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European Journal of Pharmacology 415 (2001) 209–216

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# Release of endothelial nitric oxide in coronary arteries by celiprolol, a $\beta_1$-adrenoceptor antagonist: possible clinical relevance

Kumiko Noda, Michiko Oka, Fu-H. Ma, Satoru Kitazawa, Yojiro Ukai, Noboru Toda*

*Research Laboratories, Nippon Shinyaku Co., Ltd., 14 Nishinosho-Monguchi-cho, isshoin, Minami-ku, Kyoto 601-8550, Japan*

Received 14 July 2000; received in revised form 22 January 2001; accepted 26 January 2001

**Abstract**

Mechanisms underlying celiprolol-induced vasodilatation were analyzed in isolated porcine coronary arteries. Celiprolol induced dose-related relaxation of the artery rings with endothelium, an effect which was suppressed by $N^G$-nitro-L-arginine methylester (L-NAME), nitric oxide (NO) scavenger, guanylate cyclase inhibitor, endothelium denudation, and removal of $Ca^{2+}$. L-NAME contracted, and superoxide dismutase relaxed, the arteries only when the endothelium was preserved. Neither superoxide dismutase nor β-adrenoceptor antagonists changed celiprolol-induced relaxations. Celiprolol increased the cyclic GMP content in the tissue. The release of NO from endothelium, estimated by the extracellular production of cyclic GMP in arteries incubated in medium containing guanylate cyclase and GTP, was augmented by celiprolol, and L-NAME abolished this action of celiprolol. It is concluded that celiprolol elicits relaxation by acting on sites other than β-adrenoceptors in the endothelium and by releasing NO, which activates soluble guanylate cyclase in smooth muscle and produces cyclic GMP. Scavenging of superoxide anions from the endothelium does not seem to account for the induced relaxation. 

*Keywords:* Celiprolol; β-Adrenoceptor antagonist; Nitric oxide (NO); Endothelium; Coronary artery

## 1. Introduction

Endothelium-derived relaxing factor, discovered by Furchgott and Zawadzki (1980), is now known to be nitric oxide (NO), endothelium-derived hyperpolarizing factor and prostanoids. The involvement of endothelium-derived NO in the vascular response to chemical stimuli is determined pharmacologically by susceptibility to endothelium denudation, NO synthase inhibitors, NO scavengers and soluble guanylate cyclase inhibitors and also biochemically by increased formation of cyclic GMP in vascular tissues. Many compounds, such as acetylcholine, bradykinin, substance P, histamine, $\beta_2$-adrenoceptor agonists, etc., induce vasodilatation mediated by endothelium-derived NO (Moncada et al., 1991). Recent studies have demonstrated that β-adrenoceptor agonists (Rubanyi and Vanhoutte, 1985; Iranami et al., 1996; Ferro et al., 1999) or antagonists, such as tertatolol and nebivolol (Verbeuren and Herman, 1989; Gao et al., 1991), can also be considered as endothelium-derived NO-releasing substances. NO derived from the endothelium elicits not only vasodilatation but also inhibition of platelet aggregation/adhesion and a decrease of smooth muscle proliferation or low density lipoprotein oxidation, which contribute to the prevention of thrombosis and atherosclerosis.

It is widely recognized that β-adrenoceptor-blocking agents are quite important in the treatment of cardiovascular diseases, including hypertension, angina pectoris and arrhythmia, and in the prophylaxis of attacks of myocardial infarction, although untoward actions, such as a lowered cholesterol ratio and impaired sexual function in men, are induced. β-Adrenoceptor antagonists that possess endothelium-derived NO-releasing actions are expected to augment the hypotensive action and minimize the proatherosclerotic action and impaired quality of life. The French paradox refers to the very low incidence of and mortality from ischemic heart disease in France compared to the US and other European countries, despite a similar diet high in saturated fat and cholesterol (Burr, 1995;

* Corresponding author. Tel.: +81-75-321-9098; fax: +81-75-314-3269.

