British Journal of Pharmacology (1998) 123, 344–352

The effect of chronic treatment with trandolapril on cyclic AMP- and cyclic GMP-dependent relaxations in aortic segments of rats with chronic heart failure

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1 Characteristics of cyclic GMP- and cyclic AMP-mediated relaxation in aortic segments of rats with chronic heart failure (CHF) and the effects of chronic treatment with an angiotensin I converting enzyme (ACE) inhibitor, trandolapril, were examined 8 weeks after coronary artery ligation.

2 Cardiac output indices of coronary artery-ligated and sham-operated rats were 125 ± 8 and 189 ± 10 ml min^{-1} kg^{-1}, respectively ($P < 0.05$), indicating the development of CHF at this period.

3 The maximal relaxant response of aortic segments to 10 μM acetylcholine in rats with CHF and sham-operated rats was 64.0 ± 5.7 and 86.9 ± 1.9%, respectively ($P < 0.05$), whereas the relaxant response to sodium nitroprusside (SNP) remained unchanged. Tissue cyclic GMP content in rats with CHF was lower than that of sham-operated rats.

4 In endothelium-intact segments of rats with CHF, the maximal relaxant response to 10 μM isoprenaline (44.5 ± 6.7%) was lower that sham-operated rats (81.3 ± 2.5%, $P < 0.05$) and the concentration-response curve for NKH477, a water-soluble forskolin, was shifted to the right without a reduction in the maximal response. Isoprenaline-induced relaxation of aortic segments was attenuated by N^{G}-nitro-L-arginine methyl ester (L-NAME) in sham-operated rats, but not in rats with CHF. Relaxation to 30 μM dibutyryl cyclic AMP in rats with CHF (26.8 ± 2.7%) was lower than that in sham-operated rats (63.4 ± 11.8%, $P < 0.05$).

5 Trandolapril (3 mg kg^{-1} day^{-1}) was orally administered from the 2nd to 8th week after the operation. Aortic blood flow of rats with CHF (38.5 ± 3.6 ml min^{-1}) was lower than that of sham-operated rats (55.0 ± 3.0 ml min^{-1}), and this reduction was reversed (54.1 ± 3.4 ml min^{-1}) by treatment with trandolapril. The diminished responsiveness described above was normalized in the trandolapril-treated rat with CHF (i.e., the maximal relaxation to acetylcholine, 94.7 ± 1.0%; that to isoprenaline, 80.5 ± 2.8%; that to dibutyryl cyclic AMP, 54.7 ± 6.2%). However, aortic segments of trandolapril-treated rats with CHF, L-NAME did not attenuate isoprenaline-induced relaxation and the tissue cyclic GMP level was not fully restored, suggesting that the ability of the endothelium to produce NO was still partially damaged.

6 The results suggest that vasorelaxation in CHF, diminished mainly due to dysfunction in endothelial nitric oxide (NO) production and cyclic AMP-mediated signal transduction, was partially restored by long-term treatment with trandolapril. The mechanism underlying the restoration may be attributed in part to prevention of CHF-induced endothelial dysfunction.

Keywords: Chronic heart failure (CHF); endothelium; vasorelaxation; angiotensin I converting enzyme (ACE) inhibitor; trandolapril

Introduction

Abnormal vascular responses, such as increased vasoconstriction and diminished relaxation to vasoactive stimuli, have been observed in patients with chronic heart failure (CHF) with low cardiac output (Zelis & Flaim, 1982; Parmley, 1985). It is well recognized that endothelial nitric oxide (NO) is a key transducer of the vasodilator signalling and the defect of NO production has been shown to be detrimental to the systemic circulation in patients and animals with CHF (Drexler *et al*., 1993; Katz *et al*., 1993; Teerlink *et al*., 1994). In contrast, enhanced production of NO in arteries of dogs with CHF has also been observed (Main *et al*., 1991; O'Murchu *et al*., 1994). Thus, the role of NO in the altered vascular function during CHF remains unclear. In addition, the mechanism by which endothelial dysfunction occurs during CHF is not fully understood.

Two cyclic nucleotides, guanosine 3′:5′-cyclic monophosphate (cyclic GMP) and adenosine 3′:5′-cyclic monophosphate (cyclic AMP), are known to be involved in vascular smooth muscle relaxation. Endothelium-dependent vasorelaxation is elicited mainly by endothelial production of NO, which activates cytosolic guanylate cyclase and then formation of cyclic GMP in smooth muscle cells. Cyclic AMP-mediated relaxant responses are believed to be largely endothelium-independent. However, some studies have shown that cyclic AMP-mediated relaxation is partially dependent on endothelial NO (Grace *et al*., 1988; Gray & Marshall, 1992). There is considerable 'cross-talk' between NO producing systems and cyclic AMP-mediated vasorelaxation. It has been found that the acetylcholine-induced cyclic GMP-mediated vasorelaxant response is impaired in vessels of patients with CHF (Katz *et al*., 1992; Drexler *et al*., 1993). In contrast, the β-adrenoceptor stimulant-induced cyclic AMP-mediated vascular response in CHF and the role of NO in this cyclic AMP-mediated vasorelaxation during CHF have not been fully elucidated.

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The aims of the present study were to investigate the characteristics of the vascular responsiveness to vasoactive agents, to clarify the role of NO in the CHF vessel and to assess the possible underlying mechanism. To do this, we employed the rat model with CHF following coronary artery ligation and assessed the changes in the vasodilator response to cyclic GMP- and cyclic AMP-mediated vasorelaxants in aortic segments of animals with CHF.

In addition, we determined whether the impaired vascular response of rats with CHF may be ameliorated by long-term treatment with an angiotensin I converting enzyme (ACE) inhibitor. There is ample evidence that the renin-angiotensin-aldosterone system is present in vascular tissues and regulates local vascular responsiveness under physiological conditions. This raises the possibility that angiotensin II is a critical factor in the altered vascular responsiveness of animals with CHF (Curtiss *et al*., 1978; Cody & Laragh, 1983). ACE inhibitors prevent an increase in plasma angiotensin II and augment endothelium-dependent vascular relaxation, possibly by prolongation of the half-life of bradykinin. It is hypothesized, therefore, that ACE inhibitors have a beneficial effect on altered vascular function in CHF. Despite the clinical effectiveness of ACE inhibitors, the effect of chronic treatment with ACE inhibitors on impaired vascular function of CHF is unknown. To evaluate the effect of an ACE inhibitor on vascular responses, a non-sulphydryl ACE inhibitor trandolapril was employed in the present study. Trandolapril is a prodrug which is rapidly hydrolyzed to its active metabolite trandolaprilat when administered orally (Patat *et al*., 1989) and has a long-lasting action (Chevillard *et al*., 1988).

