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Endothelin-1 Has a Unique Oxygen-Saving Effect by Increasing Contractile Efficiency in the Isolated Rat Heart

Yuzo Takeuchi, MD; Yasuki Kihara, MD, PhD; Koichi Inagaki, MD, PhD; Takeshi Yoneda, MD; Shigetake Sasayama, MD, PhD

Background—The effect of endothelin (ET)-1 on cardiac energetics is not fully understood.

Methods and Results—In isolated, coronary-perfused rat hearts, we measured left ventricular contractility index (E_max), pressure-volume area (PVA), and myocardial oxygen consumption (MV abunds) before and after administration of ET-1 (1 × 10^{-9} mol/L). ET-1 increased E_max by 48±16% (P<0.01) and the total MV abunds by 24±11% (P<0.01). The MV abunds-PVA relations were linear both before and after ET-1 (r>0.99). ET-1 shifted MV abunds-PVA upward, increasing the MV abunds intercept by 24±13%. At the same time, ET-1 decreased the slope (S), with 1/S (contractile efficiency) being 46±5% before and 56±5% after ET-1 (P<0.01). ET-1-induced increases in E_max and in contractile efficiency were abolished by an ET_a receptor blocker (S-0139) but not by an ET_b blocker (BQ-788). Although high [Ca^{2+}]_i perfusion increased E_max and the intercept to the same extent as ET-1, it did not change S. N^{G}-Nitro-L-arginine (an inhibitor of nitric oxide synthase) increased the coronary perfusion pressure as much as ET-1, but S again remained unchanged. Dimethylamyloride (Na+/H+ exchanger inhibitor) partially blocked the positive inotropic effect of ET-1 but not the ET-a-induced increase in the contractile efficiency.

Conclusions—Agonistic effects of ET-1 on the ET_a receptor economized the chemomechanical conversion efficiency of the left ventricular myocardium by a mechanism independent of the Na+/H+ exchanger. This unique oxygen-saving effect of ET-1 may play an adaptive role in the failing myocardium, in which local accumulation of ET-1 is present. (Circulation. 2001;103:1557-1563.)

Key Words: endothelin ■ oxygen ■ mechanics ■ contractility

Endothelin (ET)-1 exerts its effect through stimulation of specific receptors, ET_a and ET_b receptors, that are widely distributed in the cardiovascular system. In cardiac myocytes from various mammalian species, ET-1 acts as a positive inotropic agent.1,2 This action has been variously attributed to an increase in the activator Ca^{2+}_i or an increase in the Ca^{2+} responsiveness of the contractile proteins,3,4 although the physiological or pathophysiological significance of the inotropic effect in vivo remains obscure. Recent experiments also showed that ET-1 in the coronary endothelium influences not only coronary blood flow but also tissue oxygen consumption,5 suggesting that ET-1 may modulate the interaction between energy demand and the contractile state of the myocardium. Interestingly, McClellan et al6 showed that ET-1 decreased actomyosin ATPase activity in vitro while it increased the isometric force development. The data suggest that the chemomechanical conversion efficiency of the myocardium may be affected by ET-1. This potential regulatory role of ET-1 on cardiac energy consumption, however, has not been tested in vivo. To address this question, we used the isolated, coronary-perfused rat heart, in which the left ventricular (LV) pressure-volume-area (PVA) relationship and its oxygen consumption in the unit myocardium (MV abunds) were measured simultaneously. The MV abunds-PVA relationship based on the time-varying elastance model has been fully established in large-animal hearts.7 Recent studies by us and others demonstrated that the relationship also applies to smaller hearts, such as those of rats.8,9 Within this framework, the linear relationship between MV abunds and PVA provides information on the chemomechanical conversion rate (contractile efficiency).10 We can estimate the amount of Ca^{2+} involved in the excitation-contraction (E-C) coupling from the MV abunds axis intercept (I) (PVA-independent MV abunds).7 Here, we show that ET-1 is a potent modulator of energy efficiency involved in myocardial contractile performance.

Methods

Isolated Heart Preparation
The preparation used in this study has been described elsewhere.6 In brief, hearts isolated from 80 male Sprague-Dawley rats weighing 250 to 400 g were perfused by the Langendorff method with...
The pH was adjusted to 7.40 with NaOH at 37°C, and the solution was continuously bubbled with 100% O₂ to yield maximal line at the level of the ascending aorta and the other positioned inside.

