Nitric oxide does not modulate the increases in blood flow, O$_2$ consumption, or contractility during CaCl$_2$ administration in canine hearts

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Received 29 June 1998; accepted 12 October 1998

Abstract

Objective: Endothelium-derived nitric oxide (EDNO) has been shown to have vascular, metabolic, and contractile effects in the heart. We evaluated these effects during intracoronary (i.c.) administration of CaCl$_2$ in dogs. Methods: The left anterior descending coronary artery of nine anesthetized, open-chest dogs was perfused at controlled pressure (80 mm Hg) with arterial blood. Coronary blood flow (CBF) was measured with a Doppler transducer and segmental shortening (SS) with ultrasonic crystals. Myocardial oxygen consumption (MVO$_2$) and oxygen extraction (EO$_2$) were calculated. Responses were assessed during i.c. infusions of CaCl$_2$ (5, 10, 15 mg min$^{-1}$) before and after administration of the NO synthase inhibitor $N^\text{-}$nitro-L-arginine methyl ester (L-NAME; 300 $\mu$g min$^{-1}$ for 15 min, i.c.). Results: Before L-NAME, CaCl$_2$ caused dose-dependent, proportional increases in SS and MVO$_2$. Although CBF also increased, these responses were less than proportional to those in MVO$_2$, and thus EO$_2$ increased. L-NAME did not alter the cardiac effects of CaCl$_2$. Conclusions: (1) CaCl$_2$ had direct inotropic and coronary vasoconstricting effects. (2) The vasoconstricting effect impaired coupling of CBF to the augmented metabolic demands by local vasodilating mechanisms. (3) EDNO did not modulate the increases in CBF, MVO$_2$, or SS during administration of CaCl$_2$. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Coronary hemodynamics; Inotropic drugs; Cardiac performance; Dogs; $N^\text{-}$nitro-L-arginine methyl ester

1. Introduction

Nitric oxide (NO) is produced in the vascular endothelium from the amino acid L-arginine in a reaction catalyzed by NO synthase (NOS) and triggered by rises in intracellular Ca$^{2+}$ concentration [1]. Endothelium-derived NO (EDNO) diffuses to the underlying vascular smooth muscle, where it stimulates guanylate cyclase and production of cyclic guanosine 3'-5'-monophosphate (cGMP), which has vasorelaxing effects [1]. EDNO is released tonically in the coronary circulation, and its release is accelerated by substances, e.g., acetylcholine, which operate via specific receptors on the endothelial cell, and by mechanical factors, such as shear stress [2]. EDNO has been shown to play an important role in the reductions in coronary vasomotor tone elicited by a variety of physiological stimuli, including hypoxia [3], reduced perfusion pressure (autoregulation) [4], transient occlusion, i.e., reactive hyperemia [5], hypercapnia [6], and the increased cardiac work associated with tachycardia and increased wall tension [7]. Few studies have assessed the role of EDNO in the myocardial functional hyperemia accompanying inotropic stimulation, and they were limited to agents that operate via the $\beta$-adrenergic receptor-cyclic adenosine 3'-5'-monophosphate (cAMP) pathway, i.e., isoproterenol and dobutamine [8–10].

Calcium is an inotropic agent that acts independently of the $\beta$-adrenergic receptor-cAMP pathway [11]. In a recent study [12], we assessed the coronary vascular effects accompanying CaCl$_2$-induced augmentations in contractility in normal canine hearts. Intracoronary (i.c.) infusions of CaCl$_2$ were associated with increases in coronary blood flow, although these increases were less than proportional...
to those in myocardial oxygen consumption. This sugges-
ted that a direct constricting effect for calcium partially
antagonized the metabolic vasodilation associated with
increased cardiac work [13]. The signal-transduction path-
way underlying the coronary vasodilatation during adminis-
tration of CaCl₂ remains to be clarified. A role for the
NO-cGMP pathway is suggested by the ability of Ca²⁺
concentration to modulate NOS activity in the endothelial

cell (see above).

