Effects of low-flow ischemia on the positive inotropic action of angiotensin II in isolated rabbit and rat hearts

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Abstract

Objective: Angiotensin II (ANG II) has recently been reported to increase inotropy in adult rabbit myocytes by a mechanism of alkalinization and consequent increased myofilament sensitivity to calcium. Accordingly, we tested the hypothesis that ANG II would have a greater inotropic effect during ischemic conditions than it would during normoxia, since ischemia-induced intracellular acidosis contributes to ischemic contractile depression by decreasing myofilament calcium sensitivity. Methods: We studied the effects of ANG II in isolated, red-blood-cell-perfused, isovolumic rat and rabbit hearts during normoxic perfusion conditions and at graded reductions in coronary perfusion pressure (CPP). At each level of perfusion, ANG II was infused at progressively increasing concentrations ranging from 10^{-11} to 10^{-5} M. The maximal effective ANG II concentration was 10^{-7} M. Results: Our studies show that ANG II caused comparable absolute increases in isovolumic LV developed pressure in normoperfused and hypoperfused rabbit hearts. However, since contractile function was markedly depressed in ischemic hearts prior to ANG II administration, the relative inotropic response to ANG II was significantly greater during ischemia than normoxia. Similarly, ANG II had no positive inotropic effect in the rat during normoxia, but increased contractility during ischemia. To assess specifically the potential of ANG II to reverse the negative inotropy of acidosis, normoxic non-ischemic rat hearts were perfused with a hypercarbic acidotic perfusate (pH = 7.1). During the hypercarbic perfusion when contraction was depressed by acidosis, ANG II [10^{-7} M] increased LV developed pressure by 19% and +dP/dt by 27% (P < 0.05), in contrast to its lack of inotropic effect at a normal pH. The positive inotropic effect observed in rat hearts with ANG II during ischemia was significantly attenuated (P < 0.001) by concomitant infusion with amiloride, 5-(N-ethyl-N-isopropyl) (EIPA), a Na+/H+ exchange inhibitor. Conclusions: We conclude that during normoxia, ANG II has a different inotropic potency in rabbits from that in rats. In both species, the relative inotropic responsiveness of ANG II is potentiated during low-flow ischemia. These results are consistent with a relative intracellular alkalinization that occurs secondary to ANG II’s action to stimulate Na+/H+ exchange.

Keywords: Angiotensin II; Coronary artery tone; Contractile function; Rabbit, heart; Rat, heart; Myocardial ischemia

1. Introduction

Blockade of the renin–angiotensin system has been proven beneficial in the treatment of chronic heart failure and in groups of patients with acute myocardial infarction who were largely free of acute ischemic heart failure [21,30]. However, despite the efficacy of its inhibition in these clinical settings, the renin–angiotensin system may have positive inotropic effects which are beneficial in the setting of acute ischemic cardiac failure.

Reversal of acute ischemic failure is an important therapeutic goal in several clinical settings. Myocardial contractility is largely determined by intracellular Ca^{2+} and myofilament sensitivity to Ca^{2+}. The early decrease in contractile function with acute ischemia has been shown to result largely from a decreased myofilament Ca^{2+} sensiv-
ity secondary to ischemia-induced intracellular acidosis and/or inorganic phosphate accumulation [14,29,31]. Angiotensin II (ANG II) increases myocardial contractility by increasing both \( [Ca^{2+}]_i \) and myofilament \( Ca^{2+} \) sensitivity [23,33]. Recently, Ikenouchi et al. found that 10 nM ANG II increased inotropy in isolated rabbit hearts and dissociated myocytes without a concomitant increase in peak systolic \( [Ca^{2+}]_i \) or \( Ca^{2+} \) current \( (I_{Ca}) \) [20]. The proposed mechanism for the observed increase in inotropy was an increased myofilament \( Ca^{2+} \) sensitivity secondary to intracellular alkalosis (a 0.2 unit increase in \( pHi \), was reported). Matsui et al. recently showed that this alkalosis resulted from ANG II stimulatory effect on Na\(^+/\)H\(^+\) exchange [34]. However, higher levels of ANG II (100 nM) also increased \( I_{Ca} \) in adult rabbit myocytes [23].

In light of these previous studies, we hypothesized that ANG II would partially reverse the negative inotropic effects of ischemia, by increasing myofilament \( Ca^{2+} \) sensitivity and/or by increasing \( [Ca^{2+}]_i \). We tested this hypothesis by infusing progressively increasing concentrations of ANG II into the coronary circulation of isolated, isovolumic hearts of rabbits and rats during normoxic perfusion conditions and at graded reductions in coronary perfusion pressure (CPP). To confirm the proposed mechanism of ANG II’s improvement of ischemic contractile function we exposed the normally perfused, non-ischemic rat heart to acidosis without ischemia (hypercarbic perfusion), and in separate experiments we co-administered ANG II concomitantly with amiloride, 5-(N-ethyl-N-isopropyl) (EIPA), a specific Na\(^+\)/H\(^+\) exchange inhibitor [34].

