Intact nitric oxide production is obligatory for the sustained flow response during hypercapnic acidosis in guinea pig heart

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Received 30 August 2004; received in revised form 24 November 2004; accepted 2 December 2004

Available online 19 December 2004

Time for primary review 12 days

Abstract

Objective: The mechanisms underlying hypercapnic coronary dilation remain unsettled. This study tests the hypothesis that flow dependent NO production is obligatory for the hypercapnic flow response.

Methods/results: In isolated, constant pressure (CP) perfused guinea pig hearts a step change of arterial $p$CO$_2$ from 38.6 to 61.4 mm Hg induced a bi-phasic flow response with an early transient (maximum 60 s) and a consecutive persisting flow rise (121.6 ± 6.6 % after 10 min). In contrast, when perfused with constant flow (CF), perfusion pressure only transiently (2 min) fell by 7.4 ± 4.8 % following the step change of arterial $p$CO$_2$. In CP perfused hearts L-NAME (100 $\mu$mol/l) specifically abolished the delayed flow rise during hypercapnic acidosis (102.3 ± 7.9 % after 10 min), whereas the inhibitor had no effect on perfusion pressure response in CF perfused hearts. Under CP perfusion arterial hypercapnia resulted in a transient rise of coronary cGMP release (from 0.69 ± 0.35 to 1.12 ± 0.68 pmol/ml), which was abolished after L-NAME. Surprisingly, the K$^+$ATP channel blocker glibenclamide did not have any significant effect on the hypercapnic flow response but largely blunted reactive hyperemia after a 20 s flow stop.

Conclusions: The delayed steady state hypercapnic flow response in guinea pig heart requires intact NO production. The absence of a persisting decrease in coronary resistance under CF perfusion points to an important role of shear stress dependent NO production.

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Keywords: Hypercapnia; Acidosis; Coronary circulation; Nitric oxide; K$^+$ATP channel; Vasocostriction/dilation

This article is referred to in the Editorial by A. Cevese (pages 7–8) in this issue.

1. Introduction

Several physiological and pathophysiological conditions are associated with a rise in coronary venous $p$CO$_2$, e.g. pacing, adrenergic stimulation, exercise and myocardial ischemia [1,2]. Also in the human heart, elevation of arterial $p$CO$_2$ and/or lowering of pH is followed by a substantial increase of coronary blood flow [3]. This response is maintained in patients with coronary artery disease [4]. Although the importance of pH or $p$CO$_2$ as potential regulators of coronary flow has been studied for over a century [5], the molecular mechanisms of this flow response have not been clarified to date. During recent years two alternative mechanisms have attracted the interest in studies on coronary vessels, activation of K$^+$ATP channels and stimulation of nitric oxide production. The results from these studies are controversial. Gurevicius et al. using an in situ dog heart model concluded that hypercapnia elicits the release of NO from canine coronary vascular endothelium via a shear stress independent mechanism [6]. Phillips et al. and Song et al., using isolated rat hearts, could not confirm a role for NO as a significant mediator of the flow response.
during brief periods of hypercapnic perfusion [7–10]. Rather, these authors favored the contribution of adenosine and K\textsubscript{ATP} channels in this flow response. A role for adenosine in the hypercapnic flow response is partially supported by measurements of this nucleoside in guinea pig coronary effluent perfusate [11]. Finally, Ishizaka and associates [12,13] showed that the endothelium is unimportant for acidosis induced vasodilation using a model of isolated small coronary arteries from pig heart.

In the course of a previous study [11] we observed that the coronary flow response in isolated guinea pig hearts is clearly biphasic during continuous steady state hypercapnic acidosis. This suggested that the mechanisms involved in the mediation of the flow response may change during the course of a hypercapnic period. A potential link between an early immediate flow response and a late steady state flow regulation may be an augmented endothelial cell shear stress in response to an initial vessel dilation limited to certain vascular segments [14,15]. Thus, the present study was designed to test the hypothesis that the coronary flow response during arterial hypercapnia critically depends on the production of nitric oxide. Because the importance of nitric oxide might change during the hypercapnic period, it seemed important to pay particular attention to the kinetics of the flow response. We therefore induced hypercapnic acidosis in isolated perfused guinea pig hearts, using a constant flow (CF) as well as a constant pressure (CP) set up, respectively. To provide evidence for the importance of NO production, the effects of the NOS-inhibitors L-NAME and L-NMMA on the flow response and the cGMP release were studied. The results obtained point to the obligatory role of NO for the steady state coronary flow rise during hypercapnic acidosis, whereas K\textsubscript{ATP} channels proved to be irrelevant. Results from CF experiments indicated the importance of shear stress to trigger NO production during hypercapnic acidosis.