Methods

Experimental animals

Male Wistar rats, weighing 200–220 g (SLC Co., Ltd. Shizuoka, Japan), were used in the present study. The animals were conditioned at 23 ± 1°C with a constant humidity of 55 ± 5%, a cycle of 12 h light and 12 h dark, and had free access to food and tap water according to the Guideline of Experimental Animal Care issued from the Prime Minister's Office of Japan.

Preparation of rats with CHF and treatment with trandolapril

Myocardial infarction was produced in rats according to the method described previously (Sanbe *et al*., 1993). Briefly, the rats were anaesthetized with diethylether and a left thoracotomy was performed. The left coronary artery was ligated at approximately 2 mm from its origin with a polyester string (5-0 TICRON, Cyanamid of Great Britain, Ltd., U.K.), and the thorax was closed. Sham-operated rats were subjected to the same surgical procedure as above except for coronary artery ligation. Thereafter all rats were maintained on standard rat chow and water for a period of 8 weeks. The mortality of this procedure was 14% in the first 48 h and 43% in the second week. Two weeks after the operation, the animals with coronary artery ligation were randomly divided into two groups. In one of these groups, 3 mg kg^{-1} trandolapril suspended in 0.25% carboxymethyl cellulose sodium was administered orally from the 2nd to 8th week after the operation. In the other group, 0.25% carboxymethyl cellulose sodium was administered as vehicle. A preliminary study showed that vehicle treatment *per se* did not affect haemodynamic parameters of the rat with CHF. Sham-operated rats were treated with either the agent or vehicle in the same manner as above. Each group was divided into three subgroups for measurement of (1) systemic blood pressure, left ventricular developed pressure (LVDP) and heart rate, (2) aortic flow, and (3) isometric tension development and tissue cyclic GMP content.

Measurement of haemodynamic variables

Cardiac and haemodynamic parameters of rats were measured by the method described previously (Sanbe *et al*., 1993). Briefly, 8 weeks after the operation, rats were anaesthetized with nitrous oxide, oxygen (3 : 1) and 0.5 to 2.5% (v/v) halothane. The rats were warmed by an electronic panel heater to maintain their rectal temperature at 36 to 37°C. A microtip pressure transducer (model SPC 320, Miller Instrument, Houston, Texas) was introduced into the left ventricle through the right carotid artery to measure left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP). The pressure transducer was connected to a carrier amplifier (model AP-621G, Nihon kohden, Tokyo, Japan). The arterial blood pressure was measured through a cannula (polyethylene tube, ATOM Intravenous Catheter For Cut-Down, 3Fr, Atom Co. Ltd., Tokyo, Japan) in the right femoral artery attached to a pressure transducer (model DX-360, Nihon kohden, Tokyo, Japan) and another carrier amplifier (model AP-621G, Nihon kohden, Tokyo, Japan). Heart rate measurements were triggered from changes in arterial blood pressure (model AT-601G, Nihon Kohden, Tokyo, Japan). After equilibration for 10 min, the parameters were recorded on a thermal pen recorder (model RTA-1200, Nihon Kohden, Tokyo, Japan).

In another series of experiments, we determined aortic blood flow and cardiac output index of the rat with myocardial infarction, according to a method described previously (Sanbe *et al*., 1993). Briefly, 8 weeks after coronary artery ligation, the rat was anaesthetized with a gas mixture with nitrous oxygen, oxygen and halothane as described above. After dissection of the right thorax, an electromagnetic flow meter (model MFV-3100, Nihon Kohden, Tokyo, Japan) with a diameter of 2 to 2.5 mm, was placed around the ascending aorta to measure aortic blood flow. Cardiac output index was calculated by dividing aortic blood flow by body weight.

Determination of myocardial infarction

To determine myocardial infarction areas of coronary artery-ligated rats, myocardial slices were stained by 2,3,5-triphenyltetrazolium chloride (TTC), according to a modified method of Sanbe *et al*. (1993). Briefly, the slices were incubated with 1% TTC in saline at 37°C for 15 min and the infarcted areas were determined according to the planimetric method.

Measurement of tension development of aorta

Tension development of aortic segments were measured according to the methods described previously (Nasa *et al*., 1996). Eight weeks after the operation, the rat was anaesthetized with intraperitoneal injection of sodium pentobarbitone (50 mg kg^{-1}) and the thoracic aorta was isolated and placed in an oxygenated Krebs-Henseleit (KH) solution. The KH solution consisted of the following composition (mM): NaCl 120, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, Na_2-EDTA 0.05, $NaHCO_3$ 25 and glucose 11, pH 7.4. Aortic tissues were cut into rings at first and then cut open. Aortic segments, 6–7 mm in length and 3–4 mm in width were obtained. Care

was taken to avoid excess stretching and rubbing of the intimal surface of aortic segments. To remove the endothelium, the intimal surface was rubbed three times with a cotton stick. Aortic segments were then mounted in 30 ml organ baths filled with KH solution. Bath temperature was maintained at 37°C and the KH solution was continuously oxygenated with a gas mixture of 95% O_2 and 5% CO_2. The segments were loaded with 1.0 g of resting tension. Isometric tension development in aortic segments were measured by a force displacement transducer (TB-612T, Nihon, Kohden, Tokyo, Japan) and recorded on a pen-recorder (R-62, Rika-denki, Tokyo, Japan). After being mounted in an organ chamber, aortic segments were equilibrated for 1 h with repeated adjustments of resting tension until baseline levels were stabilized. Removal of the endothelium was assessed by ensuring the lack of relaxant response to 10 μM acetylcholine in each aortic segment precontracted with 0.1 μM noradrenaline.

