Role of ET<sub>A</sub> receptors in the regulation of vascular reactivity in rats with congestive heart failure

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Thorin, Eric, Martin Lucas, Peter Cernacek, and Jocelyn Dupuis. Role of ET<sub>A</sub> receptors in the regulation of vascular reactivity in rats with congestive heart failure. Am J Physiol Heart Circ Physiol 279: H844–H851, 2000.—Endothelium-derived nitric oxide (NO) and endothelin (ET)-1 interact to regulate vascular tone. In congestive heart failure (CHF), the release and/or the activity of both factors is affected. We hypothesized that the increased ET-1 production associated with CHF may result in a reduced smooth muscle sensitivity to NO. The aim of this study was to evaluate the effects of chronic treatment with the ET<sub>A</sub>-receptor (ET receptor A) antagonist LU-135252 (LU) on cerebrovascular reactivity to sodium nitroprusside (SNP) in the rat infarct model of CHF. Rats were subjected to coronary artery ligation and were treated for 4 wk with placebo (n = 24) or LU (50 mg·kg<sup>-1</sup>·day<sup>-1</sup>, n = 29). CHF was associated with a decreased (P < 0.05) efficacy of SNP to induce relaxation of isolated middle cerebral arteries. Furthermore, neither NO synthase inhibition with N<sup>-</sup>nitro-L-arginine (L-NNA) nor endothelial denudation affected the efficacy of SNP. Thus the endothelium no longer influences smooth muscle sensitivity to SNP. LU treatment, however, normalized (P < 0.05) smooth muscle sensitivity to SNP. Sensitivity of ET-1-induced contraction was increased in CHF only in the presence of L-NNA, whereas contraction induced by ET<sub>B</sub> receptor (receptor B) stimulation was increased (P < 0.05) in endothelium-denuded vessels. LU treatment restored these changes in reactivity and revealed a significant endothelium-dependent ET<sub>B</sub>-mediated relaxation after NO synthase inhibition. In conclusion, CHF decreases and uncouples cerebrovascular smooth muscle sensitivity to SNP from endothelial regulation. The observation that chronic ET<sub>B</sub> blockade restored most of the changes associated with CHF suggests that activation of the ET-1 system importantly contributes to the alteration in vascular reactivity observed in experimental CHF.

chronic heart failure; rat cerebral artery; endothelin receptor A; endothelin-1; sarafotoxin 6c; sodium nitroprusside; LU-135252

The hemodynamic profile of patients with congestive heart failure (CHF) is characterized by increased systemic vascular resistance with reduced peripheral perfusion. The important role of the endothelium in regulating tissue perfusion was not recognized until recently. Fundamental and clinical studies indicate that impaired endothelium-dependent relaxation is to a large extent, related to reduced nitric oxide (NO) activity. Restoration of the endothelial function is considered a desirable goal of heart failure therapy. Improvements in endothelium-dependent relaxation have been observed with a variety of interventions, such as supplementations of a precursor of NO (L-arginine), estrogen, angiotensin-converting enzyme inhibition, lipid lowering, radical scavenging by antioxidants, and physical activity (for review, see Ref. 10).

The effects of NO are, however, complex and related to at least three independent smooth muscle mechanisms: 1) activation and regulation of guanylate cyclase sensitivity (8, 19); 2) cGMP-dependent activation of intracellular relaxant mechanisms (1, 4, 7, 23); and 3) a direct activation of K<sup>+</sup> channels, leading to relaxation (5). The efficacy of NO-induced relaxation derived either from the endothelium or from exogenous sources depends, therefore, on these three mechanisms. However, Münzel et al. (21) reported that nitrate tolerance was associated with a rise in circulating levels of endothelin (ET)-1. Furthermore, the decreased vascular responsiveness to nitrate could be reversed by ET-receptor antagonism (17). Regardless of whether there is a direct relationship between ET-1 and the regulation of smooth muscle sensitivity to nitrate, these data suggested to us that other endothelium-derived factors such as ET-1 and possibly endothelium-derived hyperpolarizing factors (EDHF) (3, 26, 30) may influence smooth muscle responses to NO and its derivatives. These regulatory mechanisms may directly or indirectly regulate guanylate cyclase sensitivity to NO and modify vascular reactivity.