2. Methods

2.1. Heart isolation

Male, Wistar rats (Charles River Farms) weighing 350–450 g and male, New Zealand White rabbits (Millbrook Farms) weighing 1.4–2.1 kg were housed 1 cage under a 12 h light/dark cycle and fed ad libitum (Purina rat and rabbit chow). All rats received care in compliance with the ‘Principles of Laboratory Care’ formulated by the National Society for Medical Research. Rats were anesthetized intraperitoneally with sodium pentobarbital (0.5–1.0 ml), while rabbits were anesthetized with 0.3 ml ketamine followed by administration of intravenous sodium pentobarbital (1.5–2.0 ml) and heparin (0.1 ml). Once unresponsiveness was determined, animals were weighed and their thorax opened. Rabbits were mechanically ventilated during thoracotomy. Hearts were extracted, and within 20 s of withdrawal, mounted on a short perfusion cannula in a Langendorff fashion.

Hearts were retrogradely perfused with a red-blood-cell perfusate developed by Marshall and Zhang [35] and in our laboratory [12,13]. Briefly, fresh whole cow blood was collected at a local slaughterhouse in a vessel containing approximately 6000 units of sodium heparin and 100,000 units of penicillin per liter. The containers of blood were immediately placed on ice to facilitate rapid cooling for transportation. The whole blood was then spun in a refrigerated centrifuge (5°C) at a rotator speed of 3000 rpm for 15 min. The supernatant was aspirated and the resulting packed cells were mixed 1:1 with calcium-free Krebs-Henseleit buffer. The centrifugation and resuspension steps were repeated 3 times, resulting in packed red cells that were essentially free of white cells and platelets. The packed red blood cells were mixed 1:1 with calcium-free buffer and stored for future use at 4°C. Immediately prior to experiment, blood was once again washed and mixed in a red blood cell perfusate consisting of bovine red-blood cells at a final hematocrit of 40% suspended in a Krebs-Henseleit buffer containing in mM: NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, glucose 5.5, lactate 1, palmitic acid 0.4, heparin 60 mU/ml, and 4 g% bovine serum albumin (Sigma Chemical Co., St. Louis, MO). To mimic in situ physiological extracellular \( Ca^{2+} \) concentrations as closely as possible, the perfusate ionized \( Ca^{2+} \) concentration was adjusted to 1 mM \( Ca^{2+} \) for rats and 1.5 mM \( Ca^{2+} \) for rabbits with a calcium sensitive electrode (Nova 6, Nova Biochemical). Gentamycin (0.2 mg/dl) was added to the red-blood-cell perfusate to retard bacterial growth. The perfusate was gassed with 20% \( O_2 \), 3% \( CO_2 \), and 77% \( N_2 \), to achieve a \( P_{O_2} \) of 100–160 mmHg and a \( pHi \) of 7.35–7.4.

Following initial perfusion of the heart, a small apical drain was inserted into the left ventricle for collection of Thebesian drainage. A second cannula was inserted into apex of the right ventricle via the pulmonary artery for collection of coronary venous effluent. A pacing wire (model 59, Grass Instrument Co., Quincy, MA) and a thermistor (model 400, Yellow Springs, Boulder, CO) were inserted into the right ventricle through the superior vena cava and right atrium. A collapsed latex balloon was inserted into the left ventricle for collection of Thebesian drainage. A second cannula was inserted into apex of the right ventricle via the pulmonary artery for collection of coronary venous effluent. A pacing wire (model 59, Grass Instrument Co., Quincy, MA) and a thermistor (model 400, Yellow Springs, Boulder, CO) were inserted into the right ventricle through the superior vena cava and right atrium. 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derivative were recorded continuously on a Gould physiology recorder.

2.2. Equilibration

All hearts had an equilibration period of 30 min. During this time, coronary flow was set to a level that resulted in a coronary perfusion pressure of 80 mmHg. Rat hearts were paced at 5.5 Hz and rabbit hearts at 3 Hz. Following equilibration, baseline myocardial performance was assessed and hearts from both rabbits and rats were randomly assigned to either a normoxic or low-flow ischemia protocol. The total duration of equilibration plus either the normoxic or low-flow ischemia protocol was approximately 54–70 min.

2.3. Angiotensin II dose–response studies during normoxic perfusion levels

Rats (n = 6) and rabbits (n = 5) were perfused with a constant coronary blood flow, resulting in a coronary perfusion pressure (CPP) of 80 mmHg (CPP-80). Additional rabbit hearts were perfused with flows achieving a CPP = 120 mmHg (CPP-120; n = 3). ANG II (Sigma, St. Louis, MO) dissolved in 0.9% saline vehicle was then administered through a side-port of the aortic cannula by a constant flow pump (Harvard apparatus) in a dose–response manner yielding final blood concentrations ranging from $10^{-11}$ to $10^{-5}$ M. Preceded by a control saline infusion at 10% of the coronary flow rate, ANG II was administered at the same infusion rate of 10% of the coronary blood flow. Control rat hearts (Control; n = 5) were perfused with saline at intervals time-matched to ANG II infusions. Myocardial performance was allowed to stabilize during each infusion, and typically stabilized within 2–5 min.