The present study tested the hypothesis that EDNO
modulates the myocardial functional hyperemia during
administration of CaCl₂. This was evaluated by comparing
the CaCl₂-induced changes in coronary blood flow before
and after treatment with the NOS inhibitor N⁶-nitro-L-
arginine methyl ester (L-NAME). Recent in vitro studies
have suggested that EDNO may reduce myocardial oxygen
consumption directly via inhibition of mitochondrial en-
zymes [14] and indirectly via a negative inotropic effect
[15]. If NO has this influence in the intact heart during
exposure to CaCl₂, the increases in myocardial oxygen
consumption may be accentuated following NOS inhibi-
tion, thus providing an enhanced metabolic stimulus for
coronary vasodilatation. To distinguish between direct and
metabolism-mediated effects of L-NAME on the myocar-
dial functional hyperemic responses, the inotropicall-
duced changes in coronary blood flow were assessed in
the context of the concomitant changes in myocardial oxygen
consumption and segmental shortening.

The study was performed using an extracorporeal sys-
tem to perfuse selectively the left anterior descending
coronary artery (LAD) in situ canine hearts. This
approach facilitated the use of selective i.c. infusions of
drugs, which minimized their systemic effects.

2. Methods

2.1. Canine preparation

The investigation conforms with the Guide for the Care
and Use of Laboratory Animals published by the US
National Institutes of Health (NIH Publication No. 85-23,
revised 1985). Experiments were performed on nine
healthy mongrel dogs of either sex (weight, 20.4±22.8 kg).
Anesthesia was induced with intravenous bolus injection of
thiopental 15 mg kg⁻¹, and maintained by continuous
intravenous infusion of fentanyl and midazolam at rates of
(0.1 mg kg⁻¹ h⁻¹ and 0.6 mg kg⁻¹ h⁻¹, respectively. After
tracheal intubation and left thoracotomy in the fourth
intercostal space, the lungs were mechanically ventilated
with room air enriched with oxygen to maintain arterial
PO₂ greater than 200 mm Hg. The tidal volume and
respiratory rate were adjusted to maintain arterial PCO₂
and pH at physiological levels (PCO₂; 38±2 mm Hg; pH;
7.39±0.01). PO₂, PCO₂, and pH of arterial and venous
blood samples (see below) were measured electrometrical-
ly (model 413, Instrumentation Laboratories, Lexington,
MS). Muscle paralysis was achieved with an intravenous
injection of vecuronium bromide, 0.1 mg kg⁻¹ with sup-
plements at 0.05 mg kg⁻¹ h⁻¹. Body temperature was
maintained at 38°C with a heating pad. Lactated Ringer’s
solution was administered continuously at a rate of 5
ml kg⁻¹ h⁻¹ intravenously to compensate for evaporative
fluid losses. Heparin (400 U/kg with supplementation) was
used for anticoagulation.

The LAD was isolated for cannulation approximately 2
cm from its origin. A thin-wall stainless-steel cannula (2.5
mm inside diameter) was introduced into the isolated
segment of the artery, so that it could be perfused selec-
tively by an extracorporeal perfusion system [6,16].
The perfusion system contained a pressurized reservoir,
which was supplied with blood from the left femoral
artery.

The tubing connecting the reservoir to the LAD was
equipped with (1) a heat exchanger to maintain the
temperature of coronary arterial blood at 38°C, (2) a
Doppler flow transducer (Transonics Systems, Ithaca, NY)
to measure coronary blood flow, (3) ports for collecting
samples of coronary arterial blood and for infusing drugs,
and (4) a mixing chamber for drugs infused into the
perfusion tubing. Coronary perfusion pressure was mea-
sured through a small-diameter tube positioned at the
outlet of the perfusion cannula.

Measurements of aortic pressure, heart rate, left atrial
pressure, left ventricular +dP/dtmax, and left ventricular
−dP/dtmin were obtained using standard methods [6,16]. A
continuous record of hemodynamic variables was obtained
on a physiological recorder (model 2800S, Gould, Cleve-
land, Ohio).

2.2. Experimental measurements

2.2.1. Myocardial segmental shortening

Measurements of myocardial segmental length in the
LAD bed were obtained with a pair of ultrasonic crystals
[16]. Changes in distance between the crystals were
recorded from measurements of the ultrasonic transit time
between the crystals (Triton Technology, San Diego, CA).
The end-diastolic and end-systolic lengths (EDL, ESL)
were identified by the beginning of the rapid increase in
left ventricular pressure just before isovolumetric contrac-
tion and −dP/dt min, respectively. Segmental shortening
(SS; in %) was calculated from the formula:

