L-Arginine Potentiates Negative Inotropic and Metabolic Effects to Myocardium Partly through the Amiloride Sensitive Mechanism

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Abstract: Recently, cytokines have been proposed to cause cellular injury by nitric oxide (NO·) mediated pathway and L-arginine has been proposed to impair intracellular pH (pH) regulation via vacuolar type H⁺-ATPase in macrophages. We conducted this investigation on Langendorff perfused hearts of rabbits to elucidate the mechanisms involving the NO· precursor L-arginine on myocardial contractile function, glycolysis, mitochondrial respiration, and intracellular alkalinization and tested the effects of amiloride. L-Arginine caused a significant loss of contractile function (96±4 mmHg in control, 53±16 during L-arginine perfusion, p<0.01) and a significant increase of pH, (7.01±0.02 prearginine infusion, 7.08±0.03 at the end of L-arginine infusion, p<0.01) along with decreased oxygen consumption (MVO₂) (0.94±0.32 ml/min/g dry wt.), increased lactate release, and a loss of creatine phosphate (15% loss). Amiloride could prevent the cell alkalinization and contractile dysfunction, but not the derangement of oxidative metabolism caused by L-arginine in myocytes. We conclude that L-arginine has two distinct effects upon the myocardium: (1) an amiloride-sensitive loss of contractile function associated with intracellular alkalinization; and (2) an amiloride-insensitive inhibition of oxidative metabolism, possibly because of increased myocardial NO· production. [Japanese Journal of Physiology, 52, 207–215, 2002]

Key words: L-arginine, alkalinization, Na⁺/H⁺ exchanger, myocardium.

An essential basic amino acid, L-arginine has been known as a precursor of nitric oxide (NO) synthesis by the enzyme nitric oxide synthase (NOS). Two NOS isoforms are constitutively present in brain (NOS1), endothelial cells (NOS3), and the other isoform is inducible NOS (NOS2). Recent evidence suggests that a constitutive form of this enzyme, which exists in the heart, leads to a depression of cardiac function [1]. Arginine flux is mediated by the system (y⁺) basic amino acid transporter, which is Na⁺-independent, but voltage-dependent [2]. Increased arginine flux concomitant with hyperpolarization could conceivably play a role in modulating sustained nitric oxide release. Potential mechanisms responsible for injury to the heart by arginine include cyclic GMP-mediated negative inotropics and direct binding and inhibition of mitochondrial protein involved in oxidative metabolism, including cytochromes in the electron transport chain, and aconitase in the TCA cycle [3]. A previous report suggested that L-arginine impaired intracellular pH (pHᵢ) regulation by a vacuolar-type H⁺-ATPase through the nitric oxide generation in peritoneal macrophages [4]. The major role in pHᵢ homeostasis is thought to be via the Na⁺/H⁺ antiport, which mediates an electroneutral exchange of Na⁺ and H⁺ in mammalian cells. A similar phenomenon can be seen in cytokine-mediated action, which is related to the nitric oxide mediated inhibition of mitochondrial func-
tion and intracellular alkalinization (0.08 pH unit rise) via the activation of Na\(^+/H^+\) exchanger [5]. Moole-
naar and his associates [6] have suggested that alka-
line pH shift, mediated by Na\(^+/H^+\) antiport, may be a
common signal in the action of a platelet-derived growth factor that elicits the breakdown of inositol
phospholipids. To further elucidate these mechanisms
of injury in myocardium, we studied the effects of L-
arginine, which may be associated with NO produc-
tion and intracellular alkalinization (0.08 pH unit rise)
mediated by Na\(^+/H^+\) antiport, may be a
common signal in the action of a platelet-derived growth factor that elicits the breakdown of inositol phospholipids. To further elucidate these mechanisms of injury in myocardium, we studied the effects of L-
arginine, which may be associated with NO produc-
tion and intracellular alkalinization (0.08 pH unit rise)
mediated by Na\(^+/H^+\) antiport, which is a
Na\(^+/H^+\) exchange inhibitor on myocardial contractil-
ity, oxidative metabolism, glycolysis, and intracellular
pH in an isolated Langendorff-perfused rabbit heart.