2. Materials and methods

2.1. Animals

Healthy Hartley guinea pigs of either gender (250–350 g) were obtained from Charles River Laboratories. The care and all experiments were performed according to the animal welfare regulations of the German local authorities conforming to NIH Guidelines.

2.2. Langendorff heart preparation

Animals were stunned by a firm blow on the neck. Hearts were removed during continuous cooling (Krebs buffer 4 \textdegree C) and mounted within <1 min via the aorta vertically on a perfusion cannula. Perfusion was immediately started according to the Langendorff technique with a modified Krebs–Henseleit buffer (KHB) containing (mmol/l): NaCl 116, KCl 4.6, MgSO\textsubscript{4} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 25, CaCl\textsubscript{2} 2.5, Glucose 11 and pyruvate 2. The buffer was equilibrated with a 95% O\textsubscript{2}/5% CO\textsubscript{2} mixture (resulting pH 7.38) and maintained at 37 \textdegree C. The pulmonary artery was catheterized to permit anaerobic collection of the coronary venous effluent perfusate. Total coronary flow was measured using an ultrasonic transit-time flowmeter (T206 Transonic Systems, Ithaca, NY) inserted in the arterial perfusion line. Coronary perfusion pressure was measured through a pressure transducer (Gould Statham) connected to the perfusion cannula. Left ventricular pressure (LVP) was assessed isovolumically using a fluid-filled latex balloon (size 4, Harvard Apparatus Inc.) advanced into the ventricle via the left atrium and connected to a pressure transducer (Gould Statham). End-diastolic pressure was set to 8 ± 2 mm Hg. Heart rate was calculated electronically from the LVP recording. Measurements of coronary perfusion pressure, flow, LVP and heart rate were converted by an A/D-converter and acquired (data sampling rate 100 Hz), displayed and stored on a PC. The data were processed by this computer using AcqKnowledge™ software (Biopac Systems, CA). “Arterial” and “venous” perfusate samples collected anaerobically were analyzed for \( pO_2 \), \( pCO_2 \), pH, HCO\textsubscript{3} (AVL 990S, AVL Scientific Corporation, Roswell, GA) before, during and after each intervention.

2.3. Experimental groups

Hearts were perfused at a constant pressure of 54 mm Hg (CP, \( n=22 \)), which is close to the physiological blood pressure of this species [16], or with a constant flow at 10 ml/min (CF, \( n=7 \)). In addition to these isovolumically working hearts further experiments were carried out in empty beating hearts (mitral valve cut, no balloon, referred to as non-working (nw) hearts, \( n=8 \)), perfused at a constant perfusion pressure of 54 mm Hg (nwCP), to minimize the potential importance of a reduced ventricular pressure development during the hypercapnic challenge on the flow response.

2.4. Experimental criteria

Each heart preparation, which showed development of left ventricular pressure in the physiological range [16] was tested for the coronary vascular resistance by evoking a reactive flow response following a 20 s flow stop (CP) or by testing the drop of perfusion pressure following application of a 50 \textmu M adenosine in 50 \textmu l bolus (CF). Only hearts which exhibited at least a doubling of flow after the 20 s flow stop (CP) or showed a perfusion pressure decrease of more than 20 mm Hg following the adenosine bolus (CF) were used for the experiments.