Patients with CHF are known to be tolerant to nitrate therapy, thus explaining the considerably large doses necessary to achieve the desired hemodynamic effects (11, 12). However, the relationship between ET-1 and nitrate-induced dilation has never been investigated in CHF. Studies, however, have revealed the important influence of ET-1 in the pathogenesis of CHF (25). ET<sub>A</sub>-receptor (ET receptor A) antagonism has been shown to improve long-term survival in rats.
with CHF (20, 24) and has beneficial effects on left ventricular and myocyte function (27). Acute administration of an ET\(_A\)-receptor antagonist or a specific endothelin-converting enzyme inhibitor has been shown to improve urinary flow rate and urinary sodium excretion in association with an increase in glomerular filtration rate and renal plasma flow (33, 34). Finally, in patients already on standard heart failure therapy, short-term oral endothelin-receptor antagonist therapy improved systemic and pulmonary hemodynamics (28) and produced a significant reduction in forearm vascular resistance (18).

The improvement of cardiac function and vascular resistance by ET\(_A\)-receptor blockade in CHF is most likely smooth muscle and cardiomyocyte related, because ET\(_A\) receptors are predominantly expressed in these cell types. However, an indirect effect of ET\(_A\)-receptor blockade may involve the endothelium and its regulatory role on smooth muscle reactivity (16). Chronic ET\(_A\) inhibition further increases circulating levels of ET-1 in CHF (22, 27), which may overstimulate endothelial ET\(_B\) receptors (ET receptor B) and consequently modify endothelial cell function. Although nitrate-induced relaxation is endothelium independent, its efficacy is increased by endothelial denudation in vitro (31). We undertook this study to investigate specifically the consequences of CHF on the endothelium-dependent regulation of smooth muscle reactivity to both sodium nitroprusside (SNP) and contractile agents. To investigate the role of ET-1 in this regulatory mechanism, the effects of chronic ET\(_A\)-receptor inhibition were investigated in rats with CHF.

**METHODS**

**Induction of CHF.** Myocardial infarction was produced in male Wistar rats (200–250 g) by ligation of the proximal left anterior descending coronary artery (22). In brief, under anesthesia with ketamine-xyazine-water (5:2.5:1.5), the proximal left anterior descending coronary artery was identified and ligated with a 6-0 silk suture. Control rats underwent a similar operative procedure but without ligation of the coronary artery. Animals were maintained on standard rat chow with water ad libitum.

Two hundred and twenty rats underwent ligation. Afterward, the electrocardiogram (ECG) indicated that only 191 were occluded. After 48 h, 90 rats died. Thus 101 rats were kept. Forty-eight rats with CHF (CHF rats) have been used for these experiments.

At 4 wk after surgery, hemodynamic studies were performed, and cerebral arteries were harvested for myograph experiments.

**Chronic ET\(_A\)-receptor blockade.** One group of CHF rats was treated for 4 wk with LU-135252 (LU; Knoll), a selective ET\(_A\)-receptor antagonist (15), by gavage once daily at a dose of 50 mg/kg (CHF + LU rats, \(n = 24\)), whereas groups of CHF rats (CHF, \(n = 24\)) and sham-operated rats (SHAM, \(n = 22\)) received the vehicle (NaCl 0.9%). The treatment started 48 h after ligation of the coronary artery. Hemodynamic parameters were recorded in xylazine-xyazine-anesthetized rats at 4 wk. Treatment with LU was discontinued 48 h before anesthesia to avoid any residual influence of LU on vascular reactivity in vitro.

