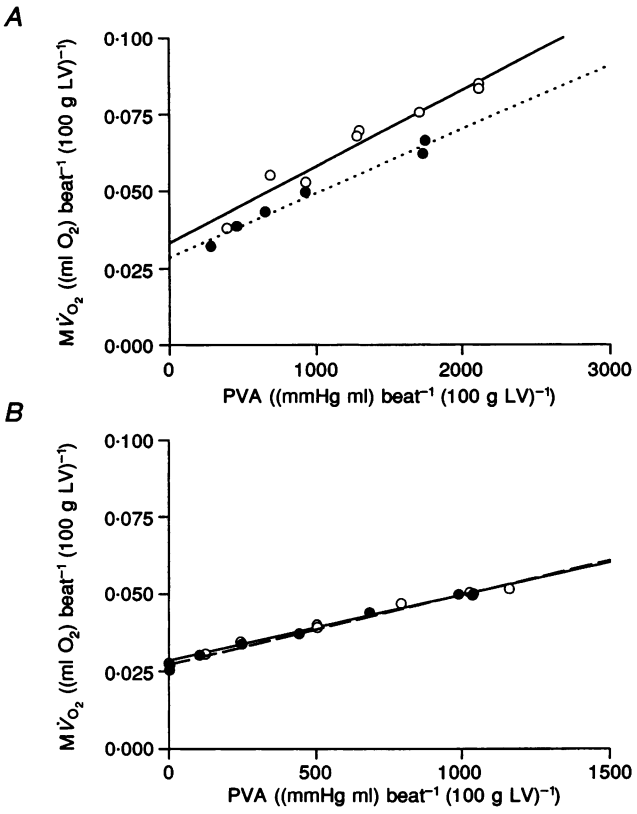

Figure 4. Two examples of the steady-state energetic analysis using the relationships between myocardial oxygen consumption ($M\dot{V}_{O_2}$) and total mechanical work (pressure–volume area, PVA)

○, before; ●, after L-NMMA. *A* shows a heart in which L-NMMA resulted in a slight downward shift in the relationship, i.e. a slight reduction in $M\dot{V}_{O_2}$ at any given total workload. This was characteristic of the majority of hearts, while three hearts demonstrated no change (*B*).

function (Table 1), the O_2 cost of contractility was unaltered (Suga, 1990).

Dose response of L-NMMA

To test whether ACh-induced flow reserve, energetics or contractile effects of NOS inhibition might be more pronounced at double or triple the L-NMMA dose (1 or 1·5 mg kg^{-1}) we examined these responses in two hearts. Coronary flow augmentation to ACh declined by 41% after the 10 mg dose, with minimal further reductions at 20 mg (−47·3%) or 30 mg (−48·5%) L-NMMA. Neither energetics (slope and intercept of the $M\dot{V}_{O_2}$–PVA relationship) nor contractility (indexed by the slope, E_{es} and intercept, V_0 of the ESPVR) were changed at the higher L-NMMA doses.

Effect of haemolysis and free plasma haemoglobin

Since whole blood was delivered to the isolated heart by peristaltic pumps, haemolysis and release of Hgb resulted in free plasma Hgb concentrations averaging 700 mg l^{-1} (11 μM) in the perfusate. To directly test whether such concentrations might influence NO-mediated flow responses in a blood-perfused preparation, we compared flow augmentation responses to 50 μM min^{-1} ACh before and after administering a similar concentration of free plasma Hgb to four additional animals *in vivo*. In the absence of haemolysis, flow increased by 165 ± 30·5%, and this did not significantly change (+142 ± 27%) with 11 μM free plasma Hgb. At a higher Hgb dose (17 μM), however, flow reserve declined to 106 ± 18% ($P < 0·05$ *versus* control).

DISCUSSION

This study investigated the effect of NOS inhibition on the dependence of basal coronary flow and LV systolic function on coronary perfusion pressure in whole-blood-perfused isolated canine hearts. Infusion of L-NMMA neither altered the basal coronary flow regulation curve, nor changed the dependence of ventricular function on myocardial perfusion. Secondly, we found that ventricular contractility as well as chemomechanical efficiency was unaltered by NOS blockade. These are the first reported data obtained in a whole-blood-perfused yet isolated mammalian heart in which chamber loading was precisely controlled. In contrast to most previous isolated heart studies, the results suggest only a minor role of NO synthesis and release in the physiological regulation of basal coronary flow, contractile function and energetics.