2.5. Experimental protocol

Following instrumentation of the heart and after a 20 min normocapnic perfusion (mean \( pO_2 \) 634 mm Hg, \( pCO_2 \)
38.6 mm Hg, pH 7.38) the reactive flow response was tested. After another 10 min, normocapnic perfusion was switched to hypercapnic perfusion (mean \( pO_2 \) 618 mm Hg, \( pCO_2 \) 61.4 mm Hg, pH 7.12) for 10 min. To test for the potential involvement of NO production or \( K_\text{ATP} \) channels in the hypercapnia induced flow response, L-NAME (100 \( \mu \text{mol/l} \); \( n=11 \)) or L-NMMA (100 \( \mu \text{mol} \); \( n=4 \)) and glibenclamide (3 \( \mu \text{mol/l} \); \( n=7 \)) were used, respectively. The applications of these blockers were started 10 min after the end of the first hypercapnic challenge. After 10 min of blocker infusion the coronary flow response following a 20 s flow stop was checked again. Then, 20 min after start of the inhibitor infusion, a second hypercapnic challenge (10 min) was performed. A diagram of the experimental procedures is given in Fig. 1.

To assure the instant change of arterial \( pCO_2 \) without an undefined delay we used a system with two separate warmed reservoirs filled with a normo- versus a hypercapnic perfusate, respectively. The perfusate containing reservoirs could be switched alternatively to the heart perfusion line via a three-way stop cock. In preliminary experiments with both columns filled with normocapnic perfusate a column switch did not lead to any relevant coronary flow response. In further pilot experiments the response time of the perfusate change was assessed by using indigo blue colored perfusate collected in fractions at a rate for 0.8 s each and measuring the color intensity off-line in cuvettes by UV-spectrometry. The response time between the stop cock switch and full intensity of the indigo blue concentration in the venous effluent perfusate of an isolated perfused heart was 2.4 s.

2.6. ELISA

NO acts via cGMP as second messenger. To provide evidence for the contribution of cGMP in the NO mediated flow response the release of cGMP was determined in the venous effluent perfusate under control conditions and after 30, 60, 90, 180, 300 and 600 s of hypercapnic perfusion in CP hearts. Effluent perfusate was collected directly from the pulmonary artery catheter and frozen at −80 °C until further analysis. A commercially available kit specific for cGMP (R&D Systems, Germany) was used for the measurements according to the manufacturer’s instructions.

2.7. Chemicals

L-NAME and L-NMMA (dissolved in KHB), glibenclamide (dissolved in DMSO) and adenosine (dissolved in isotonic saline solution) were obtained from Sigma (München, Germany). Infusion rates did not exceed 1% of the coronary flow rate for KHB or saline and were less than 1% in the case of DMSO.

2.8. Calculations and statistics

Myocardial oxygen consumption (\( V_O_2 \), \( \mu \text{mol min}^{-1} \text{g}^{-1} \)) was calculated from the arterial–venous difference of \( pO_2 \) according to Fick's principle with the use of Bunsen’s absorption coefficient (\( x'=0.036 \mu \text{mol} \cdot \text{mm Hg}^{-1} \cdot \text{ml}^{-1} \)) at 37 °C as follows: \( V_O_2 (\mu \text{mol min}^{-1} \text{g}^{-1})=(P_{aO_2}-P_{VO_2}) \cdot x' \cdot F \), where \( F \) denotes coronary flow (ml min\(^{-1}\text{g}^{-1}\)). Myocardial performance was estimated by calculating the pressure-rate product (LVPsys \( \cdot \) HR).

Data are reported as means±standard error of mean (S.E.M.). Data distribution was assessed using the Kolmogorov–Smirnov test. The differences in peak flow after a 20 s flow stop during control and after treatment were assessed by Student’s paired \( t \)-test. For statistical comparison of the control hypercapnic flow response and the flow response in the presence of L-NAME or glibenclamide, respectively, an ANOVA for repeated measures was performed. The difference in cGMP release between experimental groups was assessed by a one-way ANOVA. Statistical analyses were carried out using SPSS software for MS Windows (Release 11.0, SPSS, Chicago, IL). A \( p<0.05 \) was taken to indicate a statistical significance.