METHODS

Experimental preparation. Animal proced-
dures received institutional approval and were con-
ducted in conformity with the “Guiding Principles in
the Care and Use of Animals” of the American Physi-
ology Society and the “Guide for the Care and Use of Laboratory Animals” published by the National Insti-
tutes of Health (NIH publication No. 85-23, revised,
1985). New Zealand white rabbits (3–4 kg) were anes-
thetized with ketamine hydrochloride (50 mg/kg) and
heparinized with 1,000 U of heparin. The hearts were
crushed and perfused in crystalloid Langendorff perfu-
sion mode with 80 mmHg pressure constant at 37°C.
The composition of Krebs-Henseleit buffer (in
mmol/l) was NaCl, 140; CaCl\(_2\), 1.25; KCl, 4.7; glu-
cose, 11.0; MgSO\(_4\), 1.2; NaHCO\(_3\), 25; and NaH\(_2\)PO\(_4\),
0.5. Bovine regular insulin, 10 U/l, was also included.

Oxygen consumption, glucose uptake, and
lactate release. The myocardial oxygen con-
sumption (MVO\(_2\)) was derived from the difference of
O\(_2\) tension between the aortic perfusate and coronary
effluent (ABL-3 acid–base laboratory, Radiometer,
Copenhagen, Denmark) and the coronary flow rate.
Lactate and glucose levels were measured by enzy-
matic assay (model 2,300 STAT, YSI, Yellow Springs,
OH). Glucose uptake was determined by the differ-
ences of glucose concentration between aortic per-
fuse and coronary effluent and coronary flow. The
lactate level was determined by the calculation of
coronary flow \(\times\) lactate (\(\mu\)mol/min/g dry wt.).

Experimental protocol. All chemicals were
purchased from Sigma (St. Louis, MO). After an ini-
tial 30 min stabilization period with normal buffer,
preinfusion contractile function and metabolic para-
eters were determined. Two experimental groups
were studied. In the first \((n=6)\), L-arginine (2.5
mmol/l, pH=10 in stock solution) was infused for
2 min and 40 s followed by 30 min of washout with
functional and metabolic parameters being measured
continuously (L-arg group). In the second group of
hearts \((n=6)\), 30 \(\mu\)mol/l amiloride was infused for
5 min before and during arginine infusion as well as
during the washout period (Amilo group). The de-
veloped pressure (DevP) and end-diastolic pressure
(EDP) by intracavitary balloon and MVO\(_2\), glucose
uptake, and lactate production were evaluated.

To evaluate the effects of other basic amino acids,
such as L-lysin and D-arginine, using stock solutions
of Krebs-Henseleit (K-H) buffer with pH being ad-
justed at 10, we infused the stock solution of K-H
buffer containing 2.5 mmol/l L-lys (L-lys, pH=10) or 2.5
mmol/l D-arginine (D-arg, pH=10) for 2 min
and 40 s; we then perfused those hearts for another
30 min with normal buffer in the K-H, L-lys, and D-
arg groups \((n=5, each)\). The purpose of the experi-
ment, where pH-adjusted K-H buffer stock solution
was infused, was to test if alkaline stock solution (pH
10) may affect myocardial function and/or metabolism
in a Langendorff-perfused heart. Intracellular pH was
measured by \(^{31}\)P-NMR spectroscopy in L-arg, Amilo,
and L-lys groups by the use of other sets of hearts.

Furthermore, another 3 groups of hearts \((n=5)\)
were used to determine lactate and glucose level in
the myocardium infused with L-arginine alone,
amiloride+L-arginine, or vehicle. Heart tissues were
excised and quickly frozen in liquid nitrogen, then
prepared for the measurements via the method de-
scribed by Williamson et al. [7].