SS = [(EDL − ESL)/EDL] × 100

2.2.2. Myocardial oxygen consumption

Measurements of myocardial oxygen consumption were
obtained in the LAD perfusion territory. The anterior
interventricular vein was cannulated at the same level as
the LAD cannula for collection of regional coronary
venous effluent [17]. The venous cannula was allowed to
drain freely into a beaker to prevent venous stagnation and interstitial edema. This venous blood was returned intermittently to the dog to maintain isovolemic conditions. At specified times in the study, 1-ml blood samples were collected from the coronary venous cannula under mineral oil to maintain anaerobic conditions. These venous samples were paired with 1-ml arterial samples from the perfusion tubing, so that the coronary arterial venous difference for oxygen could be determined. Hemoglobin concentration and percent hemoglobin oxygen saturation of the blood samples were measured with a CO-Oximeter (model 482, Instrumentation Laboratories, Lexington, MS), and used to calculate oxygen bound to hemoglobin assuming an oxygen carrying capacity for hemoglobin of 1.39 ml O₂/g. The oxygen dissolved in the blood was computed (O₂ dissolved = 0.003 ml O₂ per 100 ml of blood per mm Hg) and added to the bound component to compute total oxygen content. Myocardial oxygen consumption (in ml min⁻¹ 100 g⁻¹) was computed using the Fick equation, i.e., from the product of the coronary arterial venous oxygen difference and coronary blood flow. The myocardial oxygen extraction (in %) was calculated by dividing the arteriovenous oxygen difference by the arterial oxygen content. These values are a reflection of the relationship between myocardial oxygen consumption and coronary blood flow [13], and they served as an index of the effectiveness of metabolic vasodilation during CaCl₂. The oxygen cost of inotropic stimulation was quantified by dividing the inotropically-induced percentage increase in myocardial oxygen consumption by the inotropically-induced percentage increase in segmental shortening. The resultant value was termed the oxygen cost ratio.

2.2.3. Coronary arterial ionized calcium concentration
Ionized calcium concentration ([Ca⁺⁺⁺⁺]) in the LAD blood during infusion of CaCl₂ was estimated by calculating the [Ca⁺⁺⁺⁺] added to the blood (by dividing the infusion rate for calcium by the steady state value for coronary blood flow), and adding it to the control value for [Ca⁺⁺⁺⁺]. This approach was validated in our previous study under control conditions in our previous study (1.22±0.06 mmol l⁻¹) was used in the calculations of [Ca⁺⁺⁺⁺] [12].

2.3. Experimental protocols
After >45 min for recovery from surgical preparation, control measurements for coronary blood flow, segmental shortening, oxygen consumption and oxygen extraction were obtained. Then CaCl₂ was infused into the LAD in a graded fashion (5, 10, 15 mg min⁻¹). Measurements were obtained when steady state conditions were achieved at each drug infusion rate (as indicated by stable increases in coronary blood flow and segmental shortening), which was within 2–3 min after varying the rate of infusion. The dose range for CaCl₂ was selected because it had been shown previously to span a significant portion of the dose–response curve [18]. Isotonic saline was used to dilute CaCl₂ to 5.0 mg ml⁻¹, which resulted in infusion rates over the range 1.0 to 3.0 ml min⁻¹. Preliminary studies demonstrated that infusion of the saline vehicle alone at these low flow rates had no effect on coronary blood flow or segmental shortening. After a final dose of CaCl₂, the infusion pump was stopped and at least 20 min was allowed for recovery. Then l-NAME was infused at a rate of 300 μg min⁻¹ i.c. for 15 min to inhibit production of NO from the vascular endothelium [6,10,16]. Ten min were allowed before the graded infusions of CaCl₂ were repeated. Efficacy of l-NAME was evaluated using i.c. infusions of the endothelium-dependent vasodilator acetylcholine (ACh; 20 μg min⁻¹), and of the endothelium-independent vasodilator sodium nitroprusside (SNP; 80 μg min⁻¹). The doses for ACh and SNP were the highest that could be used without causing aortic hypotension [6,10,16]. Vasodilator reserve was assessed with a maximally-dilating infusion of adenosine (8 mg min⁻¹ i.c. [6,10,16]). A coronary perfusion pressure of 80 mm Hg was used throughout the study.