3. Results

3.1. CP hearts

The baseline coronary flow in isolated hearts (\( n=18 \); ventricular mass 1.69±0.36 g) was 6.1±0.2 ml min\(^{-1}\text{g}^{-1}\) with an LVP development of 52±3 mm Hg (peak systolic ventricular pressure 60±3 mm Hg), heart rate 196±6 bpm and \( V_O_2 \) 80.6±4.3 \( \mu \text{mol min}^{-1} \text{g}^{-1} \). Interestingly, the hypercapnia-induced coronary vasodilation was biphasic. An initial rapid flow increase (maximum 60 s), was followed...
by a slower but lasting vasodilation. The maximal initial flow rise (16±6%) occurred after 28.1±7.4 s; the mean flow increase after 10 min was 21.6±6.6%. The same response we obtained in four experiments in which the hearts were paced at constant rate of 256 bpm (data not shown). An original recording is provided in Fig. 2. LVP development decreased initially (maximal decline 17.4±6% after 42.8±10 s) and recovered to 46±3 mm Hg after 10 min. Similar changes were seen for the pressure-rate product. Myocardial oxygen consumption after 10 min of hypercapnic perfusion was not significantly different from control (Table 1). Following termination of the hypercapnic challenge, all hemodynamic parameters recovered to baseline values within 10 min. Most notably, during this period the flow response was biphasic again (Fig. 2). To exclude artificial effects, e.g. time dependent deterioration, we tested whether the second hypercapnic test is different from the first in a separate group of experiments in which two consecutive hypercapnic tests were performed with a time lag of 30 min. The hypercapnic flow increase was not significantly changed (23±6% and 19±4% after 10 min hypercapnia, respectively; ANOVA for repeated measures, \( p=0.30, n=11 \)).

Table 1
Effects of hypercapnic perfusion on coronary pressure and flow, myocardial function and oxygen consumption

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Coronary flow, ml min(^{-1}) g(^{-1})</th>
<th>Coronary pressure, mm Hg</th>
<th>LVP, mm Hg</th>
<th>Heart rate, 1/min</th>
<th>PRP, mm Hg/min</th>
<th>MVO(_2), (\mu l min^{-1} g^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl.</td>
<td>6.1±0.2</td>
<td>54</td>
<td>52±3</td>
<td>196±6</td>
<td>10040±966</td>
<td>80.6±4</td>
</tr>
<tr>
<td>1 min</td>
<td>7.1±0.4</td>
<td>54</td>
<td>43±3</td>
<td>177±4</td>
<td>7651±573</td>
<td>n.d.</td>
</tr>
<tr>
<td>10 min</td>
<td>7.4±0.4*</td>
<td>54</td>
<td>46±3**</td>
<td>181±5**</td>
<td>8238±597**</td>
<td>90.3±6</td>
</tr>
<tr>
<td><strong>CP nw</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl.</td>
<td>7.0±0.6</td>
<td>54</td>
<td>–</td>
<td>166±2</td>
<td>–</td>
<td>76.3±5</td>
</tr>
<tr>
<td>1 min</td>
<td>8.3±0.9</td>
<td>54</td>
<td>–</td>
<td>169±3</td>
<td>–</td>
<td>n.d.</td>
</tr>
<tr>
<td>10 min</td>
<td>9.0±1.1**</td>
<td>54</td>
<td>–</td>
<td>165±8</td>
<td>–</td>
<td>87.0±6</td>
</tr>
<tr>
<td><strong>CF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl.</td>
<td>5.7±0.5</td>
<td>64±4</td>
<td>65±13</td>
<td>177±12</td>
<td>11498±2250</td>
<td>89.0±7</td>
</tr>
<tr>
<td>1 min</td>
<td>5.7±0.5</td>
<td>57±3</td>
<td>44±12</td>
<td>169±1</td>
<td>7383±1739</td>
<td>n.d.</td>
</tr>
<tr>
<td>10 min</td>
<td>5.72±0.5</td>
<td>59±3</td>
<td>54±9</td>
<td>165±1</td>
<td>8930±1636</td>
<td>86.3±10</td>
</tr>
<tr>
<td><strong>CP L-NAME</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl.</td>
<td>5.1±0.4</td>
<td>54</td>
<td>40±3</td>
<td>194±6</td>
<td>7864±753</td>
<td>93.7±10</td>
</tr>
<tr>
<td>1 min</td>
<td>6.1±0.5</td>
<td>54</td>
<td>35±3</td>
<td>189±11</td>
<td>6696±736</td>
<td>n.d.</td>
</tr>
<tr>
<td>10 min</td>
<td>5.2±0.1</td>
<td>54</td>
<td>38±3**</td>
<td>184±8</td>
<td>7009±767**</td>
<td>99.3±10</td>
</tr>
<tr>
<td><strong>CP Glibenclamide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl.</td>
<td>5.0±0.5</td>
<td>54</td>
<td>36±4</td>
<td>176±7</td>
<td>6501±832</td>
<td>80.8±10</td>
</tr>
<tr>
<td>1 min</td>
<td>5.6±0.6</td>
<td>54</td>
<td>30±4</td>
<td>187±20</td>
<td>5301±603</td>
<td>n.d.</td>
</tr>
<tr>
<td>10 min</td>
<td>5.8±0.6*</td>
<td>54</td>
<td>33±4**</td>
<td>171±4</td>
<td>5629±834**</td>
<td>78.4±8</td>
</tr>
</tbody>
</table>