*E-mail address:* n.toda@po.nippon-shinyaku.co.jp (N. Toda).

0014-2999/01/$ - see front matter 
PII: S0014-2999(01)00803-2

Constant, 1997). The habit of frequent drinking of red wine, which liberates endothelium-derived NO (Fitzpaatrick et al., 1993; Andriambeloson et al., 1997), would account for the paradox. This may also be the case for some drugs that liberate NO.

Therefore, the present study was undertaken to determine the mechanisms of action of celiprolol, a blocker selective for $\beta_1$-adrenoceptors with vasodilator properties (Pittner, 1983), on isolated porcine coronary artery rings with reference to endothelium-derived NO, superoxide scavenging, and β-adrenoceptor stimulation or blockade.

## 2. Materials and methods

### 2.1. Animals

Porcine hearts were obtained at the slaughterhouse. From the freshly excised heart, the left descending and circumflex coronary arteries were isolated and placed in modified Krebs–Ringer bicarbonate solution (pH 7.4) of the following composition (mM): NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $KH_2PO_4$, 1.2; $NaHCO_3$, 25.0 and D-glucose, 11.1. The arteries were cleaned of fat and connective tissues and cut into rings 3–4 mm wide.

### 2.2. Experimental protocol

#### 2.2.1. Measurement of artery mechanical response

The artery rings were carefully prepared to preserve the endothelium. In some rings, the endothelium was removed by gently rubbing the intimal surface with a cotton thread. Endothelium denudation was verified by abolishment of relaxations induced by substance P ($10^{-9}$ M). The rings were mounted between two stainless wires (diameter 200 μm) in an organ bath (20 ml) containing the bathing solution, which was maintained at $37 \pm 0.3$°C and aerated with a mixture of 95% $O_2$ and 5% $CO_2$. One of the wires was fixed, and the other was connected to an isometric force-displacement transducer and a carrier amplifier (Nihon-Kohden Kogyo, Tokyo, Japan). Isometric mechanical responses were displayed on an ink-writing recorder (Nihon-Kohden Kogyo). The resting tension was adjusted to 2.0 g, which was optimal for inducing maximal contractions. Before the start of experiments, the artery rings were allowed to equilibrate for 60 to 90 min in the bathing medium, during which time the fluid was replaced every 10 to 15 min. The contractile response to 30 mM $K^+$ was obtained first, and the arterial rings were washed three times (four washes each time) with fresh media and equilibrated. At the end of each series of experiment, papaverine ($10^{-4}$ M) was applied to attain the maximal relaxation. The artery rings were partially contracted with prostaglandin $F_{2\alpha}$ ($10^{-6}$ to $10^{-5}$ M), the contraction being in a range of 30–50% of that caused by 30 mM $K^+$. Relaxant and contractile responses induced by test drugs are expressed as relative values to the papaverine-induced relaxation and the $K^+$ (30 mM)-induced contraction, respectively. The drugs were applied directly to the bathing medium in cumulative concentrations. The preparations were treated for 20 to 30 min with blocking agents.

#### 2.2.2. Measurement of cyclic GMP

Isolated coronary arteries of similar size with an intact endothelium were separated into two groups and incubated for 60 min in tubes containing modified Krebs–Ringer bicarbonate solution, which was maintained at 37°C and aerated with a mixture of 95% $O_2$ and 5% $CO_2$. The arteries were treated for 5 min with the control bathing medium and medium containing celiprolol ($3 \times 10^{-4}$ M). The tissues were quickly frozen in liquid nitrogen and homogenized in 1.5 ml of ice-cold 6% trichloroacetic acid with a Polytron (PT 3000, Kinematica, Littau, Switzerland) and centrifuged at $1800 \times g$ for 10 min. The protein content of the pellet dissolved in 1 ml of 1 N NaOH was determined by the method of Bradford (1976), using bovine serum albumin as standard. Each supernatant fraction was extracted three times with 2 ml of ice-cold water-saturated ether. The resulting residue was stored overnight at 4°C and used for measurement of the cyclic GMP level with an enzyme immunoassay kit (Amersham, Buckinghamshire, UK).