Aortic segments with and without endothelium were contracted by the addition of 0.1 μM noradrenaline. Noradrenaline at a concentration of 10 μM produced a maximal contraction in rat aortic segments, whereas 0.1 μM noradrenaline elicited approximately 90% or greater of the maximal contraction. When the contractile response to noradrenaline was stable, either acetylcholine (0.01 – 10 μM), isoprenaline (0.01 – 10 μM), sodium nitroprusside (SNP; 0.001 – 10 μM), or colforsin dapropate hydrochloride (NKH477), a water-soluble forskolin, a stimulator of adenylate cyclase (0.001 – 1 μM), was cumulatively added into the bath medium to induce relaxation. In the case of the relaxant response to dibutyryl cyclic AMP, a single concentration of 30 μM was applied to the bath and changes in developed tension were recorded. At the final stage of the experiment, the maximal relaxation was assessed by addition of 10 μM SNP. All relaxation responses were expressed as percentages of the maximum response to SNP (Crack & Cocks, 1992). Each aortic segment was exposed to less than 4 agonists in the present study. Relaxant responses to isoprenaline were also produced in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 0.1 mM) to inhibit nitric oxide synthase.

Cyclic GMP content

The basal tissue cyclic GMP content was determined in aortic segments of these three groups. NO released from endothelial cells stimulates the soluble isoenzyme of guanylate cyclase in the vascular smooth muscle, causing an increased production of cyclic GMP. Therefore, measurement of cyclic GMP levels is an indication of NO production (Furchgott & Vanhoutte, 1989). Each aortic segment was incubated in 10 ml of KH solution oxygenated with a mixture of 95% O_2 and 5% CO_2 for 1 h at 37°C. The tissue was then quickly frozen in liquid nitrogen and stored in liquid nitrogen before biochemical analysis was performed. The frozen tissues were homogenized in ice-cold 6% trichloroacetic acid and samples were sonicated for 20 s with a sonicator (Sonifier 250, Branson, Danbury, CT, U.S.A.). The homogenate was centrifuged and the supernatant solution was washed with water-saturated ether. Cyclic GMP levels in the supernatant fluid were measured by commercially available enzyme immunoassay kits (Amersham cGMP assay system, RPN.226). The residue remaining after the centrifugation was hydrolyzed with 2 M NaOH and its protein content was determined by the method of Lowry *et al*. (1951), with bovine serum albumin as standard.

Drugs

The following agents were used in this study: acetylcholine chloride, (±)-isoprenaline hydrochloride (Nacalai Tesque, Inc., Co. Ltd., Kyoto, Japan), dibutyryl cyclic AMP, N^G-nitro-L-arginine methyl ester (L-NAME, Sigma Chemical Co. Ltd., St. Louis, MO.), 2,3,5-triphenyltetrazolium chloride (TTC), (−)-noradrenaline bitartrate, sodium nitroprusside dihydrate (SNP; Wako Pure Chemical Industries Ltd., Osaka, Japan), trandolapril, and NKH477 (6-(3-dimethyl-aminopropionyl)-forskolin). Trandolapril and NKH477 were generous gifts from Nippon Roussel Co. Ltd. (Tokyo, Japan) and Nippon Kayaku Co. Ltd. (Tokyo, Japan), respectively. All agents used in the present experiment were dissolved in distilled water just before use. Solutions were diluted with KH solution so as to make appropriate final concentrations in the organ bath.

Data analysis

The results are expressed as the means ± s.e.mean. Statistical significance between groups was estimated by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. In some cases two-way ANOVA was applied to determine whether each parameter was affected by CHF and/or treatment with trandolapril and by endothelium and/or L-NAME treatment. P values of less than 0.05 were considered to be statistically significant ($P<0.05$).

Results

Haemodynamic variables

Haemodynamic variables and myocardial infarct size of the rat 8 weeks after coronary artery ligation are shown in Table 1. Mean blood pressure and heart rate of the coronary artery-ligated rats were similar to those in the sham-operated rats. LVSP was lower in the coronary artery-ligated rat than in the sham-operated rat. A marked increase in LVEDP, a typical

Table 1 Haemodynamic variables and myocardial infarcted size of the sham-operated (Sham), coronary artery-ligated (CHF) and trandolapril-treated, coronary artery-ligated rats (CHF + Tra) 8 weeks after the operation

Group	n	*Body wt.* (g)	*MAP* (mmHg)	*Heart rate* (beats min^{-1})	*LVSP* (mmHg)	*LVEDP* (mmHg)	*MI* (%)
Sham	7	311 ± 10	105 ± 4	384 ± 12	140 ± 4	7.5 ± 1.0	ND
CHF	6	292 ± 7	101 ± 5	382 ± 12	126 ± 4*	31.0 ± 1.4*	45.4 ± 1.2
CHF + Tra	7	301 ± 8	78 ± 5†	362 ± 16	107 ± 6†	21.5 ± 2.5†	45.3 ± 1.1

Rats were subjected to coronary artery ligation, and then treated, by oral administration, with 3 mg kg^{-1} day^{-1} trandolapril from the 2nd to 8th week after the operation. Haemodynamic variables were determined at the 8th week. Values are the means ± s.e.mean. Abbreviations: MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; MI, myocardial infarction; ND, not detected. *$P<0.05$ vs sham-operated group; †$P<0.05$ vs CHF group.

symptom of CHF, was detected in the coronary artery-ligated rat. In trandolapril-treated rats with CHF mean blood pressure significantly decreased while heart rate remained unchanged as compared with that of untreated rats with CHF. The increased LVEDP in rats with CHF was significantly attenuated by treatment with trandolapril. Approximately 45% of total area was unstained by TTC in hearts of the coronary artery-ligated rats, indicating the development of myocardial infarction at this period. The size of the infarct area of rats with CHF was not altered by treatment with trandolapril.

In another set of experiments, aortic blood flows and cardiac output indices of sham-operated rats, rats with CHF and trandolapril-treated rats with CHF were determined 8 weeks after coronary artery ligation (Figure 1). Aortic blood flow and cardiac output index were significantly reduced in rats with CHF (38.5 ± 3.6 ml min^{-1} vs 55.0 ± 3.0 ml min^{-1} for sham-operated rats, $P<0.05$ and 125 ± 8 ml min^{-1} kg^{-1} vs 189 ± 10 ml min^{-1} kg^{-1} for sham-operated rats, $P<0.05$, respectively). This reduction in aortic flow and cardiac output index was reversed by treatment with trandolapril (54.1 ± 3.4 ml min^{-1} and 183 ± 12 ml min^{-1} kg^{-1}, respectively).