At the termination of each experiment, 5 ml of Evans blue dye (10 mg ml⁻¹ saline) was injected into the LAD to identify its perfusion territory. After the heart was stopped with a 10-ml bolus injection of KCl (80 mg ml⁻¹ saline) into the left ventricular cavity, it was removed and trimmed. The dyed tissue was excised and weighed so that coronary blood flow could be expressed on a per 100-g basis. The average weight of the LAD perfusion territory was 32±2 g.

2.4. Statistical analyses
A two-way analysis of variance for repeated measurements was used to assess the dose-dependent effects of CaCl₂ before and after l-NAME [19]. Post hoc comparisons were made using the Student’s t-test with the Bonferroni correction [19]. Additional statistical analyses were performed using the paired version of the Student’s t-test [19]. Data are presented as mean±standard error. A P<0.05 was considered significant throughout this study.

3. Results
Fig. 1 presents the effects of CaCl₂ on coronary blood flow, myocardial oxygen consumption, oxygen extraction, and segmental shortening before and following l-NAME. CaCl₂ caused dose-dependent increases in coronary blood flow, which at constant coronary perfusion pressure, mirrored the induced decreases in coronary vascular resistance. These increases in coronary blood flow were less than proportional to those in myocardial oxygen consumption, with the result that oxygen extraction increased. CaCl₂ caused dose-dependent increases in seg-
mental shortening, which were proportional to those in myocardial oxygen consumption; thus, oxygen cost ratio remained equal to unity (Table 1). L-NAME had no independent effect on the local cardiac variables. There was no interaction between the effects of CaCl₂ and L-NAME.

Table 2 shows that the values for calculated [Ca²⁺] varied directly with the rate of infusion of CaCl₂, and that they were not different before and following L-NAME. L-NAME itself had no effect on the baseline values for cardiac and systemic hemodynamic variables (Table 3); however, it blunted the increases in coronary blood flow by ACh (178±41% vs. 39±6%), although it had no effect on the increases in coronary blood flow by SNP (127±17% vs. 125±14%) (Fig. 2). Adenosine caused nearly five-fold increases in coronary blood flow (116±15 to 564±56 ml min⁻¹ 100 g⁻¹).

Table 4 shows the effect of the i.c. infusions of CaCl₂ before L-NAME on systemic hemodynamic and global cardiac variables. CaCl₂ had no effect on these variables.

### Table 1
Values for the O₂ cost ratio during graded intracoronary infusions of CaCl₂ before and after L-NAME

<table>
<thead>
<tr>
<th>[CaCl₂] mg min⁻¹</th>
<th>Pre L-NAME</th>
<th>Post L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.9±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>10</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>15</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SE.

### Table 2
Calculated values for ionized calcium (mmol l⁻¹) in coronary blood supply

<table>
<thead>
<tr>
<th>[CaCl₂] mg min⁻¹</th>
<th>Pre L-NAME</th>
<th>Post L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.59±0.22</td>
<td>2.72±0.24</td>
</tr>
<tr>
<td>10</td>
<td>3.66±0.38</td>
<td>3.93±0.44</td>
</tr>
<tr>
<td>15</td>
<td>4.71±0.58</td>
<td>5.09±0.67</td>
</tr>
</tbody>
</table>

Values are mean±SE.
Table 3
Baseline values for cardiac and systemic hemodynamic parameters before and following l-NAME

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre l-NAME (ml min⁻¹ 100 g⁻¹)</th>
<th>Post l-NAME (ml min⁻¹ 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary blood flow</td>
<td>106±11</td>
<td>104±14</td>
</tr>
<tr>
<td>Myocardial oxygen consumption</td>
<td>6.2±0.4</td>
<td>5.6±0.5</td>
</tr>
<tr>
<td>Myocardial O₂ extraction, %</td>
<td>43±1</td>
<td>44±2</td>
</tr>
<tr>
<td>Myocardial segmental shortening, %</td>
<td>14.9±1.7</td>
<td>14.0±1.5</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>75±7</td>
<td>76±6</td>
</tr>
<tr>
<td>Heart rate, beats per min</td>
<td>115±11</td>
<td>113±9</td>
</tr>
<tr>
<td>Mean left atrial pressure, mm Hg</td>
<td>4.4±0.8</td>
<td>4.8±0.8</td>
</tr>
<tr>
<td>Left ventricular +dP/dtmax, mm Hg/s</td>
<td>1194±77</td>
<td>1133±67</td>
</tr>
<tr>
<td>Left ventricular −dP/dtmax, mm Hg/s</td>
<td>−972±111</td>
<td>−939±115</td>
</tr>
</tbody>
</table>

Values are mean±SE.