LVP: left ventricular developed pressure, PRP: pressure-rate product, MVO\(_2\): myocardial oxygen consumption, CP: constant pressure perfusion, CPnw: constant pressure perfusion and non-working, CF: constant flow perfusion. \( n=18, 8 \) and 7 for CP, CPnw and CF, respectively. n.d.=not determined.

* \( p<0.05 \) versus control.

** \( p<0.01 \) versus control.

Table 2
Effects of L-NAME and glibenclamide on the reactive flow response

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Control</th>
<th>Intervention</th>
<th>( , p&lt; )</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NAME</td>
<td>216±10</td>
<td>209±14</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>201±4</td>
<td>137±18</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Data are given as percent peak flow versus baseline flow following a 20 s flow stop; perfusion pressure kept constant at 54 mm Hg. \( p \) indicates error probability intervention versus control.

Fig. 2. Original recording of a typical hypercapnic perfusion experiment on coronary flow, coronary pressure (CP) and left ventricular pressure (LVP). *Indicates the sampling of aliquots for \( pO_2 \) measurement. Note that CP was maintained constant during these periods. The dark bar on top indicates the 10 min period of hypercapnic perfusion (\( pCO_2 \) 61.4 mm Hg).
The potential contribution of NO was evaluated using L-NAME as an inhibitor of the endothelial NO synthase (eNOS or NOS III). During infusion of L-NAME \((n=10)\) coronary flow decreased about 16.1 \(\pm 3.7\%\) within 10 min \((p < 0.05)\). The peak reactive flow response after a 20 s flow stop was not affected (Table 2). Most notably, however, L-NAME did not change the initial flow increase \((18 \pm 6\%)\) during arterial hypercapnia, whereas the consecutive delayed flow increase was blunted (mean flow change after 10 min \(2.37 \pm 2.90\%\); Fig. 3). In four additional hearts the NO synthase inhibitor L-NMMA \((100 \mu\text{mol/l})\) was used. Coronary dilatory capacity was assessed as the flow response following application of a 50 \(\mu\text{M}\) adenosine bolus \((\text{injection volume 50 }\mu\text{l})\). The flow response during hypercapnic acidosis was identical to that shown in Fig. 3 (data not shown). These results indicate that the delayed, persisting flow increase is dependent on an intact NO production. It should be noted that similar results were obtained in 27 hearts perfused under identical conditions, in which, however, arterial acidosis \((\text{pH } 7.14 \pm 0.02, 10 \text{ min})\) was evoked by infusion of HCl \((1 \text{ N})\) via the aortic cannula. Also, in these hearts treatment with L-NAME specifically blunted the delayed coronary flow response during acidosis (data not shown).

Under control conditions, cGMP release was \(0.69 \pm 0.35 \text{ pmol min}^{-1} \text{g}^{-1}\). The release of cGMP was increased during the first 90 s \((1.12 \pm 0.68 \text{ pmol min}^{-1} \text{g}^{-1})\) of hypercapnic perfusion \((p < 0.05; \text{Fig. 4})\). Although cGMP release exhibited a considerable heterogeneity between individual experiments, the transient character of the cGMP release was evident. No significant difference was found after 3 min of hypercapnic perfusion \((\text{n.s. control versus L-NAME, Fig. 4})\). As expected, in the presence of L-NAME the basal cGMP release was decreased and, furthermore, no significant increase during hypercapnic perfusion was found \((0.57 \pm 0.30 \text{ pmol min}^{-1} \text{g}^{-1}; \text{Fig. 4})\).