NMR spectroscopy. Another 17 hearts were

used for the NMR spectroscopy measurements of the L-arg group (n=6), the Amilo group (n=6), and the L-lys group (n=5). Isovolumetric rabbit hearts were perfused in the Langendorff mode with a modified phosphate-free K-H buffer containing 1.8 mM CaCl2 and 11 mmol/l glucose. A bulb containing dimethylene phosphonic acid (DMPA) as an internal standard was placed in the right ventricle, and the right atrium was sutured closed. All experiments were performed in a 40 cm horizontal-bore, 4.7 T Brucker BioSpec spectrometer (Brucker Instruments, Inc., Billerica, MA) operating at the 31P frequency of 81 MHz. A 90° radiofrequency pulse was applied with a recycle time of 1 s and an acquisition time of 81 ms during a period of 2 min and 40 s for a total of 148 acquisitions per spectrum. Intracellular pH was determined by the following equation [8]: pH = 6.75 + log(δ – 3.37/5.69 – δ), where δ is the chemical shift difference in parts per million between Pi and phosphocreatine (PCr). Concentration measurements of each of the phosphorus-containing metabolites were estimated by an integration of the area under the individual peaks. PCr and ATP levels were expressed as a percent of the preinfusion area.

Statistical analysis. Results were expressed by mean±standard errors of the means (SEM) of the number of experiments indicated in parentheses. The data were analyzed by the use of the analysis of variance (ANOVA) with repeated measures when appropriate. Differences between specific means were tested by post hoc analysis that applied Student’s t-test with a Bonferroni correction.

RESULTS

Mechanical, metabolic, and energetic effects of D-arginine, L-arginine, L-lysin, and NaOH

DevP, end-diastolic pressure (EDP), coronary flow, MVO2, and pH data obtained from Langendorff-perfused rabbit hearts are shown in Table 1. To examine the alkaline pH effect of stock solution, we prepared and tested the stock solution of K-H buffer with pH being adjusted at 10. An insignificant change of myocardial function was attained by the infusion of pH-adjusted stock solution (pH=10). It was 99±1 mmHg of DevP and 6.8±0.5 mmHg of EDP at the end of infusion.

In L-lys group, DevP was remarkably decreased after the infusion. It was decreased from 101±8 to 30±8 mmHg at the end of the infusion. Coronary flow was markedly decreased (73±2 ml/min/g dry wt. vs. 9±5 ml/min/g dry wt. at the end of the infusion) in L-lys group, whereas it was only 50% decreased in the L-arg group. Although the pH, was significantly increased immediately after the onset of infusion in the L-arg group, there was no significant increase of pH in the L-lys group. Phosphocreatine was 94±2% of control, whereas Pi was 164±33% of control during the infusion of L-lys.

In the D-arg group, myocardial function was decreased in association with a decrease of coronary flow (6±1 ml/min/g dry wt. during infusion vs. 59±5 ml/min/g dry wt. pre-infusion). pHi was decreased after the infusion of D-arginine in association with a decrease of PCr by 28±5% of control and a contrary increase of Pi by 200±91% of control.

Cardiac function

Systolic pressure increased in the initial phase of L-arginine infusion, and EDP increased (data are not shown in the table). After about 1 min, DevP decreased to 54% of pre–L-arginine infusion (96±4 mmHg vs. 52±15 mmHg at the end of L-arginine infusion, p<0.01) (Fig. 1a). Systolic and diastolic pressures were gradually restored to the baseline during the washout of L-arginine (Figs. 2, 3). Preloading with amiloride provided no significant changes of hemodynamics. An increase of systolic pressure during the early L-arginine infusion was not prevented with 30 μmol/l amiloride (Figs. 1b, 2). The functional deterioration of developed pressure caused by L-arginine

Table 1. Mechanical and metabolic effects of L-arginine, D-arginine, L-lysin, and NaOH.

<table>
<thead>
<tr>
<th></th>
<th>DevP (mmHg)</th>
<th>EDP (mmHg)</th>
<th>Coronary flow (ml/min/g dry wt.)</th>
<th>MVO2 (ml/min/g dry wt.)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>End</td>
<td>Pre</td>
<td>End</td>
<td>Pre</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>109±6</td>
<td>52±15</td>
<td>8±0.6</td>
<td>8±2.0</td>
<td>1.8±0.05</td>
</tr>
<tr>
<td>D-Arginine</td>
<td>101±2</td>
<td>32±5</td>
<td>8±0.6</td>
<td>6±1.4</td>
<td>1.6±0.22</td>
</tr>
<tr>
<td>L-Lysin</td>
<td>101±8</td>
<td>30±8</td>
<td>8±0.5</td>
<td>7±2.0</td>
<td>—</td>
</tr>
<tr>
<td>NaOH</td>
<td>102±4</td>
<td>99±1</td>
<td>7±0.5</td>
<td>5±0.5</td>
<td>59±1</td>
</tr>
</tbody>
</table>