Fig. 2. Effects of acetylcholine (ACh) and sodium nitroprusside (SNP) on coronary blood flow before and after l-NAME. l-NAME blunted the increases in coronary blood flow by ACh but not by SNP. Values are mean±SE. * vs. Before l-NAME.

4. Discussion

4.1. Critique of methods

The baseline values for oxygen extraction in the cannulated LAD bed were lower than those usually found in anesthetized dogs with an intact coronary circulation [20]. This suggests vasodilation in the control preparation probably because of dilators released from blood cells within the extracorporeal circuit [21]. Nevertheless, vascular responsiveness to both an endothelium-dependent vasodilator (ACh) and an endothelium-independent vasodilator (SNP) was pronounced (Fig. 2). Moreover, vasodilator reserve was appreciable, as shown by the nearly five-fold increases in coronary blood flow during adenosine infusion. These latter findings indicated that the increases in coronary blood flow during CaCl₂ administration were not limited by the vasodilator reserve of the preparation.

The ability of changes in segmental shortening to reflect changes in myocardial contractility is limited by variations in heart rate and in the loading conditions of the heart [22]. The constant values for heart rate and in indices of afterload (aortic pressure) and preload (left atrial pressure) during the i.c. infusions of CaCl₂ and l-NAME suggest that this methodologic limitation did not apply to the present study.

Our baseline values for myocardial oxygen consumption

Table 4
The intracoronary infusions of CaCl₂ before l-NAME had no effect on systemic hemodynamic parameters, with the exception that they increased left ventricular dP/dtmax. Findings were similar following l-NAME

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (mg min⁻¹)</th>
<th>CaCl₂</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>75±7</td>
<td>78±6</td>
<td>77±6</td>
<td>78±6</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats per min</td>
<td>115±11</td>
<td>116±14</td>
<td>113±12</td>
<td>114±11</td>
<td></td>
</tr>
<tr>
<td>Mean left atrial pressure, mm Hg</td>
<td>4.4±0.8</td>
<td>4.3±0.8</td>
<td>4.0±0.9</td>
<td>4.0±0.9</td>
<td></td>
</tr>
<tr>
<td>Left ventricular +dP/dtmax, mm Hg/s</td>
<td>1194±77</td>
<td>1411±72*</td>
<td>1483±102*</td>
<td>1511±98*</td>
<td></td>
</tr>
<tr>
<td>Left ventricular −dP/dtmax, mm Hg/s</td>
<td>−972±111</td>
<td>−956±100</td>
<td>−961±105</td>
<td>−978±105</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SE. * P<0.05 vs. baseline.
were typical of those obtained by us in this canine preparation [6,10,12,16], but they were lower than those reported by other investigators also studying open-chest, anesthetized dogs [7,23]. This discrepancy may be due to the lower, more physiological values, for heart rate when fentanyl, rather than pentobarbital sodium, is used for general anesthesia. Fentanyl reduces heart rate by stimulation of vagal preganglionic neurons in the medulla [24], whereas pentobarbital sodium increases heart rate via a vagolytic action [25]. Our baseline values for segmental shortening were well within the range of those previously found in similar canine preparations [26,27].

\[\text{[Ca}^{++}]\] in the coronary arterial blood during infusion of \(\text{CaCl}_2\), was calculated by dividing the infusion rate for calcium by the steady state value for coronary blood flow, and assuming a constant control value for \([\text{Ca}^{++}]\), i.e., that no recirculation of the infused calcium occurred. This assumption was based on our previous finding indicating only negligible increases in \([\text{Ca}^{++}]\) within the aortic blood during a high-dose (15 mg min \(^{-1}\)) infusion of \(\text{CaCl}_2\) into the LAD [12]. The strong correlation between our calculated values for \([\text{Ca}^{++}]\) and those measured directly [12] validated our approach for estimating \([\text{Ca}^{++}]\) in the coronary arterial blood.

Intravenous infusions of NOS inhibitors cause increases in systemic vascular resistance (and concomitant increases in arterial pressure and left ventricular afterload) [1,4], suggesting that tonic release of NO may play an important role in modulating basal vascular tone in the peripheral circulation. The use of i.c. infusions of \(\text{L-NAME}\) in the present study avoided its systemic effects, which simplified interpretation of the findings. Our i.c. dose for \(\text{L-NAME}\) was adopted from previous studies [6,10,16], which showed that it produced a 70–80% attenuation of the increases in coronary blood flow without effects on systemic circulatory variables or on the increases in coronary blood flow caused by SNP. These findings were confirmed in the present study.