Experimental limitations

There are several potential limitations that should be considered. It is possible that the extent of NOS inhibition achieved in our study was insufficient to demonstrate an effect on coronary flow regulation and/or ventricular energetics. While L-NMMA is among the more specific arginine analogues for inhibiting NOS (Buxton, Cheek, Eckman, Westfall, Sanders & Keef, 1993) it is somewhat less potent than L-NAME on an equimolar basis (Rees, Palmer, Schulz, Hodson & Moncada, 1990; Richard *et al.* 1991). However, at the dose used (40 μM), the anticipated blockade of NO-dependent vasorelaxation is nearly 80% (Rees *et al.* 1990). Furthermore, doubling or tripling the dose did not appreciably alter the response. The dose–response curve for NOS inhibition could still be highly non-linear so that larger effects on both coronary flow regulation and energetics might be observed at much higher doses. However, our data are consistent with doses and responses studied in intact conscious animals and humans and are thus particularly relevant.

Haemoglobin rapidly binds NO (Wennmalm *et al.* 1993) and can act as a 'sink' for released NO. In isolated tissues, concentrations of 11 μM almost completely block vaso-relaxation induced by ACh (Martin, Villani, Jothianandan & Furchgott, 1984; Stewart, Munzel & Bassenge, 1987; Myers *et al.* 1989). Despite similar levels of free plasma Hgb due to pump-associated haemolysis in the present study, ACh flow augmentation was virtually identical to that observed in the *in vivo* hearts perfused by non-haemolysed blood and to that reported by others (Van Winkle & Feigl, 1989; Yamabe *et al.* 1992; Smith & Canty, 1993). This lack of effect was further confirmed by directly infusing reduced Hgb, I.C., at the same concentration and finding the ACh flow reserve was minimally altered. Much higher concentrations of Hgb inhibited the flow increase, but the residual response was still far greater than the near-complete blockade observed in crystalloid-perfused preparations. This disparity between the influence of free Hgb in whole-blood- *versus* crystalloid-perfused preparations is intriguing and will require further investigation.

Lastly, hydrocortisone and indomethacin were administered to the support dog early in the preparation to minimize pulmonary vascular instability during extracorporeal circulation. Although pretreatment with a cyclo-oxygenase inhibitor could lead to basal vascular constriction, resting coronary flows were within the normal range. Indomethacin should not have inhibited NO-mediated vasodilatation and it may have been useful by blocking confounding prostacyclin-mediated responses. Glucocorticoids inhibit the expression of inducible NOS but not constititutive (or endothelial) NOS (Radomski, Palmer & Moncada, 1990). Since our study primarily investigated the physiological activity of the latter forms of NOS, minimal effects would be anticipated.

Inhibition of NO production and coronary flow regulation

NOS blockade markedly lowers resting coronary blood flow in isolated crystalloid-perfused hearts (Ueeda *et al.* 1992; Amrani *et al.* 1992; Brown *et al.* 1993) and in blood-perfused rat hearts (Bouma *et al.* 1992). Such flow reduction has also been linked to increased lactate extraction (Amrani *et al.* 1992). These data suggest that NO plays an important role in maintaining basal flow in these

preparations, in striking contrast to the lack of flow change reported *in vivo* (Smith & Canty, 1993; Lefroy *et al.* 1993; Kirkebøen, Naess, Offstad & Ilebekk, 1994; Duncker & Bache, 1994; Hare, Keaney, Balligand, Loscalzo, Smith & Colucci, 1995). The present data measured in whole-blood-perfused isolated canine hearts support the latter studies.

There are several potential causes for this disparity. The smaller isolated hearts are almost always removed from the chest prior to establishing coronary perfusion and this introduces a brief but intense period of ischaemia, potentially damaging coronary vascular function. Coronary flow regulation is sensitive to the condition of the preparation (Canty, 1988) and it is markedly diminished in crystalloid-perfused hearts. Both high basal flow rates (often 4–6 ml min^{-1} g^{-1}, at a mean CPP of 90 mmHg (Ueeda *et al.* 1992; Bouma *et al.* 1992; Brown *et al.* 1993) and an increased sensitivity of coronary flow and LV function to CPP (Kitakaze & Marban, 1988; Ueeda *et al.* 1992) belie the loss of regulation. For example, Ueeda *et al.* (1992) reported a 68% rise in CBF when CPP was increased from 55 to 85 mmHg, nearly 4 times that measured in the present study. Kitakaze & Marban (1988) reported a 130% rise in developed pressure when CPP was increased from 50 to 150 mmHg, 7·5 times the change in PVA observed in our study. Thus, the present isolated heart system reflects an intermediate condition between the tight coronary flow regulation documented in conscious animals (Canty, 1988) and the minimal regulation measured in most crystalloid-perfused isolated heart preparations.