**Hemodynamic measurements and infarct-size determination.** The right carotid artery was catheterized (Intramedic, PE 50) for measurement of left ventricular (LV) and systemic arterial pressures on a physiological recorder (model no. 2200; Gould Instrument, Cleveland, OH). Mean values were determined by electronic averaging. Three milliliters of blood were withdrawn from the catheter for measurement of immunoreactive ET-1 levels, as previously described in detail (22, 29). Perimeter tracings of the entire LV and of the infarct area were used to determine infarct size by planimetry.

**Isometric recording of tension of isolated cerebral arteries.** Rat middle cerebral arteries (MCA) were harvested 4 wk after initiation of treatment and placed in ice-cold physiological salt solution (PSS), containing indomethacin (10 μmol/l), an inhibitor of cyclooxygenase, of the following composition (in mmol/l): 130 NaCl, 4.7 KCl, 1.18 KH\(_2\)PO\(_4\), 1.17 MgSO\(_4\), 14.9 NaHCO\(_3\), 1.6 CaCl\(_2\), 0.026 EDTA, and 10 glucose, aerated with 12% O\(_2\)-5% CO\(_2\)-83% N\(_2\) (pH 7.4). Two-millimeter-long segments were mounted on 20-μm tungsten wires in microvessel myographs (IMF, Univ. of Vermont) as previously described (29, 30, 32).

The endothelium was removed mechanically by gentle rubbing with a human hair. To prepare K\(^+\)-rich solutions, equimolar amounts of NaCl were replaced with KCl.

The half-maximal effective concentration (EC\(_{50}\)) of agonists was measured from each individual dose-response curve with the use of a logistic curve-fitting program (Allfit; Dr. A. Delean, Dept. of Pharmacology, Univ. of Montreal). The pD\(_2\) value is the negative log of the EC\(_{50}\) of agonists.

**Chemicals.** The following drugs were purchased from Sigma Chemical (St. Louis, MO): indomethacin, N\(^{-}\)nitro-L-arginine (L-NNA), phenylephrine (PE), and SNP. ET-1 and sarafotoxin 6c (S6c) were purchased from American Peptide (Sunnyvale, CA), anti-ET-1 antibody was from Peninsula (Belmont, CA), and \(^{[125]}\)I-labeled ET-1 was from Amersham (Oakville, Ontario, Canada). All drugs for reactivity studies were dissolved in PSS except for indomethacin, which was dissolved in ethanol; the final concentration of ethanol in the bath was 0.1% (vol/vol). Solutions were prepared fresh every day and kept on ice. LU was a generous gift from Dr. Michael Kirchengast (Knoll, Ludwigshafen, Germany).

**Statistical analysis.** Results are expressed as means ± SE. In all experiments, \(n\) represents the number of rats. Statistical differences between means were determined by analysis of variance followed by a Scheffe’s F-test. \(P < 0.05\) was accepted as significant for differences between groups of data.

**RESULTS**

**Serum ET-1 levels and hemodynamic and morphometric parameters.** Infarct rats gavaged with saline and infarct rats gavaged with LU developed CHF of similar severity; they had lowered mean arterial pressure and first derivative of the change in LV systolic pressure over time (dP/dt) and increased LV end-diastolic pressure and heart rate compared with sham-operated rats (Table 1). There was cardiopulmonary congestion as revealed by the increased lung-to-body weight ratio. The CHF + LU group, however, had higher plasma ET-1 levels and a larger infarct size, although the ratio of ischemic tissue weight to total LV (including septum) weight was similar in both groups.

**Baseline vascular parameters.** There was no difference in the external diameter of cerebral arteries isolated from sham-operated (156 ± 1 μm, \(n = 96\) seg-
The maximal contraction (E_max) induced by a depolarizing solution containing 127 mmol/l KCl was greater (P < 0.05) in sham-operated (681 ± 29 mg) than in CHF (524 ± 26 mg) and CHF+LU (579 ± 22 mg) rats. This response was decreased (P < 0.05) by endothelial denudation to a similar level of tone in sham-operated rats (470 ± 37 mg) and CHF (409 ± 30 mg) and CHF+LU (397 ± 43 mg) rats.