Relaxant response to acetylcholine

Relaxant responses to acetylcholine are shown in Figure 2a. Acetylcholine caused a concentration-dependent relaxation in endothelium-intact aortic segments of the sham-operated rat. Acetylcholine-induced relaxation was significantly attenuated in endothelium-intact aortic segments of rats with CHF (i.e., the maximal response to 10 μM acetylcholine was $64.0 \pm 5.7\%$ vs $86.9 \pm 1.9\%$ for sham-operated rats, $P<0.05$). This diminished relaxant response to acetylcholine was completely prevented by treatment with trandolapril (the maximal relaxation to 10 μM acetylcholine was $94.7 \pm 1.0\%$). Treatment with trandolapril did not alter acetylcholine-induced relaxation in sham-operated rats. Acetylcholine did not induce any relaxation in endothelium-denuded aortic segments of all groups (data not shown).

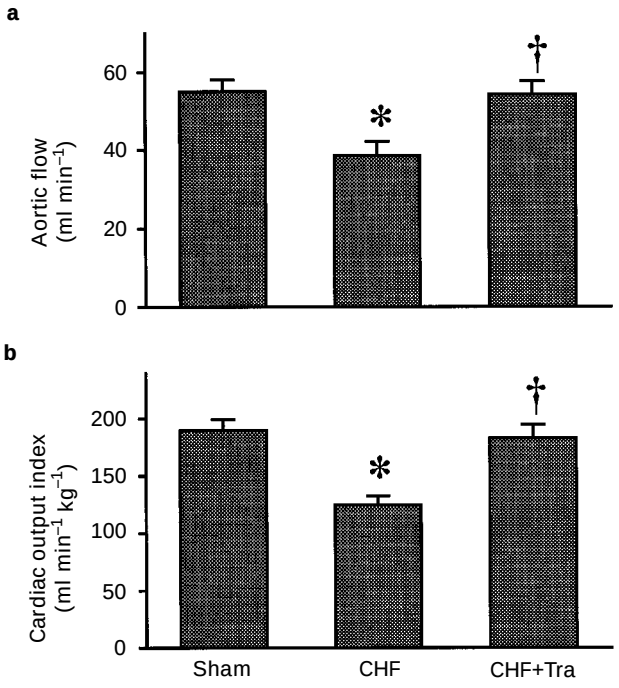

Figure 1 Aortic flow (a) and cardiac output index (b) of sham-operated rats (Sham, $n=7$), untreated rats with chronic heart failure (CHF, $n=6$), and trandolapril-treated rats with CHF (CHF + Tra, $n=7$). Rats were subjected to coronary artery ligation, and then treated, by oral administration with 3 mg kg^{-1} day^{-1} trandolapril from the 2nd to 8th week after the operation. Haemodynamic variables were determined at the 8th week. Data are shown as means ± s.e.mean. *$P<0.05$ vs Sham; †$P<0.05$ vs CHF.

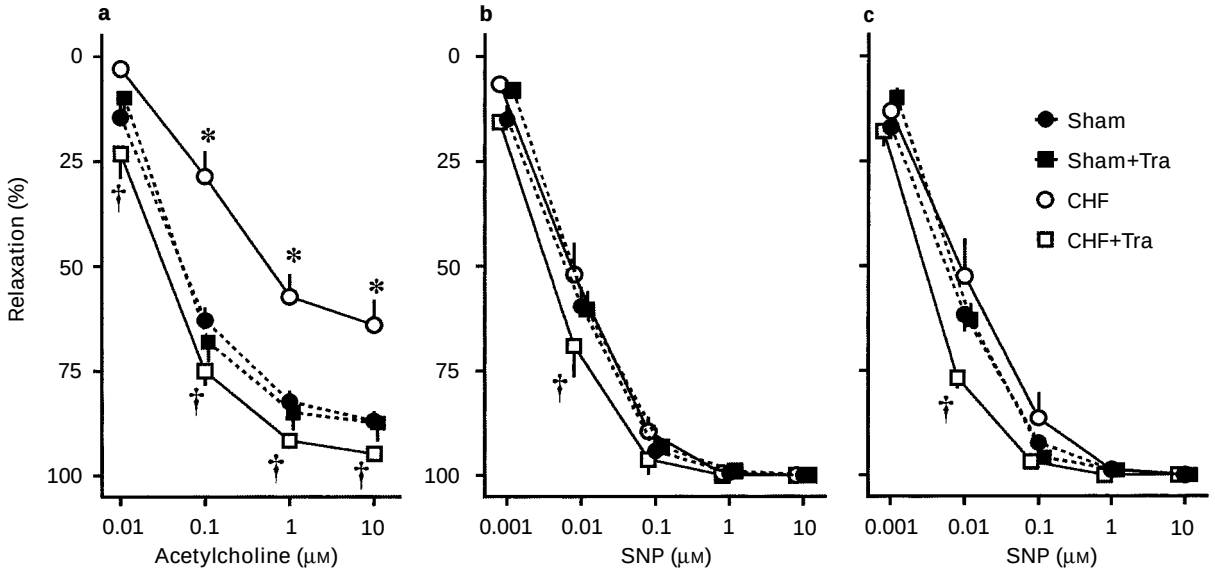

Figure 2 Concentration-response curves for acetylcholine-induced relaxation of endothelium-intact (a) aortic segments and sodium nitroprusside (SNP)-induced relaxation in endothelium-intact (b) and endothelium-denuded (c) aortic segments precontracted with noradrenaline (0.1 μM) of sham-operated rats (Sham), trandolapril-treated, sham-operated rats (Sham + Tra), untreated rats with chronic heart failure (CHF), and trandolapril-treated rats with CHF (CHF + Tra). Rats were subjected to coronary artery ligation and then treated by oral administration with 3 mg kg^{-1} day^{-1} trandolapril from the 2nd to 8th week after the operation. Values of initial contraction induced by noradrenaline in Sham, Sham + Tra, CHF, and CHF + Tra groups of each panel were 0.56 ± 0.05, 0.52 ± 0.06, 0.66 ± 0.07 and 0.56 ± 0.04 g (a), 0.62 ± 0.04, 0.58 ± 0.06, 0.71 ± 0.06 and 0.66 ± 0.04 g (b), and 0.57 ± 0.06, 0.59 ± 0.05, 0.57 ± 0.06 and 0.48 ± 0.07 g (c), respectively. Data are shown as mean ($n=6-12$ animals per group); vertical lines show s.e.mean. Symbols without s.e.mean; s.e.mean was less than 2%. *$P<0.05$ vs Sham; †$P<0.05$ vs CHF.