In another experimental series the potential role of \(K^{+}\)ATP channel opening for the hypercapnic flow response was
determined using glibenclamide \((n=8)\). To assure the efficacy of the concentration used, the reactive flow response after a 20 s flow stop was quantified before and during glibenclamide infusion. Glibenclamide reduced the basal coronary flow by 16.9% \((p<0.01)\) and reduced the reactive flow response by 60% \(Table\ 2\). In contrast glibenclamide did not have any significant effect on the time course of the hypercapnic flow response \(Fig.\ 5\).

3.2. nwCP hearts

To assess the potential importance of the transient drop of myocardial performance for the observed biphasic coronary flow response, in a separate group non-working (isotonically contracting) hearts were employed \((n=8)\). These hearts exhibited a basal flow of \(7.0\pm0.6\ \text{ml min}^{-1}\ \text{g}^{-1}\), a heart rate of \(166\pm3\ \text{bpm}\) and a \(\text{VO}_2\) of \(76.3\pm5.1\ \mu\text{mol min}^{-1}\ \text{g}^{-1}\). Similar to the hearts performing isovolumic work the hypercapnic flow response was biphasic with an early flow peak \((19\pm10\%)\) at \(20.6\pm4.3\ \text{s}\) and a delayed flow increase \((26.3\pm15.9\%\) after 10 min). Furthermore, during infusion of L-NAME the initial flow increase during arterial hypercapnia was preserved, whereas the delayed flow increase was blunted \(\text{mean flow increase after 10 min 5.2}\ %\) \(\text{Table}\ 2\). Thus, the typical flow response of guinea pig coronary vessels during arterial hypercapnia was biphasic, independent of the type of myocardial contraction \(\text{isovolumic versus isotonic}\) performed. The hemodynamic parameters and myocardial oxygen consumption data for this group of experiments are summarized in \(\text{Table}\ 1\) for hypercapnic perfusion in the absence of L-NAME.

3.3. CF hearts

Constant flow \((10 \ \text{ml/min})\) perfused hearts \((n=7);\) ventricular mass \(1.61\pm0.36\ \text{g}\) developed a coronary perfusion pressure of \(68\pm8\ \text{mm Hg}\), a mean LVP of \(66\pm13\ \text{mm Hg}\) and showed an \(\text{MVO}_2\) of \(88.7\pm6.7\ \mu\text{mol min}^{-1}\ \text{g}^{-1}\). The heart rate was \(177\pm12\ \text{bpm}\). Thus, myocardial performance and oxygen consumption were similar to those measured in CP hearts. In \(\text{Table}\ 1\) are summarized the effects of hypercapnic perfusion on hemodynamic parameters and myocardial oxygen consumption under CF. Hypercapnic perfusion induced a transient, about 2 min lasting, decrease of the arterial coronary pressure \(\left( -11\pm2.8\%;\ p<0.05 \right)\). This pressure drop, however, almost completely recovered within 3 min despite ongoing hypercapnic perfusion \(\text{Fig.}\ 7\). The LVP also showed a transient reduction during arterial hypercapnia, probably reflecting a “garden hose” effect \(\text{Fig.}\ 7\). Most importantly, the presence of L-NAME \((n=5)\) did not have any significant effect on the hypercapnia induced transient decrease of coronary perfusion pressure \(\text{Fig.}\ 7\).

4. Discussion

The major results of this study may be summarized as follows:

1) Acute arterial hypercapnic acidosis induces a biphasic flow response in the isolated, constant pressure perfused guinea pig heart.

2) The initial flow response is independent of NO production, whereas the consecutive lasting flow response requires an intact NO production.

3) The steady state decrease of coronary resistance during arterial hypercapnia requires a primary rise of flow and an intact NO production suggesting a shear stress mediated NO response.

4) \(K_{\text{ATP}}\) channels are unimportant for the mediation of the hypercapnic flow response in guinea pig heart.

5) The divergent interactions of the eNOS-inhibitor L-NAME and a \(K_{\text{ATP}}\) channel blocker glibenclamide with the hypercapnic flow response and the reactive flow response, respectively, point to different mechanisms of acidosis/reperfusion related coronary vasodilation.