2.4. Angiotensin II dose–response studies during low-flow ischemia

Following equilibration, separate groups of rat and rabbit hearts were exposed to low-flow ischemia by gradually decreasing coronary flow over a 10 min period. The final ischemic coronary flow rate was set to a level which yielded a CPP = 20 mmHg for rats (CPP-20; n = 6) and a CPP = 20 mmHg (CPP-20; n = 5) or CPP = 15 mmHg (CPP-15; n = 4) for rabbits. Myocardial performance was allowed to re-equilibrate during this time (10 min) and the functional characteristics of the heart were re-assessed. Hearts underwent an ANG II dose–response protocol as described above. Ischemic control rats (Control; n = 5) were perfused with saline at intervals time-matched to ANG II infusions. Each dose was terminated upon stabilization of myocardial performance, typically within 2–5 min of ANG II infusion.

2.5. Time-controlled ANG II infusion in rat hearts

The results of our dose–response experiments indicated that ANG II elicited a positive inotropic response in ischemic rat hearts (CPP = 20 mmHg) but did not alter contractility during normoxia (CPP = 80 mmHg) (see Section 3). To further confirm these findings, as well as to control for the variable ANG II infusion times in our dose–response experiments, additional studies were performed in isolated rat hearts. In these experiments, saline and ANG II $[10^{-7}]$M were administered for 5 min during both normoxic (CPP = 80 mmHg) (n = 4) and ischemic (CPP = 20 mmHg) (n = 8) perfusion conditions. Normoxic and ischemic hearts were infused first with saline and then $[10^{-7}]$M ANG II for 5 min at a rate equivalent to 10% of coronary flow. Each heart acted as its own control during both the normoxic and ischemic protocols, and myocardial performance during ANG II infusion was compared to its respective saline response.

2.6. Hypercarbic perfusion in non-ischemic rat hearts

Additional experiments were performed in order to test further the hypothesis that ANG II mediated its positive inotropic effect during ischemia by generating a relative intracellular alkalosis. In these studies, contractile function was depressed by perfusion with an acidic perfusate during normoxic perfusion. The acidotic perfusate was a hypercarbic blood mixture that was identical to the normoxic blood mixture with the exception that it was gassed with 10% CO₂. Rat hearts (n = 8) were first perfused with a normoxic blood mixture (as described above) during constant flow at pH 7.4 and a CPP = 85 mmHg. The hearts were then perfused with the hypercarbic blood mixture (pH = 7.13) for 15 min. Following stabilization with the hypercarbic medium, hearts were infused with $[10^{-7}]$M ANG II and saline at 10% of the coronary flow rate for 5 min. Myocardial performance during ANG II infusion was compared to its respective hypercarbic response.

2.7. Sodium–hydrogen exchange inhibition in ischemic rat hearts

In order to directly test the hypothesis that angiotensin II increases inotropy by stimulating Na⁺/H⁺ exchange, we performed additional experiments in which the Na⁺/H⁺ exchange inhibitor, EIPA [amiloride; 5-(N-ethyl-N-isopropyl), Research Biochemicals International, Natick, MA], was concomitantly infused with ANG II during ischemia. Rat hearts were allowed to equilibrate at constant flow eliciting CPP = 83 mmHg. Low-flow ischemia was imposed by reducing coronary flow to 15% of baseline for 10 min at which point hearts underwent treatment with either saline (n = 5), $[10^{-7}]$M ANG II (n = 6), $[5 \times$
Table 1
Baseline pre-drug hemodynamics of rabbit hearts during normoxia and ischemia

<table>
<thead>
<tr>
<th></th>
<th>CBF (ml/min)</th>
<th>LVDevP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LV + dP/dt (mmHg/s)</th>
<th>LV – dP/dt (mmHg/s)</th>
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<tbody>
<tr>
<td><strong>Normoxia protocol</strong></td>
<td></td>
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</tr>
<tr>
<td>CPP-120 (n = 3)</td>
<td>5.93 ± 1.06</td>
<td>104 ± 11</td>
<td>10 ± 1</td>
<td>1360 ± 140</td>
<td>1000 ± 83</td>
</tr>
<tr>
<td>CPP-80 (n = 5)</td>
<td>5.13 ± 0.56</td>
<td>90 ± 4</td>
<td>10 ± 1</td>
<td>1110 ± 81</td>
<td>942 ± 86</td>
</tr>
<tr>
<td><strong>Ischemia protocol</strong></td>
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<td></td>
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<tr>
<td><strong>Baseline (CPP = 80 mmHg)</strong></td>
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</tr>
<tr>
<td>CPP-20 (n = 5)</td>
<td>5.12 ± 0.23</td>
<td>93 ± 2</td>
<td>10 ± 1</td>
<td>1140 ± 57</td>
<td>984 ± 35</td>
</tr>
<tr>
<td>CPP-15 (n = 4)</td>
<td>5.41 ± 0.21</td>
<td>96 ± 2</td>
<td>10 ± 11</td>
<td>240 ± 69</td>
<td>1010 ± 84</td>
</tr>
<tr>
<td><strong>Ischemic perfusion</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CPP-20 (n = 5)</td>
<td>1.8 ± 0.19</td>
<td>45 ± 4</td>
<td>7 ± 2</td>
<td>556 ± 26</td>
<td>460 ± 56</td>
</tr>
<tr>
<td>CPP-15 (n = 4)</td>
<td>1.4 ± 0.07</td>
<td>38 ± 3</td>
<td>6 ± 1</td>
<td>470 ± 41</td>
<td>370 ± 55</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. CPP = coronary perfusion pressure; CBF = coronary blood flow; LVDevP = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; LV + dP/dt = maximal positive left ventricular dP/dt; LV – dP/dt = maximal negative left ventricular dP/dt; CPP-120 = group studied at CPP of 120 mmHg; CPP-80 = group studied at CPP of 80 mmHg; CPP-20 = group studied at CPP 20 mmHg; CPP-15 = group studied at CPP of 15 mmHg.