The pulmonary effluent was time-exchange inhibitor, respectively, these agents were administered 15 minutes before the ET-1 infusion (n=6 for each drug). In the third group, the effects of phenylephrine (1 μmol/L) and high [Ca²⁺] (2.0 mmol/L) in the perfusate were studied to compare their inotropic effects with that of ET-1 (n=6 for each drug). To evaluate the potentially related effects of increases in the perfusion pressure by ET-1, in the fourth group, we also treated the hearts with N²-nitro-L-arginine (L-NNA, 100 μmol/L, n=6) or pretreated the hearts before ET-1 perfusion with S-nitrosoacetylpenicillamine (SNAP, 10 μmol/L, n=6) or with adenosine (10 μmol/L, n=6).

Data Analysis
In each volume run, the LV end-diastolic and end-systolic pressures were plotted against the LV volume to construct the pressure-volume diagram. To assess the contractile state of the LV, the end-systolic pressure-volume relations (ESPVR) were fitted into a nonlinear regression analysis. The value of ESPVR curvilinearity. The total energy liberated by the ventricle under the isovolumic conditions was quantified by the PVA, the area circumscribed by the ESPVR, end-diastolic pressure-volume relations (EDPVR), and the systolic portion of the pressure-volume trajectory. The value of PVA was normalized by the wet LV mass to 1 g. The value of MVO₂ was reported as milliliters of O₂ per beat per gram of LV after the estimated right ventricular MVO₂ had been subtracted. To assess the contractile efficiency, a linear regression analysis was then performed to quantify the slope (S) and I parameters of the relationship. MVO₂=SxPVA+I.

Magnetic Resonance Spectroscopy Measurements
To test possible superimposition of tissue ischemia during the ET-1 perfusion, the isolated heart preparation (n=6) was introduced into a 17-cm-diameter horizontal bore magnet at 4.7 T (BEM 170/200, Otsuka Electronics USA) to obtain ³¹P magnetic resonance spectroscopy (MRS) at 81.01 MHz. One hundred free induction decays were averaged during 5 minutes for each spectrum. The data were sampled before and during 30-minute ET-1 infusion (n=6) and during the volume run (stepwise increases of LV end-diastolic pressure [LVEDP] to 25 mm Hg, n=6).

Chemicals
ET-1 was purchased from the Peptide Institute. S-0139 was generously supplied by Shionogi Research Laboratories. BQ-788, DMA, and L-NNA were purchased from Research Biochemicals Inc. All other chemicals were purchased from Wakenyaku Co.

Statistical Analysis
Data are expressed as mean±SD. Differences in mechanical and hemodynamic parameters between the control and the experimental drug intervention conditions were detected by Student’s t test. Differences in the MVO₂-PVA regression lines between the 2 conditions were detected by ANCOVA. Comparisons of variables among the groups were made by 1-way ANOVA. When the F test indicated a significant difference among the groups, the difference in mean values was tested by Fisher’s protected least significant difference method. Probability values of P<0.05 were considered statistically significant.

Results
Effect of ET-1 on ESPVR and MVO₂-PVA Relationship
Figure 1 shows representative tracings of CPP, LVP, the time derivative of LVP (LV dP/dt), and A-VDO₂. Perfusion with 1 nmol/L ET-1 produced sustained increases in CPP, LVP, and LV dP/dt, which were accompanied by an increase in A-VDO₂. Before and 30 minutes after the ET-1 perfusion, ET-1 increased CPP by 47% and LVP by 46% (Table). This was accompanied by an increase of 24% in the total MVO₂.
Because lactate concentration in the coronary efflux started to increase when LVEDP was >30 mm Hg (Figure 2A), we excluded 2 hearts that exceeded this diastolic pressure level during the volume run after ET-1 perfusion. Within these settings, myocardial levels of inorganic phosphate, phosphocreatinine, and ATP remained unaffected, as demonstrated by $^{31}$P MRS (Figure 2B and 2C). Figure 3A shows the effects of ET-1 on LV pressure-volume relations in a representative heart. ET-1 shifted both ESPVR and EDPVR upward. As summarized in Figure 3B, ET-1 increased Emax by 48%.