Relaxant response to sodium nitroprusside (SNP)

Relaxant responses to SNP are shown in Figure 2b and c. SNP caused a concentration-dependent relaxation in both endothelium-intact and endothelium-denuded aortic segments of the sham-operated rat to a similar degree. The maximal relaxant response to SNP, that is, the magnitude of decreased tension from baseline levels in aortic segments of sham-operated rats was not different from that of rats with CHF. SNP-induced relaxation was unaffected by CHF. In sham-operated rats, trandolapril treatment did not alter the SNP-induced relaxation. Treatment of rats with CHF with trandolapril only significantly enhanced the relaxation to 10 μM SNP.

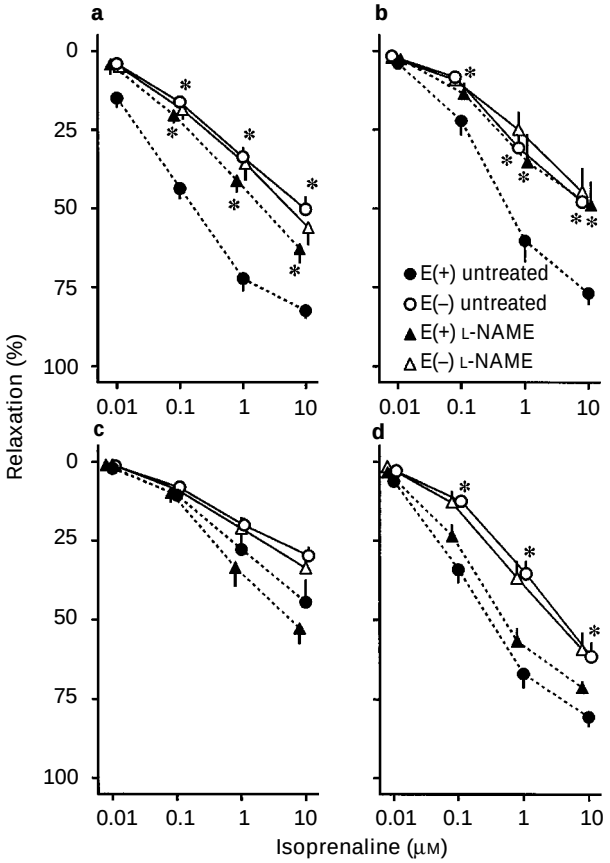

Figure 3 The effect of N^G-nitro-L-arginine methyl ester (L-NAME) on the isoprenaline-induced relaxation of endothelium-intact aortic segments precontracted with noradrenaline (0.1 μM) of sham-operated rats (a), trandolapril-treated, sham-operated rats (b), untreated rats with chronic heart failure (CHF; c) and trandolapril-treated rats with CHF (d). Responses of endothelium-intact aortic segments without L-NAME treatment (E(+) untreated), endothelium-denuded aortic segments without L-NAME treatment (E(−) untreated), endothelium-intact aortic segments with L-NAME treatment *in vitro* (E(+) L-NAME), and endothelium-denuded aortic segments with L-NAME treatment *in vitro* (E(−) L-NAME) are shown. The operation and trandolapril treatment were the same as those in Figure 2. L-NAME (0.1 mM) was applied to isolated vessels 10 min before addition of noradrenaline. Values of initial contraction induced by noradrenaline in E(+) untreated, E(−) untreated, E(+) L-NAME, and E(−) L-NAME treatment groups of each panel were 0.52 ± 0.04, 0.55 ± 0.05, 0.68 ± 0.07 and 0.66 ± 0.05 g (a), 0.50 ± 0.06, 0.58 ± 0.06, 0.68 ± 0.07 and 0.60 ± 0.04 g (b), 0.64 ± 0.07, 0.60 ± 0.05, 0.70 ± 0.08 and 0.65 ± 0.06 g (c), and 0.54 ± 0.06, 0.59 ± 0.05, 0.57 ± 0.06 and 0.55 ± 0.03 g (d), respectively. Data are shown as means ($n=6-13$ animals per group); vertical lines show s.e.mean. Symbols without s.e.mean; s.e.mean was less than 2%. $^*P<0.05$ vs E(+) untreated.

Relaxant response to isoprenaline

Relaxant responses to isoprenaline are shown in Figure 3. In both untreated and trandolapril-treated sham-operated rats, removal of endothelium and treatment with L-NAME diminished the relaxant response to isoprenaline. L-NAME did not affect the relaxation to isoprenaline in endothelium-denuded segments of these two groups. As compared with sham-operated rats, isoprenaline-induced relaxation of rats with CHF was markedly reduced in both endothelium-intact and endothelium-denuded aortic segments; the maximal relaxation to 10 μM isoprenaline was $44.5\pm6.7\%$ vs $81.3\pm2.5\%$ for sham-operated rats ($P<0.05$), $29.8\pm2.5\%$ vs $50.3\pm3.7\%$ for sham-operated rats ($P<0.05$), respectively. Unlike sham-operated rats, L-NAME did not attenuate the isoprenaline-induced relaxation in rats with CHF. In trandolapril-treated rats with CHF, the relaxant response to isoprenaline was similar to that of sham-operated rats; the maximal relaxation to 10 μM isoprenaline in endothelium-intact and endothelium-denuded segments was $80.5\pm2.8\%$ and $60.5\pm4.3\%$, respectively. In this group, isoprenaline-induced relaxation was diminished by removal of endothelium but not by L-NAME treatment. Thus, although chronic treatment with trandolapril restored the diminished relaxant response to isoprenaline in CHF, the L-NAME-inhibitable component of the isoprenaline-induced relaxation was not fully restored.