#### 2.2.3. Measurement of NO release

NO released from porcine coronary artery rings with an intact endothelium was estimated from the cyclic GMP level in the extracellular fluid after the addition of GTP, 3-isobutyl-1-methylxanthine (IBMX) and a soluble fraction of crude extract from the rat cerebellum. The soluble fraction was prepared immediately before use as follows: the rat cerebellum was dissected and homogenized with 10 volumes of ice-cold buffer (50 mM Tris–HCl containing 1 mM EDTA, 1 mM dithiothreitol and 200 mM phenylmethylsulfonyl fluoride) using a Polytron, then the homogenate was centrifuged at $30{,}000 \times g$ for 30 min and the resultant supernatant fraction was used as a source of guanylate cyclase. The procedure for the measurement of NO was as described previously (Oka et al., 2000a,b). Briefly, pieces of coronary artery ring (1.5 mm wide) were placed in a 24-well plastic plate containing 1 ml of Krebs–Ringer bicarbonate solution and pre-incubated at 37°C for 1 h under continuous aeration with a mixture of 95% $O_2$ and 5% $CO_2$. After the pre-incubation, the incubation medium was replaced by freshly prepared modified Krebs–Ringer bicarbonate solution to which 0.5 mM GTP, 10 μM IBMX and a 20-μl aliquot of supernatant fraction of the rat cerebellum (containing approximately 80 μg protein) were added, and the artery ring was incubated further for 10 or 30 min in the absence or presence of test drugs. The cyclic GMP produced in the incubation medium

was measured by using a cyclic GMP enzyme immunoassay kit. A similar procedure was performed in the presence of $10^{-4}$ M $N^G$-nitro-L-arginine methylester (L-NAME), and the difference in the extracellular cyclic GMP content determined in the absence and presence of L-NAME was regarded as the amount of NO released. At the end of experiments, tissues were dissolved in 1 ml of 1 N NaOH and the protein content was determined by the method of Bradford (1976), using bovine serum albumin as standard.

### 2.3. Statistical analysis

The results shown in the text and figures are expressed as means ± S.E. Statistical analyses were done with Student's paired or unpaired *t*-test for two groups and with Tukey's test after one-way analysis of variance for three or more groups.

### 2.4. Chemicals

Drugs used were celiprolol (Nippon Shinyaku, Kyoto, Japan), $N^G$-nitro-L-arginine methylester (L-NAME), L-arginine, D-arginine, capsaicin (Nacalai Tesque, Kyoto), carboxy-PTIO (carboxy-[2-phenyl-4,4,5,-tetramethylimidazoline-1-oxyl-3-oxide], Dojindo, Kumamoto, Japan), cyanopindolol hemifumarate (Tocris Cookson, Ballwin, MO), prostaglandin $F_{2\alpha}$ (Funakoshi, Tokyo), butoxamine hydrochloride, 1*H*-(1,2,4) oxadiazolo (4,3-*a*) quinoxalin-1-one (ODQ), 3-isobutyl-1-methylxanthine (IBMX), L-isoproterenol bitartrate, indomethacin, superoxide dismutase, sodium nitroprusside dihydrate, substance P, atropine sulfate (Sigma, St. Louis, MO), and papaverine hydrochloride (Tokyo Kasei, Tokyo). Other chemicals were all of guaranteed grade.