SNP-induced relaxation. SNP induced relaxation of isolated denuded arteries preconstricted with 10 μmol/l PE (Fig. 1). However, the pD2 value for SNP was decreased (P < 0.05) in CHF rats (7.82 ± 0.06, n = 11) compared with sham-operated rats (8.28 ± 0.04, n = 7). The treatment normalized the decreased sensitivity to SNP in CHF rats (pD2 = 8.36 ± 0.12, n = 6).

In a second series of experiments, intact arteries were preconstricted with 40 mmol/l KCl under control conditions after blockade of NO formation (l-NNA, 100 μmol/l) and after endothelial denudation. None of these conditions affected the contractile response to the high K+ (40 mmol/l) of sham-operated rats (Fig. 2A). Contractile responses, however, were lower (P < 0.05) in CHF rats, LU treated or not, compared with sham-operated rats (Fig. 2A). Blockade of NO formation potentiated (P < 0.05) the contractile response induced by high K+ in CHF rats. This potentiation was lower (P < 0.05) in the LU-treated CHF group and similar to the response of sham-operated rat arteries. In the absence of endothelium, the contraction was similar in sham-operated and CHF rat MCA but less (P < 0.05) in LU-treated CHF rats. However, in both CHF groups, the amplitude of the response after endothelial denudation was reduced (P < 0.05) compared with the response obtained in the presence of an intact endothelium combined with NO synthase inhibition. Further-
more, in CHF rats, the contraction obtained after denudation remained higher (P < 0.05) compared with the control response obtained in the presence of an intact endothelium.

Under these depolarized conditions, SNP (0.1 μmol/l) induced relaxation (Fig. 2B). The potency of SNP-induced relaxation was as follows: sham operated = CHF < CHF+LU. Inhibition of NO synthase (L-NNA, 100 μmol/l) or endothelial denudation potentiated (P < 0.05) the relaxation induced by SNP. This was significantly blunted in CHF rats but restored in the CHF+LU group. Consequently, in the CHF rats, the relaxation was decreased compared with the two other groups under both experimental conditions, i.e., after NO synthase blockade and after endothelial denudation.

To evaluate the influence of the endothelium on SNP-induced smooth muscle relaxation, we expressed the above results (Fig. 2, A and B) as a ratio of the relaxation to contraction in each condition (Fig. 3). We hypothesized that in the absence of endothelium and under depolarized conditions, SNP-induced relaxation would depend on cGMP formation and thus be a functional index of guanylate cyclase sensitivity to SNP, assuming that cGMP-induced relaxation is not affected. In the absence of endothelium, this ratio was ~1 in the sham-operated rat MCA. The value of this ratio was <1 (P < 0.05) in the presence of the endothelium, revealing the low efficacy of SNP-induced relaxation and thus a low guanylate cyclase sensitivity. The opposite occurred after NO synthase inhibition. This was significant (P < 0.05) the relaxation induced by SNP. This was significantly blunted in CHF rats but restored in the CHF+LU group. Consequently, in the CHF rats, the relaxation was decreased compared with the two other groups under both experimental conditions, i.e., after NO synthase blockade and after endothelial denudation.

Fig. 3. Expression of the ratio of relaxation (Rel; SNP, 0.1 μmol/l) to contraction (Cont; 40 mmol/l KCl solution) as an index of the endothelium-dependent regulation of smooth muscle sensitivity to SNP: for a ratio <1, the efficacy of SNP-induced relaxation is low, the reference sensitivity being without endothelium (~1). Experiments were performed with endothelium (+E; n = 15, 11, and 17, respectively), after blockade of NO synthase (+L-NNA, 100 μmol/l; n = 8, 10, and 8, respectively), or without endothelium (-E; n = 11, 10, and 8, respectively). Results are expressed as means ± SE. *P < 0.05 vs. Sham. †P < 0.05 vs. +E. ‡P < 0.05 vs. CHF.