Relaxant response to NKH477

Relaxant responses to NKH477 are shown in Figure 4. NKH477 produced a slowly developing, concentration-dependent decrease in tension in both endothelium-intact and endothelium-denuded aortic segments. In sham-operated rats, NKH477-induced relaxation was diminished by removal of the endothelium. In rats with CHF, relaxant responses to

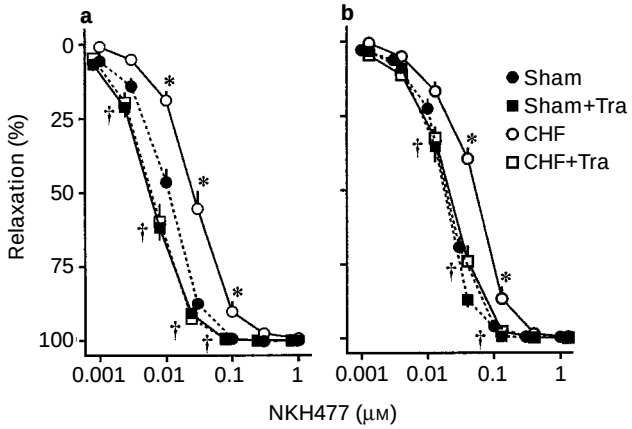

Figure 4 Graphs showing concentration-response curves for NKH477-induced relaxation of endothelium-intact (a) and endothelium-denuded (b) aortic segments precontracted with noradrenaline (0.1 μM) of sham-operated rats (Sham), trandolapril-treated, sham-operated rats (Sham + Tra), untreated rats with chronic heart failure (CHF), and trandolapril-treated rats with CHF (CHF + Tra). The operation and drug treatment were the same as those in Figure 2. Values of initial contraction induced by noradrenaline in Sham, Sham + Tra, CHF and CHF + Tra groups of each panel were 0.63 ± 0.06, 0.59 ± 0.06, 0.63 ± 0.07 and 0.59 ± 0.05 g (a) and 0.64 ± 0.06, 0.61 ± 0.06, 0.68 ± 0.07 and 0.59 ± 0.06 g (b) respectively. Data are shown as means ($n=7-11$ animals per group); vertical lines show s.e.mean. Symbols without s.e.mean; s.e.mean was less than 2%. $^*P<0.05$ vs Sham, †$P<0.05$ vs CHF.

NKH477 at concentrations ranging from 0.01 to 0.1 μM were significantly attenuated in endothelium-intact aortic segments, although the maximal relaxation was achieved at the highest concentration used (1 μM) in all aortic segments studied. Long-term treatment with trandolapril almost completely reversed the diminished relaxation in both the endothelium-intact and endothelium-denuded segments of rats with CHF.

Relaxant response to dibutyryl cyclic AMP

Relaxant responses to 30 μM dibutyryl cyclic AMP are shown in Figure 5. We measured the time course of changes in the relaxant response to 30 μM dibutyryl cyclic AMP in the three groups. Dibutyryl cyclic AMP at concentrations of 0.3 μM to 10 μM elicited a slowly developed relaxation (2.1 ± 1.1 to 38.3 ± 3.6%, respectively) which was sustained for 1 h or longer in both endothelium-intact and endothelium-denuded aortic segments. In endothelium-intact segments of rats with CHF, relaxation to 30 μM dibutyryl cyclic AMP was significantly reduced (26.8 ± 2.7%) compared to that in segments of sham-operated rats (63.4 ± 11.8%). The reduced vascular relaxation in rats with CHF to dibutyryl cyclic AMP was reversed by long-term treatment with trandolapril (54.7 ± 6.2%). Removal of the endothelium significantly attenuated the relaxation to dibutyryl cyclic AMP in both sham-operated rats and trandolapril-treated rats with CHF.

Cyclic GMP contents

Basal cyclic GMP content in aortic segments of rats with CHF was significantly lower than that of sham-operated rats. The decrease in cyclic GMP content of rats with CHF was significantly attenuated by treatment with trandolapril (Figure 6).

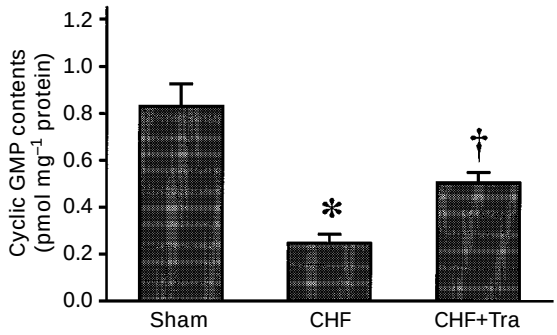

Figure 6 The basal cyclic GMP contents in aortic segments of sham-operated rats (Sham, $n=9$), untreated rats with chronic heart failure (CHF, $n=7$), and trandolapril-treated rats with CHF (CHF + Tra, $n=11$). The operation and drug treatment were the same as those in Figure 2. Data are shown as means $\pm$ s.e.mean. $^*P<0.05$ vs Sham; $\dagger P<0.05$ vs CHF.