## 3. Results

### 3.1. Mechanical response to celiprolol

In porcine coronary artery rings partially contracted with prostaglandin $F_{2\alpha}$, the addition of celiprolol ($10^{-5}$ to $3 \times 10^{-4}$ M) induced a concentration-related relaxation. Removal of the endothelium or treatment with $10^{-4}$ M L-NAME abolished the response to celiprolol at doses up to $10^{-4}$ M and markedly inhibited the relaxation at $3 \times 10^{-4}$ M (Fig. 1). The substance P ($10^{-9}$ M)-induced relaxation (86.9 ± 2.0% of the papaverine-induced relaxation, $n = 16$) was also abolished by endothelium-denudation. The dose–response curve of celiprolol was reproducible. Typical tracings of the response to celiprolol before and after L-NAME are illustrated in Fig. 2, upper tracings. A similar magnitude of inhibition was also obtained with $10^{-5}$ M L-NAME, and the response was completely restored by the addition of L-arginine ($10^{-3}$ M) (Fig. 3, upper left). D-NAME ($10^{-5}$ M) was without effect ($n = 5$). Attenuation of the response to celiprolol by carboxy PTIO ($3 \times 10^{-4}$ M) and ODQ ($10^{-6}$ M) or by removal of $Ca^{2+}$ from the bathing medium is summarized in Fig. 3. Indomethacin ($10^{-6}$ M) did not alter the response ($n = 5$). Treatment with celiprolol ($10^{-4}$ M) did not affect the relaxation induced by sodium nitroprusside; mean EC50 values before and after treatment were 6.33 ± 3.18 and 2.66 ± 0.82 × $10^{-7}$ M ($n = 6$), respectively.

The relaxant response to celiprolol ($10^{-5}$ to $3 \times 10^{-4}$ M) was reproduced after repeated rinsing (3 times, every 10 min), as indicated in Fig. 1, right and Fig. 4, right. In

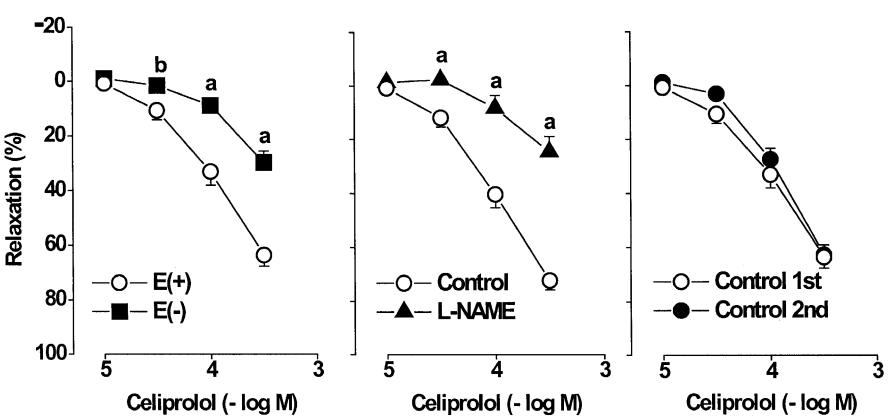


Fig. 1. Concentration–relaxant response curves for celiprolol in porcine coronary artery rings contracted with prostaglandin $F_{2\alpha}$, as affected by endothelium denudation (left figure, E + and −) and L-NAME ($10^{-4}$ M; center). The right figure includes control curves obtained in the 1st and 2nd trials (time control). The ordinate indicates celiprolol-induced relaxations relative to those elicited by $10^{-4}$ M papaverine. Significantly different from E(+) for left figure and from control for middle figure, [a] $P < 0.01$; [b] $P < 0.05$ (unpaired *t*-test). Sixteen rings were obtained from different pigs. Three different series of experiments were performed with the rings from the same pig. Vertical bars represent S.E.