The residual ACh-induced increase in coronary blood flow following \(\text{L-NAME}\) raises the possibility that our dose for \(\text{L-NAME}\) was not adequate for a complete blockade of the NO-cGMP pathway. Although we cannot definitively rule this out, it seems unlikely since previous investigators using an i.c. dose for \(\text{L-NAME}\) four-times our dose (and sufficient to cause increases in arterial pressure) reported similar results [8]. Indeed, the failure of NOS inhibitors, including \(\text{L-NAME}\), to completely abolish ACh-induced coronary vasodilation is a consistent finding in the literature [5–10,16,23], and it is likely explained by the ability of ACh to cause release of a hyperpolarizing factor, in addition to NO, from the vascular endothelium [28].

4.2. Role of nitric oxide in cardiac effects of calcium chloride

Coronary blood flow is normally matched to the prevailing myocardial oxygen demand by local adjustments in coronary vasomotor tone mediated by metabolic control mechanisms [13]. This local control of coronary blood flow tends to maintain coronary venous \(\text{PO}_2\) and, therefore oxygen extraction, constant. The increases in oxygen extraction during administration of \(\text{CaCl}_2\) in the present study reflected increases in coronary blood flow that were less than proportional to the induced augmentations in myocardial oxygen consumption (secondary to calcium’s positive inotropic effect). These findings are consistent with a direct vasoconstrictor effect for calcium, which was capable of partially antagonizing, but not completely overriding, metabolic vasodilation. These findings in the basal preparation confirm findings from our previous study [12].

Our previous study in the same canine model using dobutamine provides a reference for assessing the effects of \(\text{CaCl}_2\) [29]. Dobutamine caused increases in myocardial contractility that were associated with proportional increases in myocardial oxygen consumption and coronary blood flow with the result that oxygen extraction remained constant. These findings implied that metabolic control of coronary blood flow remained intact during dobutamine infusion, and thus that this agent had no direct vasomotor effect on coronary resistance vessels [13]. They also ruled out the possibility that the increase in oxygen extraction (and the apparent direct vasoconstrictor effect) during \(\text{CaCl}_2\) was an obligatory response to inotropic stimulation in our canine cardiac preparation.

It has been established that endothelial NO synthase (so-called constitutive NO synthase) is \(\text{Ca}^{++}\)-dependent, and that production of EDNO is modulated by intracellular \(\text{Ca}^{++}\) concentration [1]. Yet, we showed that \(\text{L-NAME}\) did not blunt the increases in coronary blood flow or alter their relationship to myocardial oxygen consumption, i.e., the increases in oxygen extraction during \(\text{CaCl}_2\), thus suggesting that EDNO did not contribute to the coronary vasodilating response. We can propose two potential explanations for this finding: (1) The increases in \(\text{Ca}^{++}\) concentration in the plasma were not reflected within the vascular endothelium. (2) The ability of \(\text{Ca}^{++}\) to modulate EDNO production in the coronary resistance vessels, like that in isolated aortic segments [30], does not apply to concentrations above the physiological range. Of course, we cannot rule out the possibility that EDNO was indeed involved in the coronary vasodilating response during \(\text{CaCl}_2\), but that an alternate vasodilating factor, e.g., an augmented adenosine release, compensated for its inhibition.

The failure of NOS inhibition to reduce baseline coronary blood flow has been demonstrated previously [5,6,8–10,16,31], and it has been explained by the emergence of an alternate metabolic mechanism, which preserves myocardial oxygen supply/demand balance when the tonic influence of EDNO is blocked. In support of this view, Kostic and Schrader [32] reported augmented release of
adenosine from isolated guinea pigs hearts following administration of l-NAME.