**ET-1- and S6c-induced contraction.** ET-1 (300 nmol/l) induced a maximal contraction in all groups (Fig. 4), with similar pD2 values in sham-operated rats, CHF rats, and CHF+LU rats (Table 2). NO synthase inhibition increased (P < 0.05) the sensitivity to ET-1 in CHF rats but had no effect in sham-operated and LU-treated CHF rat arteries (Table 2). In the absence of endothelium (Fig. 5), pD2 values for ET-1 were similar in all groups (Table 2).

S6c had no effect on baseline tension of intact arteries (data not shown). After NO synthase inhibition, baseline tension increased similarly in arteries from sham-operated (40 ± 4% Emax, n = 38 segments), CHF (39 ± 4% Emax, n = 44 segments), and CHF+LU (44 ±

**Table 2. pD2 values for ET-1**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CHF</th>
<th>CHF+LU</th>
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<tbody>
<tr>
<td>+E</td>
<td>8.25±0.16(7)</td>
<td>8.16±0.07(8)</td>
<td>8.42±0.15(11)</td>
</tr>
<tr>
<td>+E+L-NNA</td>
<td>8.59±0.13(7)</td>
<td>8.94±0.25(7)</td>
<td>8.76±0.29(9)</td>
</tr>
<tr>
<td>-E</td>
<td>8.53±0.09(8)</td>
<td>8.79±0.17(10)</td>
<td>8.31±0.20(6)</td>
</tr>
</tbody>
</table>

Data are means ± SE; n is shown in parentheses. Experiments were performed in the presence of an intact endothelium (+E), after nitric oxide synthase inhibition (+E+L-NNA), and after endothelial denudation (-E). L-NNA, N^o-nitro-L-arginine. *P < 0.05 vs. +E.
3% \( E_{\text{max}} \), \( n = 34 \) segments) rats. Under these conditions, S6c induced a relaxation of sham-operated rat arteries at low concentrations followed by a contraction at high concentrations (Fig. 6). In CHF rat arteries, the dilatory component of S6c was absent, whereas in CHF+LU rats, the relaxant component of S6c was potentiated. Finally, in the absence of endothelium, S6c induced a greater contraction in CHF compared with sham-operated rat cerebral arteries (Fig. 7); the treatment reversed this effect of S6c except for the highest concentration tested (300 nmol/l). In the absence of maximal contraction, we could not measure EC50 for S6c.

DISCUSSION

In the present study, we report that CHF alters vascular smooth muscle reactivity to SNP and the endothelium-dependent regulation of smooth muscle reactivity. Despite a lack of effect of a chronic inhibition of ETA receptors on cardiac function, this treatment normalized almost all measured vascular responses. Surprisingly, however, it is the endothelium-dependent regulation of smooth muscle reactivity that was altered in CHF. Furthermore, ETA-receptor antagonism modified the endothelium-dependent regulation of smooth muscle reactivity rather than smooth muscle reactivity per se.

Responses to SNP. Patients with CHF are known to be tolerant to nitrate therapy, thus explaining the considerably large doses necessary to achieve the desired hemodynamic effects (11, 12). As shown in Figs. 1 and 3, sensitivity to SNP is decreased in CHF, which may explain this tolerance. Most interestingly, the results suggest that smooth muscle sensitivity to SNP remains low and unaffected regardless of the experimental conditions (Fig. 3); compared with the sham-operated group, CHF is associated with a lack of endothelium-dependent regulation of smooth muscle sensitivity to SNP. It is therefore clear that CHF uncouples smooth muscle sensitivity to SNP from endothelial regulation.