Fig. 1. ANG II dose–response curves in rabbit hearts. Isolated rabbit hearts were perfused throughout a range of coronary perfusion pressures (CPP = 120, 80, 20, and 15 mmHg) with coronary flow held constant during each experiment. ANG II was added at progressively increasing concentrations. At all CPPs concentrations of 10^{-5} M ANG II and above caused similar absolute changes in LV developed pressure and +dP/dt. ANG II had no effect on LV end-diastolic pressure or coronary perfusion pressure at any CPP. Values are mean ± s.e.m. * Indicates (P < 0.05) vs. baseline. + Indicates (P < 0.05) vs. baseline in CPP = 80 mmHg.
10^{-5} M EIPA (n = 6) or [10^{-7} M ANG II + [5 \times 10^{-5} M EIPA (n = 6). All treatments were infused at 5% of coronary flow. Control vehicles were infused during both equilibration and ischemia at 5% of coronary flow.

2.8. Data analysis

Data are reported as the mean ± s.e.m. Baseline characteristics of rabbits perfused at different coronary perfusion pressures were compared with one-way analysis of variance (ANOVA). Baseline characteristics of rats infused with ANG II and saline were compared with unpaired t-tests. Myocardial performance during ANG II infusion was tested by two-way ANOVA with repeated measures. When ANOVA indicated overall significance of groups or interaction, values at specific time points were examined by the method of least significant differences [44]. Rat hearts undergoing time controlled ANG II or saline infusions were analyzed with paired t-tests. Rat hearts perfused with a ANG II during hypercarbia were compared with ANOVA with repeated measures and Student-Neumann-Keuls multiple comparison tests. Sodium/hydrogen exchange inhibition experiments were analyzed with one-way analysis of variance and Student-Neumann-Keuls post-hoc analysis. All data were deemed significant at a P < 0.05 level.

3. Results

3.1. Baseline hemodynamic characteristics of rabbit hearts

Baseline hemodynamics of rabbit hearts are listed in Table 1. All experimental groups had comparable baseline function prior to ANG II administration. In hearts subjected to low-flow ischemia (groups CPP-20 and CPP-15) left ventricular developed pressure decreased promptly to about 40% of the baseline value after flow reduction (Table 1).

3.2. ANG II dose–response in rabbit hearts

Fig. 1 shows the hemodynamic responses of rabbit hearts subjected to ANG II at various levels of CPP. ANG II acted as a positive inotrope at all levels of coronary perfusion. This effect was demonstrated by significant positive changes in left ventricular developed pressure and +dP/dt at all levels of CPP (P < 0.05). The peak inotropic effect for all groups was most prominent at an ANG II concentration of 10^{-7} M. This increase in inotropy was of greatest relative value in ischemic hearts (CPP = 20 and 15 mmHg) where left ventricular developed pressure increased 33 ± 9% and 37 ± 9%, respectively (LV developed pressure increased 19 ± 3% at CPP = 80 mmHg and 12 ± 2% at CPP = 120 mmHg; P < 0.05 vs CPP-20, CPP-15). In all groups, the increase in inotropy was attained without any significant changes in left ventricular end-diastolic pressure or coronary perfusion pressure.

3.3. Baseline hemodynamic characteristics of normoxic and ischemic rat hearts

The hemodynamic performance of normoxic rat hearts treated with ANG II was similar to saline controls at baseline (see Table 2). Baseline myocardial performance was also similar between saline controls and ANG II treated hearts undergoing the ischemic protocol. Hearts in the ANG II group had slightly lower baseline coronary flow rates during ischemia than saline controls (P < 0.05), but had similar myocardial performance.