Investigators using a variety of isolated cardiac preparations and experimental approaches have demonstrated that the NO has the capability to reduce myocardial contractility via an increase in cGMP, and, in turn, a stimulation of cGMP-dependent protein kinase [15], although this effect appears limited to concentrations of NO that greatly exceed physiological values [33]. Interestingly, Kojda et al. [34] in a recent study in isolated ventricular myocytes, showed that NO, in low concentrations, may paradoxically increase myocardial contractility via its ability to inhibit cAMP phosphodiesterase, leading to increased levels of cAMP, and stimulation of cAMP-dependent protein kinase. Previous in vivo studies have consistently shown no influence of EDNO on contractility in non-stimulated myocardium [16,23,31], while some [15], but not others [10], have demonstrated a negative inotropic effect in myocardium stimulated with a β-receptor agonist. The present study extends these latter observations to myocardium stimulated with CaCl₂. The lack of influence of EDNO on myocardial contractility in vivo has been attributed to the ability of hemoglobin and myoglobin to bind NO, thus restricting its access to the cardiomyocytes [16].

Our findings pertain strictly to NO synthesized in the coronary endothelium via the constitutive NOS pathway. Another form of NOS (so-called inducible NOS) has been identified in a variety of cell types, e.g., neutrophils, vascular smooth muscle, and cardiac myocytes, following induction by immunological stimuli, such as cytokines and endotoxin [1]. NO production is more extended and at much higher concentrations via the inducible NOS pathway [1]. Evidence for expression and activity of inducible NOS has been obtained in myocardial samples from patients with dilated cardiopathy, ischemic heart disease, valvular heart disease, and cardiac allograft rejection [35–37]. Studies conducted in various isolated tissues, including cardiac muscle slices and hepatocytes, have demonstrated that the elevated NO concentrations associated with the inducible NOS pathway may have a direct depressive effect on tissue oxygen use [14,38,39]. This effect has been attributed to the binding of NO to the heme moiety of cytochrome enzymes in the mitochondrial electron-transport chain.

Recent studies have sought to determine whether this inhibitory metabolic effect extends to physiological-relevant concentrations of NO in the normal heart in vivo [5–7,10,16,23,31,40,41]. To date, the preponderance of evidence has indicated otherwise. Neither NOS inhibitors, NO donors, nor ACh affected basal myocardial oxygen consumption [5–7,16,23,31,40,41]. Furthermore, NOS inhibitors did not alter the increase in myocardial oxygen consumption caused by atrial pacing, aortic constriction, isoproterenol, or dobutamine [7,10]. The present findings extend these latter observations to the increase in myocardial oxygen consumption caused by CaCl₂. They indicate that CaCl₂ administration caused proportional changes in segmental shortening and myocardial oxygen consumption whether or not tissue NO concentrations were decreased with l-NAME. This implies that basally-released EDNO had no direct effect on the rate of myocardial oxidative metabolism.

Bernstein et al. [41] recently reported that an intravenous infusion of an NOS inhibitor increased myocardial oxygen consumption at various levels of cardiac work in exercising, chronically instrumented dogs. The apparent discrepancy between these findings and those from the present study may be attributable to methodological differences, including the absence or presence of general anesthesia, the route and dose of the NOS inhibitor, and the levels of myocardial oxygen consumption studied. Another factor may be the difficulty in distinguishing changes in myocardial oxygen consumption due to direct effects on the mitochondria from those due to variations in the hemodynamic determinants of cardiac workload, e.g., the increases in aortic pressure, during exercise in the study of Bernstein et al. [41]. Our use of i.c. infusions of CaCl₂ to alter the level of cardiac work precluded this potential pitfall.

We conclude that: (1) CaCl₂ had a vasoconstricting effect and a positive inotropic effect in the normal heart in vivo. This vasoconstricting effect impaired coupling of coronary blood flow to the augmented myocardial oxygen demand by metabolic vascular control mechanisms. (2) EDNO did not modulate the increases in coronary blood flow, myocardial oxygen consumption, or myocardial contractility during administration of CaCl₂.

From a clinical point of view, the present findings suggest that CaCl₂ may be risky as an inotropic agent following termination of cardiopulmonary bypass, when the generation of metabolic vasodilators, e.g., adenosine, may be decreased due to a diminished cardiac responsiveness. This could unmask the coronary vasoconstricting action of CaCl₂, which could precipitate myocardial ischemia. Our findings also imply that the myocardial functional hyperemia during CaCl₂ should be normal in the patient in which endothelial function is impaired because of chronic disease, e.g., atherosclerosis [42], or acute insult, e.g., ischemia and reperfusion [43].

Acknowledgements

The authors thank Derrick L. Harris, B.S., for his expert technical assistance.

References