Values are represented by mean±SE. * p<0.05 vs. D-arginine and L-lysin; # p<0.05 vs. preinfusion.
was completely prevented (Fig. 2), whereas EDP was significantly higher in the Amilo group during the infusion period and washout period (Fig. 3). This may be related to a higher cytosolic calcium concentration in the Amilo group.

Oxygen consumption (MV$_{O_2}$)

No significant difference was noted between the L-arg group and the Amilo group in MV$_{O_2}$ before the L-arginine infusion. MV$_{O_2}$ significantly decreased by the end of the arginine infusion (1.80±0.05 ml/min/g dry wt. vs. 0.94±0.29 ml/min/g dry wt., p<0.01) (Fig. 4) and was gradually recovered by 30 min of washout in the L-arg group. It was quite constant in the Amilo group, however, this was significantly different from the L-arg group during the infusion and early washout period (1.59±0.15 ml/min/g dry wt. at the end of the infusion).

L-Arginine effect on intracellular pH

A significant increase of intracellular pH occurred, to 7.08±0.03 pH units, from 7.01±0.02 (p<0.01), by 2.5 mmol/l L-arginine infusion, showing a cytosolic alkalinization by L-arginine. And the pH then rapidly declined to 6.87±0.03 pH units during the early washout period, with a gradual recovery of pH units within 30 min of washout (Fig. 5). In the presence of amiloride, however, pH did not significantly increase (7.01±0.03 to 7.04±0.03 pH units) during the L-argi-
nine infusion or decrease during the washout period (Fig. 5) \( (p > 0.05) \).

**High-energy phosphates and inorganic phosphates**

In the L-arginine treated hearts, a significant decline in PCr (15% loss) and a significant increase of Pi (57% increase) (Table 2) were noted, followed by the complete recovery of PCr and Pi after 5 min of washout. It is possible to say that a 57% increase of Pi is almost equivalent to the 15% loss of PCr in terms of moles. Although amiloride prevented intracellular alkalization and loss of contractile function, it did not prevent the decline of PCr with the reciprocal increase of Pi during arginine infusion. A 16% loss of PCr level and a 39% increase of Pi level were seen in the Amilo group (Table 2). In contrast, the ATP level was nearly constant in the L-arg and Amilo groups. These data suggested that L-arginine could affect the high-energy phosphate potential and that amiloride did not improve this energetic insult.

**Lactate production**

L-Arginine caused a gradual increase of lactate release during the infusion (6.9±2.7 μmol/min/g dry wt.) as well as early washout (9.6±7.2 μmol/min/g dry wt.). In contrast, in the presence of amiloride a markedly augmented release of lactate was observed during the L-arginine infusion (29±4.7 μmol/min/g dry wt.). This lactate level rapidly returned to baseline during early washout (2.4±0.9 μmol/min/g dry wt.) (Table 3). The myocardial tissue lactate level was also

![Fig. 5. Effect of L-arginine on intracellular pH. L-arg, L-arginine group; Amilo, L-arginine + amiloride group; pre, preadministration of L-arginine with or without amiloride. A significant alkalinization was seen with L-arginine infusion followed by a significant drop of intracellular pH. This was protected by amiloride treatment.](image)