| Changes in Cardiac Mechanoenergetics Before and After Drug Interventions |
|--------------------|----------------|--------------------|----------------|--------------------|
|                     | ET-1 (n=8)  | High [Ca$^{2+}$] (n=6) | L-NNA (n=6)  | L-NNA With High [Ca$^{2+}$] (n=6) |
| ESP, mm Hg          | 85.7±6.8   | 126.0±17.0*        | 91.7±9.2     | 121.2±8.8*         |
| EDP, mm Hg          | 3.8±0.8    | 6.1±1.6*          | 5.3±1.4      | 5.0±1.4†           |
| dP/dt$\text{max}$, mm Hg/s | 1777±353  | 2438±645*        | 1714±315     | 2281±314*          |
| CBF, mL/min         | 11.2±1.4   | 10.8±1.2*        | 11.5±0.8     | 11.3±0.8           |
| CPP, mm Hg          | 116.0±11.3 | 171.4±12.2*      | 112.7±8.1    | 116.0±9.3†         |
| MV$\text{O}_{2}$/beat·g$^{-1}$ | 588±61    | 731±89*         | 569±90       | 703±110*           |
| Emax, mm Hg·g·mL$^{-1}$ | 504±84    | 748±126*        | 622±102      | 815±101*           |
| PVA, mL·O$_2$/beat·g$^{-1}$ | 8.6±2.0   | 14.2±5.3*       | 8.4±1.0      | 11.3±0.8*          |
| I, mL·O$_2$/beat·g$^{-1}$ | 463±71    | 576±77*         | 453±84       | 542±102*           |
| Eff, %               | 46.3±4.9   | 56.1±5.3*        | 48.6±5.1     | 47.6±6.8†          |

ESP indicates end-systolic pressure; EDP, end-diastolic pressure; dP/dt$\text{max}$, maximum positive value of time derivative of LV pressure; CBF, coronary blood flow; Emax, slope of the ESPVR; Eff, contractile efficiency; and I, MV$\text{O}_{2}$ intercept. Values are mean±SD. There was no statistically significant difference in the variables of standard conditions among the interventions.

*P<0.05 vs control; †P<0.05 vs ET-1.

Figure 2. A, Data from a preliminary study that showed relationships between LV preload (LVEDP) and myocardial ischemia (lactate production measured in time-collected coronary effluent) in our isolated heart preparations. Both in control (open squares, n=8) and during ET-1 perfusion (solid squares, n=8), no increases in lactate production were detected when EDP was <30 mm Hg. B and C, Representative traces of $^{31}$P MRS of isolated hearts during stepwise increments of LVEDP to 25 mm Hg (n=6) (B) and during ET-1 (1 nmol/L) infusion for 30 minutes (n=6) (C). Under both conditions, there were no changes in area under signals of inorganic phosphate (Pi), phosphocreatine (PCr), or ATP. Calculated tissue pH was 7.04±0.02.
Figure 4A shows the \( MV_O2 \)-PVA plots before and after the ET-1 perfusion in a representative heart. \( MV_O2 \) was linearly and tightly correlated with the corresponding PVA in each state (\( r > 0.99 \)). ET-1 shifted the \( MV_O2 \)-PVA relation upward, with a 24% increase in the \( MV_O2 \) intercept (I) (Figure 4B), while it decreased the slope (S). Consequently, ET-1 significantly increased 1/S (the contractile efficiency) by 21% (Figure 4C). We tested the effects of high [Ca\(^{2+}\)], an established positive inotropic agent, in our experimental setting. Figure 5 shows the effect of an increase of perfusate [Ca\(^{2+}\)] to 2 mmol/L on the LV pressure-volume and \( MV_O2 \)-PVA relations in a representative study. As summarized in the Table, the high [Ca\(^{2+}\)] induced a parallel shift of the \( MV_O2 \)-PVA relationship, which was consistent with its known effects in other experimental settings. The perfusion with phenylephrine (1 \( \mu \)mol/L) also produced a parallel shift of the relationship (data not shown). Thus, the ET-1–induced shift of S was not due to our specific experimental conditions. To assess the effects of coronary vasoconstriction on myocardial contractile efficiency, we further examined the effects of L-NNA. L-NNA increased CPP to the same extent as ET-1, whereas it decreased LVP by 4% and E\(_{\text{max}}\) by 18% (Table). L-NNA shifted the \( MV_O2 \)-PVA relation downward, but S remained unchanged (\( P = 0.79 \)). Furthermore, L-NNA with high [Ca\(^{2+}\)] (2.4 mmol/L) increased both CPP and E\(_{\text{max}}\) to the same extent as ET-1. This condition, however, did not affect the contractile efficiency. These results suggest that the increase in perfusion pressure that occurred during ET-1 perfusion did not affect, at least directly, the contractile efficiency. Interestingly, pretreatments with both adenosine and SNAP abolished the ET-1–induced increases in CPP, whereas their suppressive effects on ET-1–induced increases in

Figure 3. A, Effect of ET-1 on LVP–LV volume (LVV) relations of a representative heart. Symbols show individual data points, and superimposed lines show nonlinear regressions for each group. Correlation coefficient was 0.99 for both control and ET-1. From regression curve, E\(_{\text{max}}\) was determined (see Methods for details). B, Mean values of E\(_{\text{max}}\) of control and ET-1 perfusion groups. Values are mean±SD. n=8 per group. *\( P < 0.01 \) vs control.
in LVEDP were markedly different (% increase in LVEDP: 4.13% by ET-1 with SNAP, 53.18% by ET-1 with adenosine, P<0.05). Thus, the upward shift of EDPVR with ET-1 was not the consequence of changes in CPP.