<table>
<thead>
<tr>
<th>Normoxia protocol (CPP = 80 mmHg)</th>
<th>CBF (ml/min)</th>
<th>LVDevP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LV +dP/dt (mmHg/s)</th>
<th>LV–dP/dt (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>2.64 ± 0.26</td>
<td>106 ± 7</td>
<td>10 ± 1</td>
<td>2720 ± 185</td>
<td>1740 ± 160</td>
</tr>
<tr>
<td>CPP-80 (n = 6)</td>
<td>3.72 ± 0.59</td>
<td>109 ± 12</td>
<td>10 ± 1</td>
<td>2492 ± 222</td>
<td>1842 ± 190</td>
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</table>

<table>
<thead>
<tr>
<th>Ischemia protocol (CPP = 80 mmHg)</th>
<th>CBF (ml/min)</th>
<th>LVDevP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LV +dP/dt (mmHg/s)</th>
<th>LV–dP/dt (mmHg/s)</th>
</tr>
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<tr>
<td>Control (n = 5)</td>
<td>3.44 ± 0.12</td>
<td>109 ± 4</td>
<td>10 ± 1</td>
<td>2570 ± 223</td>
<td>1970 ± 179</td>
</tr>
<tr>
<td>CPP-20 (n = 6)</td>
<td>3.13 ± 0.23</td>
<td>97 ± 5</td>
<td>10 ± 1</td>
<td>2383 ± 233</td>
<td>1592 ± 98</td>
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<tr>
<th>Ischemic perfusion (CPP = 20 mmHg)</th>
<th>CBF (ml/min)</th>
<th>LVDevP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LV +dP/dt (mmHg/s)</th>
<th>LV–dP/dt (mmHg/s)</th>
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<tbody>
<tr>
<td>Control (n = 5)</td>
<td>1.00 ± 0.06</td>
<td>45 ± 3</td>
<td>10 ± 1</td>
<td>1220 ± 102</td>
<td>700 ± 45</td>
</tr>
<tr>
<td>CPP-20 (n = 6)</td>
<td>0.76 ± 0.06</td>
<td>39 ± 3</td>
<td>11 ± 1</td>
<td>1025 ± 101</td>
<td>558 ± 52</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. CPP = coronary perfusion pressure; CBF = coronary blood flow; LVDevP = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; LV +dP/dt = maximal positive left ventricular dP/dt; LV–dP/dt = maximal negative left ventricular dP/dt; CPP-80 = group studied at CPP of 80 mmHg; CPP-20 = group studied at CPP of 20 mmHg.

* P < 0.05 vs CPP-20.
3.4. ANG II dose–response in normoxic rat hearts

The hemodynamic responses of rat hearts to ANG II and saline were similar during normoxia (CPP = 80 mmHg). Hemodynamic performance (i.e., left ventricular developed pressure) declined approximately 10 mmHg relative to baseline in both saline controls and ANG II treated hearts during the 24–40 min dose–response protocol. There were no significant differences between saline controls and ANG II treated hearts for left ventricular developed pressure, + dP/dt, – dP/dt, left ventricular end-diastolic pressure or coronary perfusion pressure.

3.5. ANG II dose–response in ischemic rat hearts

Fig. 2 demonstrates the hemodynamic performance of ischemic rat hearts during ANG II infusion. ANG II infusion significantly increased myocardial contractility at concentrations of $10^{-9}$ M and greater. ANG II elicited significant positive changes in left ventricular developed pressure, + dP/dt, and – dP/dt vs. saline controls ($P < 0.01$). During ischemia, ANG-II-treated hearts demonstrated a trend towards higher left ventricular end-diastolic pressure which was not statistically significant. Coronary perfusion pressure at a constant flow rate, significantly increased with ANG II vs. saline control ($P < 0.01$), indicating an increase in this index of coronary resistance.

3.6. Five-minute infusions with $[10^{-7}]$ M ANG II

Five-minute infusion experiments in the rat demonstrated results similar to the dose–response experiments reported in Fig. 2. During ischemia (CPP = 20 mmHg), 5 min of ANG II infusion resulted in an 11% increase in left ventricular developed pressure and + dP/dt. Left ventricular developed pressure significantly increased from $34 \pm 1$ mmHg during saline infusion to $38 \pm 1$ mmHg with ANG II ($P < 0.001$), and + dP/dt significantly increased from $937 \pm 40$ mmHg/s during saline infusion to $1040 \pm 46$ mmHg/s with ANG II ($P < 0.01$). During this relatively
Normoxia HC HC+ HC+ (PH=7.4) (PH=7.1) ANG II Saline

Fig. 3. Changes in hemodynamic performance in rat hearts (n=8) perfused with a hypercarbic blood perfusate at CPP = 80 mmHg. Rat hearts were first infused with a normoxic blood mixture at pH = 7.4. Hearts were then transferred to a hypercarbic blood (HC) mixture at pH = 7.1. Left ventricular developed pressure and + dP/dt significantly decreased (P < 0.001) with hypercarbia vs. normoxia. The addition of ANG II [10^{-7}]M (HC + ANG II) caused a positive inotropic response in hypercarbic perfused hearts (P < 0.05). Saline infusion (HC + Saline) had no inotropic effect. Values are mean ± s.e.m. * Indicates (P < 0.05) vs. hypercarbia and saline. ** Indicates (P < 0.001) vs. normoxia. *** Indicates (P < 0.001) vs. normoxia.

brief ischemic period, ANG II did not affect left ventricular end-diastolic pressure. ANG II infusion had no effect on hemodynamic performance during normoxia. However, the prolonged infusion of the high dose ANG II resulted in coronary vasoconstriction during both normoxia and ischemia. CPP increased 14.5 ± 5.2% with ANG II vs. saline during normoxia (P < 0.05) and 11 ± 2% vs. saline during ischemia (P < 0.001).