Intact NO production was important for steady state coronary flow increase during arterial acidosis in guinea pig heart \((\text{Figs.}\ 3, 4\ \text{and}\ 6)\). However, Song et al. studying the effects of respiratory and metabolic acidosis on the coronary flow regulation of rat heart came to the conclusion that nitric oxide is unimportant for the respective flow increase \(\text{[9]}\). In contrast to the present study these authors studied only rather short lasting periods of acidosis \((2\ \text{min})\). Thus, the potential effect of enhanced nitric oxide production on the hypercapnic flow response was possibly missed. As shown in \(\text{Fig.}\ 3\), the effect of nitric oxide production is only evident during the persistent flow rise, i.e. later than 2 min of hypercapnic perfusion. The NO-dependent flow response in our experiments was dependent on intact NO production as well as a primary flow increase. The mediating mechanism of this primary flow increase in guinea pig
heart remains to be determined, but is not due to K$_{ATP}$ channel activation as indicated by the ineffectiveness of glibenclamide to change the hypercapnic flow response in guinea pig heart (Fig. 5). The importance of the NO mediated decrease of coronary resistance is stressed by the fact that the flow increase during hypercapnic acidosis vanishes completely, if either NO synthases were inhibited or an increase of shear stress prevented. In line with the results of L-NAME and L-NMMA on coronary resistance is the increased cGMP release during hypercapnic acidosis (Fig. 4). Although cGMP production is stimulated by other factors such as ANP or BNP, the fact that the release is suppressed after L-NAME strongly indicates that increased cGMP release is due to increased NO production. The only transient rise of cGMP release despite ongoing vasorelaxation is expected based on previous studies. An increase of cGMP was shown to activate phosphodiesterase 5, favoring cGMP metabolism [17]. In contrast, protein phosphorylation by cGMP-dependent protein kinases, which mediate the cellular effects of cGMP [18], has been shown to persist a transient rise of the nitric oxide concentration [19]. In the present study we did not assess NO/NOx production directly. However, we believe that the effectiveness of 2 different NO synthase blockers (L-NAME, L-NMMA) and the measurement of cGMP release as a substitute for NO release provide compelling evidence for the involvement of NO in the mediation of the sustained flow response during hypercapnic acidosis in guinea pig heart.

Phillis et al. have provided evidence that in rat heart the flow increase during acute coronary acidosis is blunted in the presence of glibenclamide (5 μM) [10]. Another study (Ishizaka and Kuo) showed that in pig coronary microvessels of 40–110 μm diameter glibenclamide blocked the vessel dilation during acidosis, whereas the removal of the endothelium had no effect on the vessel response during acidosis [12]. In agreement with this, blockers of NO synthase(s) and cyclooxygenase did not have any effect on the vessel dilation during acidosis [12]. A cytosolic pH sensitivity of K$_{ATP}$ channels has recently been shown on the example of smooth muscle cells isolated from rat mesenteric arteries [20]. Surprisingly, in isolated perfused guinea pig heart, glibenclamide failed to have any significant effect on the hypercapnia induced flow increase (Fig. 5). However, in the same experiments glibenclamide significantly reduced the basal coronary flow by 15% and the reactive flow response after a 15 s lasting flow stop by 60% (Table 2), which demonstrated the effectiveness of the inhibitor. In experiments conducted on the isolated perfused mouse heart we have observed a partial inhibition of the hypercapnic flow response by glibenclamide, while inhibition of NO production also decreased the flow rise during hypercapnic acidosis significantly [21]. Thus, it seems that considerable species differences exist with respect to the importance of K$_{ATP}$ channels in the mediation of the coronary flow response during hypercapnic acidosis.

A previous study has addressed the potential importance of nitric oxide for the flow response during hypercapnic acidosis in dog heart in situ [6]. In this study a rise of coronary arterial pCO$_2$ from 39 to 60 mm Hg resulted in a coronary flow increase 154% above control flow, a response considerably larger than that observed in the present study (22% flow increase). A possible reason for this difference is that cardiac preload and afterload remained unchanged in the study by Gurevicius et al., because these authors employed regional hypercapnic perfusion of the LAD vascular bed [6]. In contrast, global heart hypercapnic perfusion was induced in the present study, which led to a fall of global myocardial performance. This may have limited the hypercapnic flow increase. However, it should be noted that Gurevicius et al. did not report data on regional myocardial shortening [6]. Thus, the true myocardial work of the LAD dependent myocardium during hypercapnic perfusion remains unclear in that study. The Gurevicius et al. [6] study and ours agree that a rise of coronary arterial pCO$_2$ from 40 to 60 mm Hg results in a substantial flow increase that reaches a steady state after 5–10 min and which is in part dependent on NO production. In contrast to the present study, however, in the dog heart in situ model the NO dependence was still found under constant flow conditions. This led the authors to conclude that in dog heart NO release is triggered directly by the acidosis or hypercapnia, rather than by an increase of shear stress. This conclusion is opposite to that obtained from the results of the present study. It should be noted, however, that apart from the discussed differences, it might also result from the fact that we employed a buffer perfused isolated heart preparation whereas Gurevicius et al. [6] used a blood perfused in vivo model.