**Effects of S-0139, BQ-788, and DMA on ET-1–Induced Increases in Contractile Efficiency, Emax, and MVO2 Intercept**

To explore the subcellular mechanism by which ET-1 increases contractile efficiency, we further examined the effects of pretreatment of the preparations with S-0139, BQ-788, or DMA. None of these agents alone showed a statistically significant effect on the basal contractile efficiency, Emax, and I (Figure 6). With BQ-788, ET-1 increased Emax, I, and contractile efficiency to the same extent as ET-1 alone. In contrast, with S-0139, ET-1 did not increase Emax, I, or contractile efficiency. With DMA, ET-1 increased Emax and I to a level smaller than with ET-1 alone, but it increased contractile efficiency to the same extent. Thus, the positive inotropic effect of ET-1 is due, at least in part, to the ET A-mediated activation of the Na+/H+ exchanger. The improved chemomechanical conversion efficiency of the contractile proteins, however, is independent of the Na+/H+ exchange mechanism.

**Discussion**

**Possible Superimposition of Myocardial Ischemia During ET-1 Perfusion**

ET-1 has been shown to have a positive inotropic effect in isolated papillary muscle as well as in isolated ventricular...
myocytes.\textsuperscript{1,16} In contrast, negative inotropic actions were reported in in vivo intact circulation.\textsuperscript{17,18} This discrepancy was explained by the vasoconstrictive property of ET-1, whereby myocardial ischemia may override the intrinsic positive inotropic effect. In this study, we used constant coronary flow conditions to avoid the effect of vasoconstriction and the subsequent reduction in myocardial flow. In our study, the ET-1 (1 nmol/L) perfusion for 30 minutes did not induce myocardial lactate production as measured in the coronary effluent (Figure 2A). We further tested whether the ET-1 perfusion at a constant flow rate precluded myocardial ischemia by introducing the same system into an MRS apparatus.\textsuperscript{15} As clearly shown in Figure 2C, ET-1 perfusion did not affect the $^{31}$P spectroscopic profile, whereas it consistently caused increases in LVEDP. Thus, we concluded that at least in our experimental conditions, it was ET-1 itself and not the tissue hyperperfusion that caused the increase in LVEDP.

**Positive Inotropic Effect of ET-1**

In the present study, ET-1 clearly showed a potent positive inotropic effect. At the same time, ET-1 shifted the MVO$_2$ I upward, which is consistent with the increase in the amount of activator Ca$^{2+}$ for E-C coupling. Furthermore, a Na$^+/H^+$ exchange inhibitor, DMA, partially inhibited the ET-1--induced increases in E$_{cm}$ and in the MVO$_2$ I. The data indicate that the positive inotropic effect of ET-1 is, at least in part, mediated by the increase of activator Ca$^{2+}$ through the Na$^+/H^+$ exchange mechanism.\textsuperscript{3,4}