3.7. Hypercarbic perfusion in non-ischemic rat hearts

Fig. 3 shows that perfusion with the hypercarbic medium (pH = 7.13) resulted in a prompt decrease in left ventricular developed pressure from 117 ± 6 mmHg during normoperfusion to 86 ± 5 mmHg (P < 0.001). Positive dP/dt also decreased significantly from 2562 ± 151 mmHg/s during normoperfusion to 1782 ± 106 mmHg/s with hypercarbia (P < 0.001). Infusion with [10^{-7}]M ANG II increased left ventricular developed pressure by 17 mmHg to 103 ± 10 mmHg (P < 0.05) and + dP/dt to 2270 ± 262 (P < 0.05). A matched saline infusion had no effect on inotropy. Hypercarbia caused an insignificant increase in left ventricular end-diastolic pressure from 11 ± 1 mmHg during normoperfusion to 14 ± 1 mmHg during hypercarbia. ANG II infusion did not affect left ventricular end-diastolic pressure. Coronary perfusion pressure was relatively constant throughout normoperfusion and hypercarbia (85 ± 2 mmHg during normoperfusion; 80 ± 2 mmHg during hypercarbia; 84 ± 4 mmHg with saline) and increased slightly with ANG II (90 ± 4 mmHg) (P = n.s.).

3.8. Sodium–hydrogen exchange inhibition in ischemic rat hearts

Fig. 4 shows that during low-flow ischemia [10^{-7}]M ANG II significantly increased LV developed pressure (P < 0.001), but concomitant infusion with EIPA significantly attenuated this inotropic effect (P < 0.001). In this series of experiments, at baseline CPP was approximately 85 mmHg in all groups (saline, 87 ± 3 mmHg; EIPA, 86 ± 3 mmHg; ANG II, 86 ± 4 mmHg; ANG II + EIPA, 82 ± 4 mmHg; P = ns) and left ventricular developed pressure was approximately 130 mmHg and similar in all groups (saline, 132 ± 15 mmHg; EIPA, 137 ± 11 mmHg; ANG II, 144 ± 4 mmHg; ANG II + EIPA, 105 ± 21 mmHg). During low-flow ischemia when CPP was reduced to comparable levels in all groups (saline, 15 ± 2 mmHg; EIPA, 15 ± 2 mmHg; ANG II, 14 ± 1 mmHg; ANG II + EIPA, 12 ± 1 mmHg) left ventricular developed pressure fell to approximately 35 mmHg and was similar in all groups (saline, 39 ± 5 mmHg; EIPA, 33 ± 3 mmHg; ANG II, 39 ± 2 mmHg; ANG II + EIPA, 31 ± 2 mmHg).

Fig. 4. Changes in left ventricular developed pressure in rat hearts during low-flow ischemia following a 5 min infusion with saline (n=5), ANG II [10^{-7}]M (n=6), EIPA [5–10^{-5}]M (n=6), or ANG II [10^{-7}]M + EIPA [5–10^{-5}]M (n=6). ANG II significantly increased left ventricular developed pressure relative to saline control and EIPA alone (P < 0.001) The positive inotropic action of ANG II was significantly reduced by concomitant infusion with EIPA (P < 0.001). Values are mean ± s.e.m. * Indicates (P < 0.001) vs. all other groups.
4. Discussion

The importance of the renin-angiotensin system in cardiac disease has recently become apparent. The renin-angiotensin system contributes significantly to the pathophysiology of several aspects of heart failure, remodeling, and myocardial loading conditions [33]. Angiotensin-converting-enzyme inhibition has successfully been used to improve both systolic and diastolic dysfunction and reduce left ventricular dilation after myocardial infarction [13,33].

However, a potentially important role for the renin-angiotensin system in acute ischemic syndromes has received relatively little attention. In the current study, we utilized a model of low-flow ischemia, where the myocardium is hypoperfused but still functional, albeit at a reduced level. Such a low-flow ischemic condition is frequent and common to several coronary artery disease syndromes such as angina pectoris, unstable angina, myocardial infarction, and cardiogenic shock. The interaction between low-flow ischemia and the inotropic action of ANG II is therefore of potential clinical and physiologic significance.

4.1. Mechanism of inotropic action

Recently, 10 nM ANG II has been shown to exert a positive inotropic action in the rabbit by a mechanism of intracellular alkalization and an increased myofilament sensitivity to calcium, rather than by increasing peak systolic [Ca^{2+}$_i$], or $I_{Ca}$ [20,34], whereas higher concentrations of ANG II (100 nM) also increased $I_{Ca}$ [23]. Since the ischemic condition has been reported to acutely decrease myocardial contractility by decreasing myofilament sensitivity to calcium, at least in part by intracellular acidosis [8,14,31], we hypothesized that ANG II may potentially reverse the ischemia-induced depression of calcium sensitivity and have a more pronounced inotropic effect during ischemia.