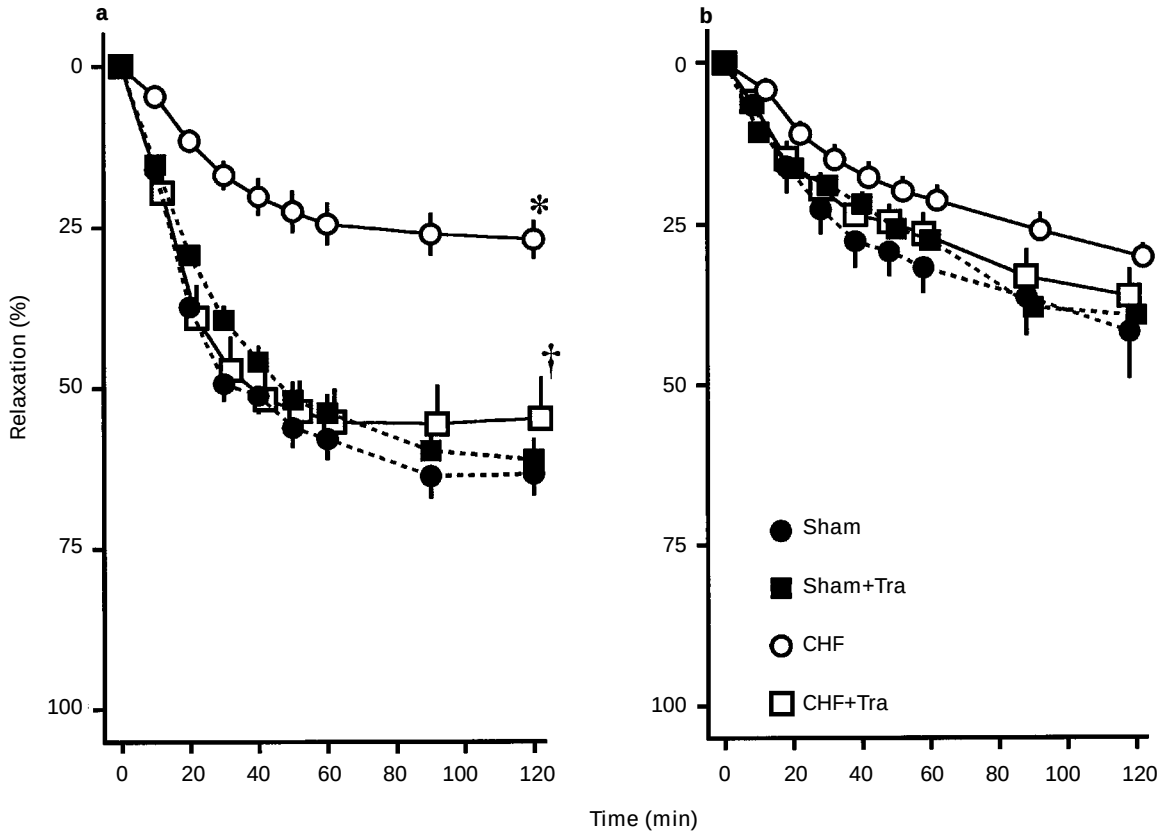

Figure 5 Graphs showing the time course of changes in 30 μM dibutyryl cyclic AMP-induced relaxation of endothelium-intact (a) and endothelium-denuded (b) aortic segments precontracted with noradrenaline (0.1 μM) of sham-operated rats (Sham), trandolapril-treated, sham-operated rats (Sham + Tra), untreated rats with chronic heart failure (CHF), and trandolapril-treated rats with CHF (CHF + Tra). The operation and drug treatment were the same as those in Figure 2. Values of initial contraction induced by noradrenaline in Sham, Sham + Tra, CHF and CHF + Tra groups were 0.61 ± 0.09, 0.58 ± 0.05, 0.62 ± 0.07 and 0.60 ± 0.05 g (a) and 0.68 ± 0.06, 0.66 ± 0.08, 0.56 ± 0.07 and 0.59 ± 0.08 g (b) respectively. Data are shown as means ($n=4-11$ animals per group); vertical lines show s.e.mean. Symbols without s.e.mean; s.e.mean was less than 2%. $^*P<0.05$ vs Sham; $\dagger P<0.05$ vs CHF.

Discussion

In the present study, we observed that relaxation to acetylcholine was diminished in endothelium-intact aortic segments of rats 8 weeks after coronary artery ligation (Figure 2). This is compatible with the findings of other investigators in peripheral vessels of canines with heart failure induced by rapid pacing (Kaiser *et al*., 1989) and in vessels of patients with severe heart failure (Kubo *et al*., 1991; Katz *et al*., 1992; Drexler *et al*., 1993). Endothelial dysfunction seems to contribute to the altered vascular response to acetylcholine in rats with CHF (Ontkean *et al*., 1991; Lindsay *et al*., 1992; Teerlink *et al*., 1993; Nasa *et al*., 1996). In contrast, SNP-induced relaxation remained unchanged in arteries of rats with CHF in the present study (Figure 2). SNP releases NO and increases cyclic GMP in smooth muscle cells (Ignarro *et al*., 1980). Thus, responsiveness of the vascular smooth muscle cell to NO does not appear to be impaired. Our findings suggest that release of NO following activation of muscarinic receptors in endothelial cells is blunted, whereas the cyclic GMP-mediated signal transduction system and contractile apparatus in vascular smooth muscle cells are functionally preserved in rats with CHF. Mathew *et al*. (1993) have shown the preservation of cyclic GMP-mediated relaxation and reduction of cyclic AMP-mediated relaxation in pulmonary arteries of dogs with heart failure induced by rapid pacing. These observations suggest that there are at least two signal transduction systems for relaxation in arteries, which possess different susceptibilities to heart failure.

In general, isoprenaline-induced relaxation responses are not dependent on the presence of the endothelium (Moncada *et al*., 1991). The present study showed that isoprenaline-induced relaxation of the aortic segment was, at least in part, endothelium-dependent because the relaxant response of the aortic segment of sham-operated rats to isoprenaline was markedly attenuated by removal of endothelium (Figure 3). Furthermore, we observed that in the sham-operated rat relaxant responses to the adenylate cyclase activator NKH477 and membrane permeable cyclic AMP analogue dibutyryl cyclic AMP were reduced in endothelium-denuded aortic segments, as compared with those in endothelium-intact aortic segments (Figures 4 and 5). These findings suggest that adenylate cyclase activity and cyclic AMP formation in endothelial cells play a role in eliciting the isoprenaline-induced relaxation in the normal rat aorta. In addition, since the relaxant response to isoprenaline was attenuated by the NO synthase inhibitor L-NAME in endothelium-intact aortic segments of the sham-operated rat (Figure 3), it is likely that endothelial NO may contribute to the relaxation. This is compatible with the hypotheses that, in vascular tissue, there is a link between increased cyclic AMP and activation of NO synthase in endothelial cells (Gray & Marshall, 1992) and an increase in endothelial cyclic AMP amplifies an agonist-induced rise in intracellular calcium, which secondarily enhances the release of NO (Graier *et al*., 1992).