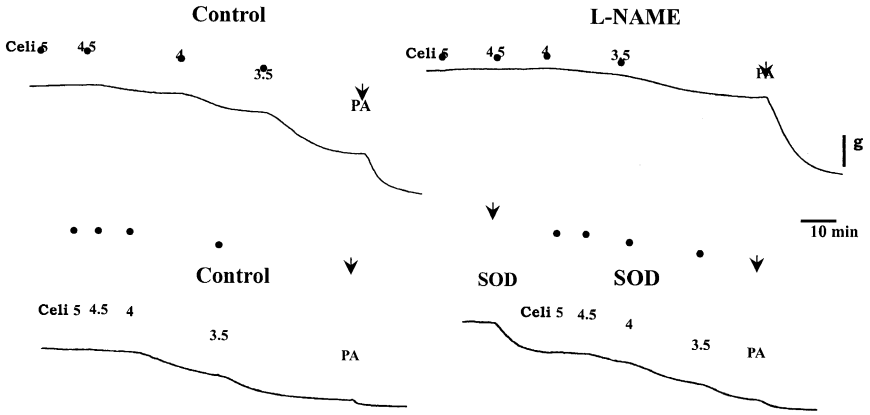


Fig. 2. Tracings of the response to celiprolol (Celi) before and after treatment with L-NAME ($10^{-4}$ M; upper tracing) or superoxide dismutase (SOD, 200 U/ml; lower tracing) in porcine coronary artery rings contracted with prostaglandin $F_{2\alpha}$. Concentrations of celiprolol from 5 to 3.5: $10^{-5}$, $3\times10^{-5}$ ($10^{-4.5}$), $10^{-4}$ and $3\times10^{-4}$ ($10^{-3.5}$) M. PA represents $10^{-4}$ M papaverine, which produced the maximal relaxation.

contrast, the concentration–response curve for isoproterenol was shifted to the right in the rings that had been repeatedly rinsed with the control medium after the dose–response curve for celiprolol up to $3\times10^{-4}$ M had been recorded (Fig. 4, left). The EC50 values of the β-adrenoceptor agonist before and after treatment with celiprolol were $[4.09\pm1.27]\times10^{-8}$ M and $[4.99\pm2.90]\times10^{-6}$ M ($n=4$), respectively, suggesting that the $\beta_1$ adrenoceptor-blocking action of celiprolol remains even after repeated rinsing of the preparations. Isoproterenol-induced relaxations were unaffected by endothelium denudation ($n=4$). Relaxations in response to celiprolol were not reduced by butoxamine (Fig. 5, left) applied in a concentration ($10^{-5}$ M) sufficient to significantly inhibit the response mediated by $\beta_2$ adrenoceptors (Toda and Okamura, 1990). Cyanopindolol ($10^{-6}$ M), a $\beta_3$ adrenoceptor antagonist (Brawley et al., 2000) (Fig. 5, right), atropine ($10^{-6}$ M, $n=4$) and capsaicin ($10^{-6}$ M, $n=4$) did not affect the celiprolol-induced relaxation.

In the artery rings with an intact endothelium, superoxide dismutase (200 U/ml) produced relaxations (Fig. 2, lower right tracing), which were abolished by endothelium denudation (Fig. 6, left). Treatment with superoxide dismu-