The effects of NO synthase inhibition and endothelial denudation on nitrate-induced relaxation in normal vessels are well known: the sensitivity to guanylate cyclase increases (8, 19), as illustrated by Fig. 3, confirming our previous results (31). However, the observation that the sensitivity to SNP is lower in the absence of endothelium than after NO synthase inhibition suggests that endothelium-derived NO is not the only factor involved in the regulation of smooth muscle sensitivity to SNP. ET-1 may be involved in this regulatory pathway, because chronic ETA-receptor blockade restored the sensitivity of SNP-induced relaxation in CHF (Fig. 1). The origin of these changes may be secondary to the rise in circulating levels of ET-1. As recently demonstrated (17, 21), ET-1 may generate free...
radicals that are known to interfere with NO-mediated effects. For comparison, nitrate tolerance is a condition where ET-1 levels increase: blockade of ET receptors restores the normal dilatory response to nitrate (17). In nitrate tolerance, however, the maximal dilatory response to nitrate is reduced, whereas in CHF, there is a decrease in sensitivity without a change in maximal response (Figs. 1 and 3). Thus ET-1 may reduce NO-mediated relaxation by both generating free radicals (reducing the maximal response) and decreasing guanylate cyclase sensitivity. The former effect of ET-1 may be achieved for higher concentrations of ET-1.

**Contractile responses to high K**⁺. Contractile responses induced by a depolarizing solution appear to be independent of endothelial regulation in cerebral arteries of control rats, as shown by Fig. 2A. It is most likely that under normal conditions, cerebrovascular contractile responses are regulated by the endothelium to be homogeneous regardless of whether NO is present. We have previously reported that endothelial mechanisms compensate for a lack of NO in physiological conditions via the intervention of other factors such as EDHF (30). CHF, however, compromises this mechanism (Fig. 2A). Contractions are decreased under basal experimental conditions and potentiated by NO synthase inhibition. Smooth muscle responses, however, are similar to those obtained in sham-operated rats after endothelial denudation. Thus NO exerts a greater inhibitory influence on contractions induced by high K⁺. The mechanisms involved in endothelium-dependent regulation of smooth muscle contractility under depolarized conditions are unclear. First, blockade of NO synthase induced a similar increase in tension in the three groups. Thus basal NO-dependent regulation of tone is not affected. This does not mean that in the presence of stimuli, NO production is not affected: we previously reported that after endothelial injury, stimulated but not resting endothelial function was altered (31). Likewise, in CHF, high external K⁺ seems to facilitate NO release, because endothelial denudation did not reduce the contraction to the level of the control conditions, i.e., with endothelium. The mechanisms responsible for the changes in contractile response to high K⁺ may be related to a change in the mechanisms by which smooth muscle membrane potential regulates endothelial responses. Work by Dora et al. (9) suggests that smooth muscle depolarization increases endothelial intracellular Ca²⁺ via gap junctions in resistance arteries. More recently, it has been shown that contractions induced by high K⁺ and norepinephrine were increased by a gap junction inhibitor in an endothelium-dependent manner (14). Alternatively, Fleming et al. (13) reported that smooth muscle contraction increased endothelial NO synthase activity in a Ca²⁺-independent process associated with tyrosine kinase activation. These mechanisms are most likely essential for vascular control, and myoendothelial communications may be one of the vascular targets of CHF. Smooth muscle depolarization will trigger NO release from the endothelium. This may represent a compensatory mechanism for the lower guanylate cyclase sensitivity, possibly in relation to the change in ET-1 metabolism.

Although LU treatment in CHF partially restored the relaxant responses to SNP (Figs. 1 and 2B) and, overall, increased vascular sensitivity to SNP (Fig. 3), it did not abolish the NO-dependent regulation of the contractile response by high external K⁺ (Fig. 2A). The amplitude of the contractile response was, however, reduced with and without endothelium compared with sham-operated rats. This suggests that ETΑ-receptor blockade reduces smooth muscle sensitivity to high external K⁺. It is therefore likely that ET-1 plays a role in regulating smooth muscle reactivity and may be involved in regulating myoendothelial coupling.