**Table 2. Phosphocreatine and inorganic phosphate level.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Preinfusion</th>
<th>L-Arginine infusion</th>
<th>2.5 min of wash</th>
<th>5 min of wash</th>
<th>10 min of wash</th>
<th>20 min of wash</th>
<th>30 min of wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphocreatine (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amilo</td>
<td>100</td>
<td>84±11*</td>
<td>90±11</td>
<td>99±7</td>
<td>101±9</td>
<td>99±8</td>
<td>101±9</td>
</tr>
<tr>
<td>L-arg</td>
<td>100</td>
<td>85±4*</td>
<td>82±4*</td>
<td>93±4</td>
<td>101±4</td>
<td>96±4</td>
<td>96±3</td>
</tr>
<tr>
<td>Inorganic phosphate (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amilo</td>
<td>100</td>
<td>139±42*</td>
<td>87±23</td>
<td>81±36</td>
<td>88±20</td>
<td>103±11</td>
<td>130±39</td>
</tr>
<tr>
<td>L-arg</td>
<td>100</td>
<td>157±12*</td>
<td>149±19</td>
<td>103±18</td>
<td>120±13</td>
<td>124±20</td>
<td>122±28</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. preinfusion. L-arg, only L-arginine–treated group; Amilo, L-arginine and amiloride–treated group.

**Table 3. Lactate and glucose levels in myocardium.**

<table>
<thead>
<tr>
<th>Group</th>
<th>L-Arg</th>
<th>Amilo</th>
<th>K-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate release (μmol/min/g dry wt.)</td>
<td>L-Arginine infusion</td>
<td>6.9±2.7</td>
<td>29±4.7*</td>
</tr>
<tr>
<td>Early wash</td>
<td>9.6±7.2</td>
<td>2.4±0.9*</td>
<td>—</td>
</tr>
<tr>
<td>Tissue lactate (μmol/min/g dry wt.)</td>
<td>Pre</td>
<td>2.1±0.3</td>
<td>3.6±0.5*</td>
</tr>
<tr>
<td>Glucose extraction (μmol/min/g dry wt.)</td>
<td>L-Arginine</td>
<td>184±45</td>
<td>183±31</td>
</tr>
<tr>
<td>5 min of wash</td>
<td>226±52</td>
<td>324±8*</td>
<td>—</td>
</tr>
<tr>
<td>30 min of wash</td>
<td>141±51</td>
<td>126±9</td>
<td>—</td>
</tr>
<tr>
<td>Tissue glucose (μmol/min/g dry wt.)</td>
<td>230±38</td>
<td>201±29</td>
<td>—</td>
</tr>
</tbody>
</table>

Pre, pre–L-arginine infusion; L-Arginine, at the end of L-arginine infusion; K-H, modified Krebs-Henseleit buffer. *p < 0.05 vs. L-arginine alone; #p < 0.05 vs. K-H.

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significantly higher in the Amilo group (3.6 ± 0.5 μg/g dry wt.) than in the L-arg group (2.1 ± 0.3 μmol/min/g dry wt.) at the end of the L-arginine infusion (Table 3).

**Glucose uptake and tissue glucose**

Glucose uptake did not significantly change in the L-arg group, whereas a significant increase was seen in the Amilo group at the end of the infusion (Table 3). Tissue glucose level was significantly higher in the L-arg group (16.9 ± 1.9 μmol/g dry wt.) than in the other groups, such as the K-H group (11.5 ± 0.6 μmol/g dry wt., p < 0.01 vs. L-arg group) and the Amilo group (7.8 ± 0.4 μmol/g dry wt., p < 0.01 vs. L-arg and K-H groups).

**DISCUSSION**

The effect of L-arginine was unique on pH and energy metabolism. L-Arginine has an action of intracellular alkalinization as a result of the activation of an amiloride-sensitive Na\(^+\)/H\(^+\) antiport because amiloride could prevent the cell alkalinization attained by L-arginine. However, other basic amino acids such as D-arginine and L-lysine did not have this action. L-arginine also affected the energy metabolism, especially the mitochondrial respiratory chain. Amiloride could prevent the cell alkalinization attained by promoting anaerobic glycolysis.

The maintenance of intracellular pH within physiological range is essential for cell function and survival, and active processes have been evolved to extrude excess H\(^+\), which are driven electrophoretically into the cell or generated metabolically therein. One of the primary mechanisms involved in the extrusion of H\(^+\) from mammalian cells is the Na\(^+\)/H\(^+\) antiport. It plays a major role not only in intracellular pH regulation [8–11], but also in volume regulation [10, 11] and transepithelial iron transport [12]. The antiport activated by a variety of growth factors, including angiotensin, interleukin 1 [12, 13], insulin [13, 14], platelet-derived growth factor [15], thrombin [16], and other bioactive compounds such as okadecic acid [17], protein kinase C [18–21], and phorbol esters [22] were well reported in several cell types. Swallow and his associates [5] suggested that L-arginine probably resulting from the production of NO· impairs intracellular pH regulation by vacuolar-type H\(^+\) ATPase in peritoneal macrophages. The mechanism of intracellular alkalinization attained by L-arginine may include (1) an elevation in intracellular guanosin 3′,5′-monophosphate (cGMP) and (2) Na\(^+\)/Ca\(^{2+}\) antiport.