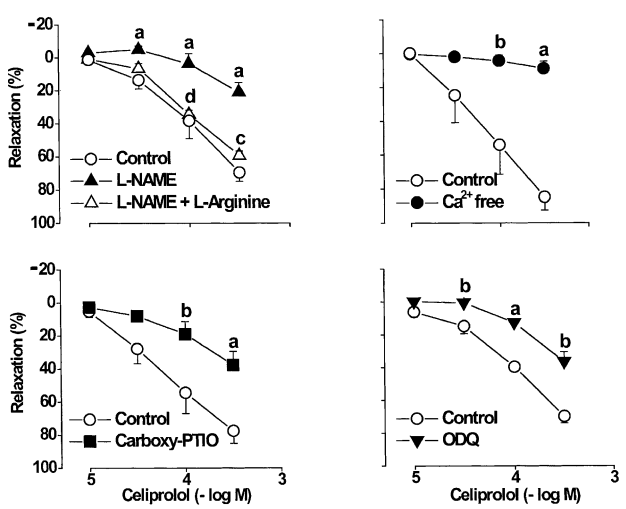


Fig. 3. Modification by L-NAME ($10^{-5}$ M) and L-arginine ($10^{-3}$ M) (upper left figure), removal of external $Ca^2$ (upper right), carboxy-PTIO ($3\times10^{-4}$ M, lower left) and ODQ ($10^{-6}$ M, lower right) of the response to celiprolol in prostaglandin $F_{2\alpha}$-contracted porcine coronary artery rings with an intact endothelium. The ordinate indicates celiprolol-induced relaxations relative to those elicited by $10^{-4}$ M papaverine. Significantly different from control, $^{a}P<0.01$; $^{b}P<0.05$ (Tukey's test for the upper left, and Student unpaired *t*-test for the others); significantly different from the value with L-NAME, $^{c}P<0.01$; $^{d}P<0.05$ (Tukey's test for the upper left). Four rings were used from different pigs. Vertical bars represent S.E.

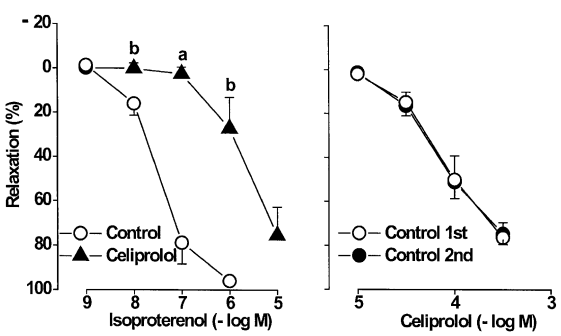


Fig. 4. Modification by celiprolol of the response to isoproterenol (left figure) and celiprolol (right) in prostaglandin $F_{2\alpha}$-contracted porcine coronary artery rings with an intact endothelium. Relaxations induced by $10^{-4}$ M papaverine were taken as 100%. In the left figure, after the dose–response curve for isoproterenol was determined to be reproducible, the celiprolol dose–response curve with doses from $10^{-5}$ to $3\times10^{-4}$ M (as in the right figure) was recorded. Then, the rings were rinsed three times with fresh medium and equilibrated to obtain another dose–response curve for the amine (solid triangles). The right figure compares the first and second dose–response curves for celiprolol in the same rings. The second curve was obtained under the influence of celiprolol as applied for the first curve. Significantly different from control, $^{a}P<0.01$, $^{b}P<0.05$ (unpaired *t*-test). Four rings were used were from different pigs. Vertical bars represent S.E.

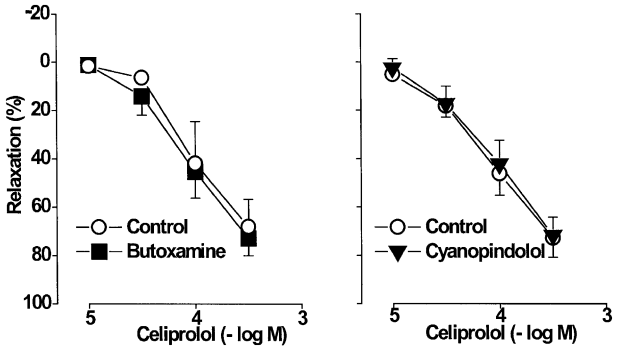


Fig. 5. Effects of butoxamine ($10^{-5}$ M; left figure) and cyanopindolol ($10^{-6}$ M; right) on the response to celiprolol in prostaglandin $F_{2\alpha}$-contracted porcine coronary artery rings with an intact endothelium. The ordinate represents celiprolol-induced relaxations relative to those elicited by $10^{-4}$ M papaverine, which produced the maximal relaxation. Four rings were used were the left figure and five for the right figure. All rings were from different pigs. Vertical bars represent S.E.