In experiments conducted on isolated perfused ferret hearts a close relationship between left ventricular pressure development and cytosolic pH has been shown [22]. Interestingly, the kinetics of pressure development resembled those found in the present study (Fig. 2). PRP decreased in agreement with results reported [22] within 1 min of hypercapnic perfusion (Table 1). Although there is a trend, there are actually no significant differences in myocardial oxygen consumption between control and 10 min hypercapnic perfusion. The slight albeit significant decrease of PRP during 10 min of hypercapnic perfusion may be interpreted in two ways: these are most likely random changes that could be explained by interindividual variability, or alternatively, myocardial efficiency changed indeed, because oxygen consumption remained unchanged while PRP slightly decreased. A final conclusion on this issue cannot be derived from the present experiments.

Mechanisms that might favor NO production, aside from shear stress [14,23], or prolong its action during hypercapnic acidosis remain unclear at present. Studies on isolated endothelial cells have indicated that alkalinization may enhance NO production [24,25]. This, however, is in contrast to what would be expected, if changes in pH were
important for triggering NO production during hypercapnic perfusion. However, acidification was reported to increase the vascular dilatory response during stimulation with NO-donors [26]. In isolated rat thoracic aorta acetylcholine induced relaxation is augmented when the pH of the bathing solution was lowered from 7.4 to 7.0 [26]. This increased relaxation is associated with increased cGMP levels during acidification but unrelated to potassium channel dependent relaxation. Whether this effect observed in rat thoracic aorta is of relevance for coronary resistance vessels remains to be shown.

Acidosis is an important component of myocardial ischemia/reperfusion processes. It might therefore be expected that the mechanisms controlling myocardial blood flow in response to myocardial ischemia and coronary hypercapnic perfusion are similar. However, the reactive flow response was largely reduced after application of glibenclamide, whereas the flow response during hypercapnia was not. The importance of KATP channels for acidotic flow regulation in guinea pig may capnia was not. The importance of KATP channels for glibenclamide, whereas the flow response during hypercapnic perfusion. However, acidification was reported to increase nitric oxide limits coronary vasoconstriction by a shear stress-dependent mechanism. Am J Physiol Heart Circ Physiol 2001;281:H796–803. 12. Ishizaka H, Kuo L. Acidosis-induced coronary arteriolar dilation is mediated by ATP-sensitive potassium channels in vascular smooth muscle. Circ Res 1996;78:50–7. 13. Ishizaka H, Gudi SR, Frangos JA, Kuo L. Coronary arteriolar dilation to acidosis: role of ATP-sensitive potassium channels and pertussis toxin-sensitive G proteins. Circulation 1999;99(2–2):558–63. 14. Stepp DW, Merkus D, Nishikawa Y, Chilian WM. Nitric oxide limits coronary flow regulation during conditions associated with acidosis. In conclusion, shear stress dependent NO production was shown to be of critical importance for the steady state flow increase during hypercapnic acidosis of guinea pig heart, whereas no evidence was found for importance of KATP channels. Thus, acidic flow regulation in guinea pig may significantly differ from that of rat and pig, in which involvement of KATP channels has been shown [7,12,20]. Future studies must address whether this difference results from an insensitivity of the guinea pig KATP channel for cytosolic hydrogen ions or from differences in cytosolic buffer capacity.

Acknowledgments

The study was supported by an intramural grant within the MedDrive programme 2002 established by the Medical Faculty Carl Gustav Carus. The expert technical assistance of Sandra Tuchscheerer-Hofmeister during the experiments is gratefully acknowledged.

References