**Effect of ET-1 on Contractile Efficiency**

The most important finding of the present study is that ET-1 decreased S of the MVO$_2$-PVA relation, which indicates an increase in the chemomechanical conversion efficiency. Changes in S of the MVO$_2$-PVA relation could be interpreted as reflecting alterations in myofibrillar energy efficiency,\textsuperscript{7} but several alternative explanations should be considered. Substantial changes in the CPP may alter S of the MVO$_2$-PVA relation. We tested the effects of increased perfusion pressure by L-NNA (an inhibitor of nitric oxide synthase) infusion. L-NNA increased the CPP to the same extent as ET-1. However, S of the MVO$_2$-PVA relation remained unchanged. Recent reports clearly showed that nitric oxide was a determinant of MVO$_2$\textsuperscript{19} whereas it did not change S of the MVO$_2$-PVA relation.\textsuperscript{20,21} Taken together, it is not likely that the ET-1--induced increase in CPP could be the primary cause of the change in S. The nonlinearity of the ESPVR may induce overestimation of PVA, resulting in an underestimate of S. In the present study, we calculated PVA using a parabolic fit of ESPVR. This fitting procedure was used as in our previous study, in which we made a comparative examination between the linear versus nonlinear fit of ESPVR in the isolated heart preparation.\textsuperscript{8} The data indicated that the nonlinearity of ESPVR varied depending on the disease state of the rat heart, so that nonlinear regression analysis was more suitable to intact hearts. Actually, in the present study, the regression coefficient ($r$) decreased to $<0.6$ when linear fitting was used. In addition, only with nonlinear fitting did we find that the estimated values of unstressed volume could be kept constant before and after the ET-1 perfusion (data not shown). Therefore, the decreased S of the MVO$_2$-PVA relation should not be attributed to the nonlinear fitting of the ESPVR that we used in this study. According to these critical considerations, the most likely explanation for the ET-1--induced decrease in S of the MVO$_2$-PVA relation is the intrinsic change in the energy utilization of the contractile proteins. The observed increase in contractile efficiency might reflect either an increase in the V$_{0}$-to-ATP efficiency or an increase in the ATP-to-PVA efficiency.\textsuperscript{7,10} As shown previously, a shift from an aerobic to an anaerobic state was precluded. Changes in the preference of metabolic substrates have been reported not to affect the MVO$_2$-PVA S.\textsuperscript{22} From these lines of evidence, the increased contractile efficiency is most likely due to an increased efficiency of the ATP-to-PVA conversion. In the present study, the underlying mechanism of this ET-1--mediated increase in contractile efficiency (the ATP-to-PVA efficiency) is not clear. We believe, however, that the increase may be best explained by a direct effect of ET-1 on cross-bridge formation at the level of the actin-myosin reaction. Goto et al\textsuperscript{13} reported that in hyperthyroid rabbit heart, there was a substantial decrease in contractile efficiency, which was associated with an increased V$_{0}$/V, ratio of the myosin isoform component and hence, an increased rate of ATP hydrolysis by myosin. McClellan et al\textsuperscript{8} made an intriguing observation with cryostat sections of quickly frozen rat hearts: ET-1, in concentrations of 0.1 to 10 nmol/L, decreased actomyosin ATPase activity while increasing the isometric force. Thus, ET-1 may increase contractile efficiency by decreasing myofibrillar ATPase activity through a posttranslational mechanism. The ET-1--mediated change in the contractile proteins is most likely the result of at least 1 phosphorylations. In isolated cardiac myofibrils and myocytes, protein kinase C phosphorylates the same sites as protein kinase A on C protein but different sites on troponin I, which results in inhibition of actomyosin ATPase.\textsuperscript{23} There is also a body of evidence that troponin T is phosphorylated by protein kinase C in vitro. Noland and Kuo\textsuperscript{24} showed that protein kinase C--induced phosphorylation of troponin T resulted in a depression of the ATPase activity. Taken together, it is conceivable that ET-1 decreases the myofibrillar ATPase activity through the activation of protein kinase C, which leads to phosphorylation of contractile proteins such as troponin I, C protein, and troponin T and therefore increases the contractile efficiency. Conversely, we also observed that phenylephrine, another established protein kinase C activator, did not change the contractile efficiency. Recent studies have shown that there are some differences in the isoform pattern and the time course of protein kinase C activation between ET-1 and phenylephrine that may reflect heterogeneous activations of the protein kinase C isofoms by ET-1 and phenylephrine.\textsuperscript{25,26} Therefore, ET-1 presumably phosphorylates different sites of contractile proteins from those phosphorylated by phenylephrine. Further studies will be needed to elucidate the relation between the change of contractile efficiency and the pattern of phosphorylation in contractile proteins.
Clinical Implications

In summary, our study demonstrated that ET-1 exerts a positive inotropic effect mainly through the activation of the ET₃ receptor–mediated Na⁺/H⁺ exchanger. At the same time, ET-1 also improves the chemomechanical transduction efficiency through the ET₄ receptor–mediated but Na⁺/H⁺ exchanger–independent mechanism. The former leads to an oxygen-wasting effect, whereas the latter may exert an oxygen-saving effect. When these 2 pathways through ET-1 are finally dissected, the unique oxygen-saving effect may have a great therapeutic impact on various oxygen-wasting states, such as myocardial infarction and congestive heart failure. In fact, ET-1 may play an adaptive role in the failing myocardium, in which local accumulation of ET-1 is present and there is an increase of contractile efficiency.¹¹,²⁷

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