NO· will rapidly diffuse both within the endothelial cells and across the plasma membrane to nearby cells [23] and induce a rise in the concentration of cGMP [24, 25] mediated by guanylyl cyclase that results in smooth muscle relaxation. NO· released from endocardial cells by the Ca\(^{2+}\)-dependent NO· synthetase [26] plays a role in the physiological modulation of myocardial contractility by increasing the level of cyclic GMP in cardiac muscle, and this exerts a negative inotropic effect [15, 27]. This action is thought to be a possible mechanism that explains how NO· is effective to protect ischemia/reperfusion injury [28–30]. Swallow et al. [5] suggested that nitroprusside caused a rapid inhibition of intracellular pH homeostasis in association with a marked increase of cGMP levels, and the incubation of macrophages with the membrane-permeant form of cGMP, 8-bromo cGMP, impaired H\(^+\)-ATPase-mediated intracellular pH regulation. The activation of a cGMP-dependent kinase appears to be a signal transduction pathway in other cell types [28, 31–34]. Furthermore, Semrad and his associates [35] have shown that 8-bromo cGMP inhibits Na\(^+\)/H\(^+\) exchange in chicken enterocytes, resulting in cytoplasmic acidification. CgMP increased by nitroprusside-reduced lactate accumulation in hypoxic rat myocardium was also reported [36].

Another plausible mechanism of cell alkalinization is that the influx of Ca\(^{2+}\) through the Na\(^+\)/Ca\(^{2+}\) antiport causes a counterdecrease of intracellular Na\(^+\), which stimulates the Na\(^+\)/H\(^+\) antiport to maintain its intracellular Na\(^+\) level. This leads to the observed decrease in intracellular H\(^+\). The major driving force for Na\(^+\)/Ca\(^{2+}\) antiport is the sodium gradient, [Na\(^+\)]/[Na\(^+\)]o, which is established by Na\(^+\)/K\(^+\) ATPase. Moolenaar and his associates [6] have suggested that the alkaline pH shift, mediated by Na\(^+\)/H\(^+\) antiport, may be a common signal in the action of platelet-derived growth factor that elicits the breakdown of inositol phospholipids. Phosphoinositide cascade evokes a wide variety of responses in many kinds of cells, such as glycogenolysis in liver cells and smooth muscle contraction [36]. The intracellular messenger formed by the activation of this pathway arises from phosphatidyl inositol 4,5-bisphosphate (PIP2), a phospholipid in the plasma membrane. The binding of a hormone such as serotonin to a cell surface receptor leads to the activation of phosphoinositide-dase (phospholipase C). Two messengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), are formed by the cleavage of PIP2. Submicromolar levels of IP3 mobilize Ca\(^{2+}\) from intracellular stores by directly opening Ca channels in the membrane of the endoplasmic reticulum and the sarcoplasmic retic-
ultram. So an increase in $[Ca^{2+}]$, promotes $Na^+$ accumulation via the $Na^+\!/H^+$ antiport, leading to the observed decrease in intracellular $H^+$ (intracellular alkalinization). Although we did not determine the $[Ca^{2+}]$ level, our data showing an increase of systolic pressure followed by an increase of end-diastolic pressure may imply an increase of $[Ca^{2+}]$, influx (Fig. 1). This possible mechanism is supported by the fact that amiloride did not prevent this augmentation of LV systolic pressure. Amiloride is known to increase the intracellular magnesium ($[Mg^{2+}]$) level by a direct inhibition of $Na^+/Mg^{2+}$ exchange or another $Mg^{2+}$ pathway, which is amiloride insensitive and blocked by $Ca^{2+}$ [37]. They also found that the removal of the external calcium results in a larger increase in $[Mg^{2+}]$, [37], suggesting an interaction between $[Ca^{2+}]$, and $[Mg^{2+}]$. This may potentially induce an increase of $[Ca^{2+}]$, and lead to a protrusion of intracellular $H^+$, which might be seen in the Amilo group.