While NO inhibition would appear to minimally influence basal coronary flow, prior studies performed in conscious animals (Smith & Canty, 1993; Duncker & Bache, 1994) have suggested that autoregulation becomes compromised at higher perfusion pressures. Thus, NO could play an important role in reducing coronary vascular resistance when CPP is compromised. However, in both studies, NOS inhibition led to an increase in peripheral vascular load and this itself could raise the CPP at which flow regulation diminishes (Mosher *et al.* 1963; Dole, 1987; Feigl *et al.* 1989). The present data support this interpretation, since when $M\dot{V}_{O_2}$ and PVA were held absolutely constant, NOS inhibition did not influence the CPP–CBF relationship.

Basal NO and LV contractility

Data regarding the importance of NO production to basal contractility have yielded varying results. Brady, Warren, Poole-Wilson, Williams & Harding (1993) reported that unloaded cardiac myocyte shortening could be attenuated by superfusion with NO solution, acting via intracellular production of guanosine 3′,5′-cyclicmonophosphate. Similar results were reported by Balligand, Kelly, Marsden, Smith & Michel (1993). In both instances, the effects of NOS inhibition were dependent upon the presence of enhanced β-adrenergic stimulation or Ca^{2+} activation. Adrenergic stimulation was also important for observing a depressant effect of NO in intact animals (Hare *et al.* 1995), in contrast to the minimal changes observed under basal conditions (Chu *et al.* 1991). In a recent clinical study, Paulus *et al.* (1994) performed bi-coronary sodium nitroprusside infusion and reported a 9% decline in peak-systolic pressure and a 3% reduction in the systolic time period under basal conditions. These were interpreted as indicating a significant effect of NO on LV contractile function. However, these small changes could not be entirely separated from peripheral vascular effects. Furthermore, dP/dt_{max}, another measure of contractile function, was unaltered.

The present data also found an insignificant influence of NOS inhibition on contractile function when measured 15–20 min after L-NMMA administration. We found a very small decline in contractile function as measured by the ESPVR later in the protocol. This may have resulted from a delayed effect of NOS inhibition, or from slight deterioration in the preparation over time; however, this change (~6%) was also unlikely to be of any physiological significance. By stabilizing the condition of the support dog, the isolated heart is less sympathetically driven, as indicated by an end-systolic elastance of 2–3 mmHg ml^{-1}, similar to values measured in autonomically blocked intact animals (Liu, Tunin & Kass, 1993). Reduced cardiac sympathetic stimulation would be expected to yield less change in contractile function after NOS inhibition, contributing to a lack of effect in the present study.

Effect of NOS inhibition on LV energetics

No previous study has directly assessed the influence of NOS inhibition on myocardial energetics. Two recent preliminary studies (Davis, Harris, Quinn, Ahlin & Klocke, 1994; Bernstein, Shen, Xu & Hintze, 1994) suggested that NOS inhibition could lower $M\dot{V}_{O_2}$ in response to isoprenaline, or at comparable levels of cardiac workload. However, workload was indexed to the double or triple product; thus systemic effects on peripheral vascular load due to NOS inhibition may not have been accurately indexed. The present study employed the $M\dot{V}_{O_2}$–PVA relationship to provide a more load-independent assessment of chamber energetics (Suga *et al.* 1983; Suga, 1990). In particular, the reciprocal of the slope of this relationship was unaltered by L-NMMA. The very small, but statistically significant, parallel downward shift ($< -6\%$) does indicate a slight fall in $M\dot{V}_{O_2}$ at a matched workload. However, this is also compatible with the small similar decline in contractile function observed over the same time period.

Conclusion

Inhibition of NOS in the coronary vasculature of whole-blood-perfused canine hearts contracting at constant workload does not have a significant effect on the perfusion pressure-dependent regulation of basal coronary flow, nor on LV contractile function and energetics. Previous *in vitro* or *in vivo* data indicating greater influences of NO blockade on basal coronary flow and autoregulation are likely to

reflect alterations in vascular tone and endothelial function, or influences of NOS inhibitors on the peripheral vasculature. While NO may play a more prominent role in hearts operating under particularly high inotropic stimulation, or in the presence of a stimulus for vasodilatation, it does not appear to be a major modifier of basal cardiac function or perfusion.

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