**Contractile responses to S6c.** CHF strongly increased S6c-dependent contractions in endothelium-denuded arteries (Fig. 6). This observation has been previously reported in heart failure (6). This contractile response was, however, efficiently prevented by the endothelium: S6c induced no contraction in its presence and a similar contraction after NO synthase inhibition in CHF and sham-operated rats (Fig. 5). Furthermore, the exaggerated contractile response observed in denuded arteries was normalized by LU in CHF rats except for the highest concentration of S6c tested (300 nmol/l). This suggests that blockade of ETΑ receptors modifies ETB-receptor number and/or sensitivity. It is unlikely that the decreased contractile response to S6c observed in CHF+LU is due to the blockade of ETB receptors by the ETΑ antagonist for two reasons: first, LU was withdrawn from the rat diet 48 h before anesthesia, and, second, its increased endothelial ETB-receptor activity that is observed (Fig. 5) rather than a decrease (see below). We know also that ETΑ receptors are not expressed on endothelial cells (2). Therefore, chronic ETΑ-receptor antagonism increases smooth muscle ETB-receptor-mediated responses.

This upregulated response to S6c was not limited to smooth muscle. After NO synthase inhibition, which per se induced a similar tone in all groups, S6c relaxed vessels from LU-treated CHF rats up to a concentration of 3 nmol/l. This relaxation is most likely EDHF dependent, because both NO and prostanoid productions were inhibited. Thus endothelial ETB-receptor stimulation may trigger EDHF release: this is abolished in CHF but upregulated by chronic ETΑ-receptor inhibition. An increase in acetylcholine-induced EDHF production and/or effect has been recently reported in the aortas of rats treated with LU for 2 wk (16). The increase in circulating ET-1 levels observed with LU could be the signal leading to the change in ETB-receptor function.

A contraction still developed for a concentration of S6c of 30 nmol/l and above, suggesting that there is no interaction between the endothelial dilatory and the smooth muscle contractile responses mediated by ETB-receptor activation.

**Contractile responses to ET-1.** In sham-operated animals, the sensitivity of ET-1-induced contraction was affected neither by NO synthase inhibition nor by endothelium removal. pD₂ values were similar in the
three groups of animals (Table 2). In CHF, however, the sensitivity of ET-1 was increased by NO synthase inhibition compared with the control experimental conditions (Table 2). These data are in agreement with the increased responses to high external K⁺ after NO-formation blockade (Fig. 2A), confirming that CHF is associated with an upregulation of NO-dependent inhibition of smooth muscle cell contraction. Interestingly, LU treatment prevented the changes in ET-1 sensitivity associated with CHF. Therefore, SNP-induced relaxation and S6c-, ET-1-, and high external K⁺-induced contraction all appear to be under a tight endothelium-dependent regulation. CHF seems to alter this regulatory mechanism, which is, however, unclear. The lack of increase in smooth muscle sensitivity to SNP after NO synthase inhibition in CHF rats could favor smooth muscle contractility, an effect reversed by the LU treatment and compensated by an over production of NO during smooth muscle stimuli. This decrease in smooth muscle sensitivity to ET-1 in LU-treated CHF rats could also be related to the upregulation of ETB-receptor-mediated vasorelaxation. Altogether, LU therapy in CHF normalized ET-1 sensitivity: this suggests that the beneficial effects of ETA-receptor blockade in CHF may be partly endothelial via an upregulation of the ETB-dependent pathway and an improvement of the regulatory mechanisms of smooth muscle sensitivity to NO.

Clinical relevance. The reduced sensitivity to SNP may explain the reported greater tolerance to nitrates and the unreported but frequently observed reduced incidence of headaches in patients with CHF. Consequently, higher dosages of nitrates are necessary to obtain clinical benefit (11, 12) and may precipitate the incidence of headaches in patients with CHF. The observation that chronic ETA-receptor inhibition reduced relaxation and S6c-, ET-1-, and high external K⁺-induced contraction could also be related to the upregulation of ETB-receptor-mediated vasorelaxation. Altogether, LU therapy in CHF normalized ET-1 sensitivity: this suggests that the beneficial effects of ETA-receptor blockade in CHF may be partly endothelial via an upregulation of the ETB-dependent pathway and an improvement of the regulatory mechanisms of smooth muscle sensitivity to NO.

Furthermore, inhibition of smooth muscle ETA receptors has a profound influence on vascular reactivity, possibly by increasing ETB-receptor stimulation on endothelial cells.

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