The cyclic AMP-mediated signal transduction mechanism seems to be critical in the altered vascular response in rats with CHF (Nasa *et al*., 1996). We observed that relaxant responses to isoprenaline and NKH477 were diminished in endothelium-intact aortic segments of rats with CHF as compared with those of sham-operated rats (Figures 3 and 4). In addition, these responses were further diminished in endothelium-denuded aortic segments of the rat with CHF. Since endothelium-independent relaxation to SNP was not altered in CHF (Figure 2), decreased responsiveness of the contractile apparatus of vascular smooth muscle cells is not a cause of the diminished relaxation in endothelium-denuded aortic segments. Thus, the function of the β-adrenoceptor and adenylate cyclase in both endothelial and smooth muscle cells may be diminished in CHF. Furthermore, it is likely that the mechanism underlying the diminished relaxation to isoprenaline in CHF is attributed to dysfunction of adenylate cyclase rather than that of the β-adrenoceptor, because the relaxation induced by the adenylate cyclase activator, NKH477, was also reduced (Figure 4).

In contrast to the reduced relaxation to isoprenaline and NKH477, in both endothelium-intact and endothelium-denuded segments of rats with CHF, relaxation to dibutyryl cyclic AMP was attenuated only in endothelium-intact segments. This finding suggests that the cyclic AMP-mediated signal transduction pathway is impaired in endothelial, but not smooth muscle cells in CHF. This may be attributed in part to the reduced production of NO in endothelial cells as suggested above. Taken together, it appears that β-adrenoceptors and the adenylate cyclase system in endothelial and smooth muscle cells are impaired, while the cyclic AMP-initiated signal transduction in vascular smooth muscle cells is preserved in CHF.

The most notable finding in the present study is that the diminished relaxation to vasodilators in aortic segments of the rat with CHF was almost completely restored by chronic treatment with trandolapril. ACE inhibitors are known to have beneficial effects in patients and animals with CHF (Pfeffer *et al*., 1985; Guyatt, 1986; Richer *et al*., 1992). Despite wide use of ACE inhibitors for treatment of CHF, the effects of ACE inhibitors on vascular responses in CHF are not well understood. ACE inhibitors act mainly via inhibition of circulating ACE or local vascular ACE, resulting in a decreased production of angiotensin II (Hirsch *et al*., 1990; 1991). Furthermore, several lines of evidence suggest that ACE inhibitors affect the ability of endothelial cells to produce NO *in vitro* (Wiemer *et al*., 1991; Mombouli *et al*., 1992). ACE inhibitors also decrease the degradation of bradykinin by kininase II and the resultant increase in bradykinin secondarily enhances production and/or release of NO (Wiemer *et al*., 1991; Feletou *et al*., 1992). Therefore, long-term treatment of rats with CHF with trandolapril appears to restore, at least in part, vascular endothelial production of NO by local accumulation of endothelium-derived bradykinin. This is compatible with the finding that trandolaprilat, an active form of trandolapril, enhances endothelium-dependent, bradykinin-induced relaxation of canine coronary and femoral arteries (Vidal & Vanhoutte, 1988). However, we did not confirm the possible contribution of bradykinin to the effect of long-term treatment with trandolapril on CHF in the present study.

In this study, the diminished cyclic AMP-mediated relaxation response in aortic segments of rats with CHF was restored by treatment with trandolapril (Figures 3, 5 and 6). This finding is notable because it has been observed that β-adrenoceptor-mediated relaxation in normal rat aorta is not affected by captopril (Kikta & Fregly, 1982). Furthermore, in sham-operated rats treatment with trandolapril did not affect the relaxant response to isoprenaline. The present results suggest that the endothelium-dependent portion of the β-adrenoceptor-mediated relaxation is restored by treatment with trandolapril, through increased responsiveness of β-adrenoceptor and/or the receptor-linked signal transduction in endothelial cells.

Our findings showed that L-NAME inhibition of isoprenaline relaxation was attenuated in aortic segments of rats with CHF and this was not completely restored by trandolapril treatment (Figure 4). This suggests that the site of action of L-

NAME, presumably endothelial NO synthase, may still be damaged following ACE inhibitor treatment. This is supported in part by the fact that basal cyclic GMP content was still decreased in trandolapril-treated rats with CHF. Nevertheless, the results demonstrate that the reduced vascular responsiveness to isoprenaline, NKH477 and dibutyryl cyclic AMP in rats with CHF was restored almost to the level of sham-operated rats (Figures 3, 4 and 5). Therefore, it seems likely that restoration of vascular responsiveness was due to both restitution of NO production in endothelial cells and other effects of the ACE inhibitor. Since trandolapril restored endothelium-independent relaxation to isoprenaline and NKH477 (Figures 3 and 4), the ACE inhibitor may also act by enhancing cyclic AMP-mediated signalling in smooth muscle cells.

Another possible mechanism for the effect of trandolapril treatment on vasorelaxation in CHF is a change in the systemic circulation. Chronic reduction of blood flow has been shown to cause a reduction in EDRF production in endothelial cells (Langille & O'Donnell, 1986). Furthermore, an increase in blood flow in femoral arteries of fistula-operated dogs was shown to enhance the production of EDRF (Miller & Vanhoutte, 1988). The current observations are consistent with the relationship between endothelium-mediated vasorelaxation of aortae and aortic blood flow. We observed a reduced aortic blood flow in rats with CHF and restoration of aortic flow by ACE inhibitor treatment (Figure 1). Furthermore, recent observations have suggested that ACE inhibition enhances flow-dependent, endothelium-mediated vasodilatation by a bradykinin-dependent mechanism (Hornig *et al*., 1997). Thus, the restoration of blood flow may have contributed to the protection of endothelial function in CHF. Inversely, improved endothelial function may have led to an overall improvement of the systemic circulation and cardiac contractility. The present study did not address the cause-effect relationship between these two variables.

In conclusion, the present study demonstrated that cyclic GMP-mediated vasorelaxation was diminished in aortic segments of rats with CHF. This was mainly due to endothelial dysfunction. Isoprenaline-induced, cyclic AMP-mediated vasorelaxation was also diminished in the aortae of rats with CHF, apparently due to reduced endothelial NO production and dysfunction of the adenylate cyclase in smooth muscle cells. Since chronic treatment with trandolapril reversed the diminished vascular relaxation and normalized aortic blood flow in CHF, the mechanism underlying this reversal may also be attributed in part to restoration of the endothelial NO production.

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(Received June 10, 1996
Revised April 28, 1997
Accepted October 24, 1997)