This study also supports the hypothesis that myocardial cells treated with L-arginine exhibited the inhibition of the mitochondrial respiratory chain because MVO$_2$ rapidly decreased with L-arginine administration to 52%, accompanying the decrease of the contractile function. This phenomenon is also accompanied by an increased lactate production, which indicated the inhibition of oxidative phosphorylation and the acceleration of anaerobic glycolysis. At the end of L-arginine infusion, the rate of lactate release into the coronary effluent had increased to 6.9±2.7 ($\mu$mol/min/g dry wt.) and the tissue lactate also significantly increased, suggesting the stimulation of anaerobic glycolysis. Although the ATP level stayed nearly constant, PCr was decreased by 15% because of the anaerobic condition where glycolysis was the only pathway for energy production. Anaerobic glycolysis is less effective (only 2 ATP molecules) than that from the aerobic glucose combustion (36 ATP molecules). Geng and his associates [38] reported that INF-$\gamma$+TNF-$\alpha$ promote anaerobic glycolysis via the L-arginine--NO· pathway in isolated vascular smooth muscle cells.

L-Arginine caused a temporal inhibition of mitochondrial respiration. One possible mechanism of inhibition of the mitochondrial respiratory system by L-arginine might be NO·-mediated injury, since the NO·-mediated inhibition of mitochondrial respiration was reported in activated macrophages [3, 38, 39] and isolated rat pancreatic islets [40]. NO· readily forms complexes with the transition metal iron, nonheme transition metal complex, as well as heme-containing proteins, such as hemoglobin [40] and myoglobin [41]. Its reactivity toward iron-sulfur centers in proteins, which are in complex I and complex II in the mitochondrial electron transport system, and/or aconitase in TCA cycle [38, 39,42], might be especially important for the NO·-dependent inhibition of myocardial respiration and glucose oxidation. This is supported by data revealing that reconstitution of the iron-sulfur complex restores aconitase activity in NO·-exposed tumor cells [43]. However, we could detect no increase of NO·-related products by L-arginine infusion in coronary effluent by HPLC or myocardium by EPR. The crystalloid perfusion system may be an explanation for nondetectable NO·-related products. Because of no significant change of the nitroso compound signal in myocardium measured by EPR, the NO·-dependent pathway may not be involved in this mechanism of mitochondrial respiratory inhibition.

Amiloride, an aminoguanidine derivative, prevented the deterioration of oxygen consumption by L-arginine and attained good cardiac contractility (129±5 mmHg in the Amilo group vs. 53±16 mmHg in the L-arg group). However, the PCr level decreased (16% loss). The NO·-dependent inhibition of energy production pathway, most likely in the TCA cycle [42, 44, 45], might not be protected by amiloride. Regarding glucose and lactate metabolism, total lactate production, i.e., lactate release and tissue lactate level, was significantly higher in the Amilo group than in the L-arg group. Glucose metabolism was also augmented in amiloride-treated heart, suggesting a promotion of anaerobic glycolysis by amiloride. This might be a potential benefit that will help to maintain better myocardial function. This lactate accumulation may also be associated with the protection of cell alkalinization attained by L-arginine.

In summary, we studied the effects of a basic semiessential amino acid, L-arginine, on cardiac function, myocardial metabolism, and intracellular alkalinization by using crystalloid perfused rabbit hearts in a Langendorff mode. Our data suggest that L-arginine has two distinct effects on the myocardium: (1) a novel, amiloride-reversible (Na$^+$/H$^+$ antiport mediated) loss of contractile function associated with intracellular alkalinization; and (2) an amiloride-insensitive inhibition of oxidative metabolism, possibly because of increased myocardial NO· production. Since NO was not detected in coronary effluent or tissue, however, the mechanism of myocardial injury by L-arginine may be something else. Further studies may add even more insight into this mechanism.

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