Our results support this hypothesis. The results of our rabbit experiments show that although ANG II increased developed pressure and +dP/dt by a similar absolute amount at all CPPs in the rabbit, its effect was of a greater relative degree in hypoperfused rabbit hearts. The increased contractility observed with ANG II in the current study was not accompanied by significant changes in coronary perfusion pressure or isovolumic left ventricular end-diastolic pressure, suggesting that the duration of ischemia is critical in determining the extent of diastolic dysfunction reported to occur with ANG II in prior studies of longer periods of ischemia [36]. Our observations of enhanced inotropy with ANG II are consistent with previous reports in rabbits [9,20,34], humans [37], and most other species [5,11,33].

Our results provide new information by showing that ANG II augments contractility to a relatively greater extent during low-flow ischemia than during normoperfusion in the rabbit heart. Furthermore, in the rat, ANG II had a positive inotropic effect only during ischemia or during non-ischemic acidosis, but in contrast with the rabbit, not during normoxic perfusion at normal pH. The mechanisms responsible for these differences in inotropy between species and perfusion conditions are probably related to differences in one or more steps in the ANG II pathway.

However, the positive inotropic response of ANG II was somewhat less than that of dobutamine in this model. Recent studies from our laboratory have shown that 10^{-5}M dobutamine increases left ventricular developed pressure by approximately 20 mmHg during ischemic (CPP = 20 mmHg) and normoxic (CPP = 80 mmHg) perfusion conditions [7]. Similar results have been reported in the rat [26].

ANG II initiates its physiological cascade by receptor stimulation of well-defined ANG II receptors [22,39,41,42,49]. Following receptor activation, ANG II acts through the second messengers inositol 1,4,5-triphosphate (IP$_3$) and diacylglycerol, which increase contractility by increasing sarcolemmal $I_{Ca}^{2+}$ and [Ca$^{2+}$], [1,16,24] and/or by alkalinizing the intracellular milieu of the myocyte via protein kinase C stimulation of Na+/H+ exchange [20,34] (Fig. 5). It is well recognized that changes in pH$_i$ can alter the inotropic state of the heart, with intracellular alkalinization providing a positive inotropic effect via an enhanced sensitivity of troponin C to Ca$^{2+}$ [8].

![Mechanisms of action for ANG II in a cardiac muscle cell.](image-url)
Since pH is known to be reduced during ischemia [10,14,31], we hypothesized that the inotropic effects of ANG II during ischemia were secondary to ANG II's alkalinizing actions. We addressed this hypothesis by assessing the potential of ANG II to reverse the negative inotropy associated with non-ischemic acidosis.

4.2. Effects of ANG II in ischemic, non-ischemic, acidicotic, and normal pH rat hearts

At normal perfusate pH (10^-7 M) ANG II did not affect developed pressure, but in non-ischemic acidicotic rat hearts (hypercarbic perfusate) it caused a 19% increase, similar to that observed during low-flow ischemia. The lack of an ANG II effect at normal pH suggests that, in the rat, ANG II stimulates Na+/H+ exchange significantly only when Na+/H+ exchange has been already stimulated by intracellular acidosis. Thus during ischemia and its associated acidosis ANG II's effects on pH and myofilament Ca2+ sensitivity were probably partly responsible for its greater inotropic effect relative to normoperfusion. ANG II alone (i.e., in the absence of intracellular acidosis) may not be capable of stimulating the rat's Na+/H+ exchange because the rat's relatively high intracellular Na+ concentration opposes Na+ entry more than the lower intracellular Na+ of the rabbit [43] (see discussion below).

To test whether ANG II increased inotropy by inducing intracellular alkalosis by extrusion of protons via stimulation of Na+/H+ exchange, we infused the Na+/H+ exchange inhibitor EIPA during low-flow ischemia. EIPA negated the inotropic action of ANG II. These results suggest that stimulation of Na+/H+ exchange mediates the positive inotropic response of ANG II in ischemic rat hearts.

The greater inotropic potency of ANG II in the ischemic rat heart compared to the non-ischemic state may also be related to the effects of coronary turgor on myocyte stretch. During normal perfusion, coronary flow and vascular distension contribute to preload and sarcomere length via the effect of coronary turgor [17,27,40,48]. Li et al. [32] have shown that rat papillary muscles preloaded to maximal length have an attenuated force production with ANG II, whereas if muscle length falls on the ascending limb of the length–tension relationship, ANG II induces a positive inotropic effect. Thus during ischemia, when coronary flow and turgor were reduced, myocyte stretch probably decreased and may have shifted to a point on the ascending limb of the length–tension curve where ANG II increases myocardial contractility.

4.3. Rat–rabbit differences

Both ANG II and endothelin increased contraction amplitude of adult rabbit myocytes, concomitant with an increase in intracellular pH and with no change in the Ca2+ transient. However, both ANG II and endothelin decreased the contraction magnitude in neonatal rat myocytes together with a reduced inward Ca2+ transient and intracellular acidification [25]. These observations are consistent with our results where the normoxic adult rabbit heart had a greater inotropic response than the adult rat heart.

Species differences in myocyte Na+ and Ca2+ regulation may be responsible for this differential inotropic response. Although intracellular pH is similar in rat and rabbit myocytes [20,28], Shattock and Bers [43] have shown that intracellular Na+ is higher in rat than in rabbit myocytes. Therefore Na+/H+ exchange stimulation by ANG II may be relatively ineffective during normoxia and normal pH in the rat because Na+ entry is opposed by a relatively higher intracellular [Na+]. However, an increased intracellular H+ concentration may provide a further stimulus to Na+/H+ exchange to which the ANG II effect is additive, resulting in ANG II's positive inotropic action during ischemia, or during non-ischemic acidosis, as in our hypercarbia experiments.

Rat–rabbit differences in Na+/Ca2+ exchange may also contribute to the differential inotropic response to ANG II between these species. The increase in intracellular Na+ resulting from Na+/H+ exchange stimulation by ANG II can increase contractile function by increasing intracellular Ca2+ via Na+/Ca2+ exchange. The rates and direction of Na+/Ca2+ exchange are quite different in the rat and rabbit heart [6,43]. During twitching Na+/Ca2+ exchange is in the direction of Ca2+ uptake in the rabbit, but in the direction of Ca2+ release in the rat, with the reverse occurring at rest. In our experiments at relatively rapid pacing rates, Na+/Ca2+ exchanges of the twitch state may predominate and contribute to the species differences in inotropic response to ANG II; ANG II may be able to increase intracellular Na+ and secondarily increase Ca2+ entry in the rabbit, but not in the rat where Na+/Ca2+ exchange is operating in the direction of Ca2+ efflux. In the study by Ikenouchi et al. [20] an increase in [Ca2+], was not observed with 10^-8 M ANG II, but Ca2+ influx did increase at 10^-7 M ANG II in the rabbit, consistent with increased Na+/Ca2+ exchange [23]. Other species differences may be related to ANG II receptor subtypes [4,22]. ANG II binding capacity [33,39,49], and/or second messenger cross-talk [2]. Rat hearts have been reported to express two AT1 receptors (AT1a and AT1b) [22] and have greater ANG II binding capacities than rabbit [39,49]. Also, rat sarcolemma contains ANG II receptors that are negatively coupled to adenylate cyclase [2], which may thereby decrease cAMP levels and reduce contractile function in the rat heart.

4.4. Study limitations

Since peak systolic [Ca2+] and I_{Ca,v} were not measured in our study, we cannot assess a role for reverse-mode Na+/Ca2+ exchange or for involvement of the Na+/H+...
antiporter in regulating L-type Ca\(^{2+}\) current [23] and contractility in experiments where we used 10^{-7} M ANG II. However, Ikenouchi et al. did not report any changes in peak systolic [Ca^{2+}], or I_{Ca^{2+}} with 10^{-8} M ANG II in rabbit myocytes, thereby suggesting that the role of Na\(^+/\)Ca\(^{2+}\) exchange and L-type Ca\(^{2+}\) flux are not required for mediating ANG II’s effect in the rabbit [20,34]. Experiments which have examined the actions of endothelin-1 on [Ca^{2+}], pH_i, and cell contracture have reported similar results [25]. Endothelin-1 has an intracellular cascade similar to ANG II and has been shown to increase cell contraction and pH_i without altering [Ca^{2+}], in adult rabbit myocytes [25] as well as stimulating Na\(^+\)/H\(^+\) exchange by a protein kinase C mediated pathway in adult rat ventricular myocytes [28]. In a recent study in adult rat cardiomyocytes [46] endothelin increased [Ca^{2+}], in a dose-dependent manner, but ANG II did not, consistent with our observed lack of inotropy under normoxic and normal pH conditions in the rat.

4.5. Potential significance

In our studies, ANG II’s action of increasing contractility during low-flow ischemia may represent a compensatory mechanism which improves global contractile function during periods of limited coronary supply. Similarly, rats exposed to chronic ischemia (1 week) have a 3–4-fold increase in the expression and density of left ventricular ANG II receptors. Subsequent ANG II exposure to these myocytes produced a relatively large increase in contraction magnitude with concomitant increases in peak systolic [Ca^{2+}], [19]. Thus the renin–angiotensin system may oppose ischemic contractile dysfunction by virtue of an inotropic effect which becomes amplified during both acute and more chronic ischemia. These observations should be considered as anti-renin–angiotensin therapies are expanded in the treatment of cardiac disease.

However, our results do not contradict the reported benefit of angiotensin-converting-enzyme inhibitors in large trials of patients with myocardial infarction where hypotension was absent in patients receiving these agents [30]. Our results suggest that angiotensin II may be useful when diffuse myocardial ischemia is present (e.g., with severe hypotension or cardiogenic shock).

While human hearts express cardiac Na\(^+\)/Ca\(^{2+}\) [45] and Na\(^+\)/H\(^+\) [15] exchangers, it is difficult to determine whether ANG II mediates its inotropic effects in human myocardium via these subcellular pathways. ANG II receptor binding sites have been reported in human hearts [38,47], but in the few studies which have addressed the inotropic effects of ANG II in human myocardium [18,37,47], the positive inotropic action of ANG II appears to be more pronounced in atrial than in ventricular tissue [18,37]. Thus, while ANG II tends to increase contractility in the human heart, further study is needed to understand the inotropic action of ANG II in humans.

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References