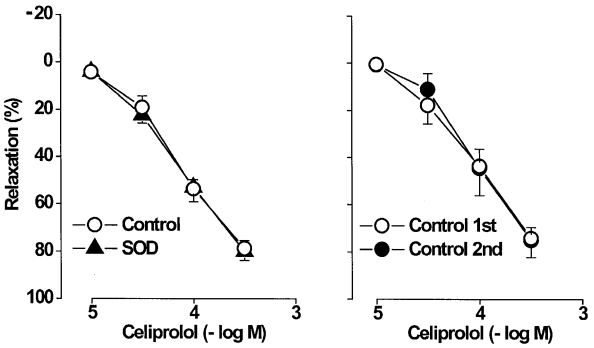


Fig. 7. Effects of superoxide dismutase (SOD, 200 U/ml; left figure) on the response to celiprolol in prostaglandin $F_{2\alpha}$-contracted porcine coronary artery rings with an intact endothelium. The right figure shows the first and second dose–response curves for celiprolol without any treatment (time control). The same rings were used for both curves. The ordinate indicates celiprolol-induced relaxations relative to those caused by $10^{-4}$ M papaverine, which produced maximal relaxation. Six rings were used from different pigs. Vertical bars represent S.E.

tase failed to inhibit the celiprolol-induced relaxation (Fig. 7, left); the right panel of this figure shows the reproducible response to celiprolol in the same rings. In these preparations, L-NAME elicited an endothelium-dependent contraction (Fig. 6, right), suggesting that there was a basal release of NO from the endothelium.

### *3.2. Measurement of cyclic GMP in the tissue and external medium*

Contents of cyclic GMP were compared in porcine coronary arteries with an intact endothelium under control conditions and those exposed to celiprolol ($10^{-4}$ M). The cyclic GMP contents were $0.39 \pm 0.061$ and $0.71 \pm 0.140$ pmol/mg protein ($n = 8$), respectively, the difference ($92.3 \pm 36.0\%$) being statistically significant ($P < 0.05$, paired comparison).

Release of NO from the coronary artery was assayed by measurement of cyclic GMP in the incubation medium, which contained the crude extract of soluble guanylate cyclase obtained from the rat cerebellum, GTP and IBMX. Cyclic GMP accumulated in the external medium was $2.24 \pm 0.22$ pmol/mg protein ($n = 10$), which was decreased to $0.74 \pm 0.08$ pmol/mg protein ($n = 10$) by treatment with L-NAME ($10^{-4}$ M). Approximately 67% of cyclic GMP seemed to be attributable to NO released from the endothelium under control conditions. Fig. 8 summarizes the data from the arteries with and without stimulation of celiprolol or substance P ($10^{-9}$ M) in the absence and presence of L-NAME. Celiprolol ($3 \times 10^{-4}$ M)

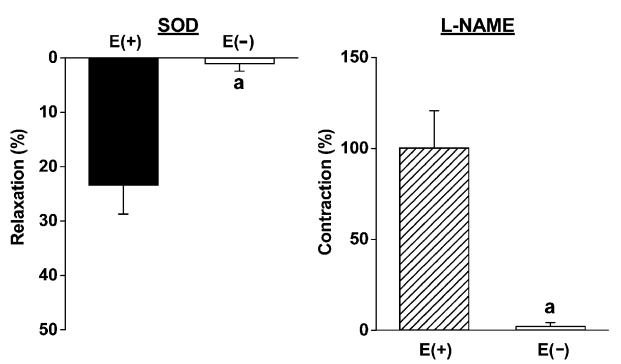


Fig. 6. Modification by superoxide dismutase (SOD, 200 U/ml; left figure) and L-NAME ($10^{-4}$ M; right) of the basal tone in prostaglandin $F_{2\alpha}$-contracted porcine coronary artery rings with (E+) or without (E−) an intact endothelium. The ordinate for the left figure indicates superoxide dismutase-induced relaxation relative to that caused by $10^{-4}$ M papaverine. The ordinate for the right figure indicates the L-NAME-induced contraction relative to that elicited by 30 mM $K^+$. Significantly different from the value in endothelium-intact rings, $^aP < 0.01$ (unpaired *t*-test). Six rings were used from different pigs. Vertical bars represent S.E.

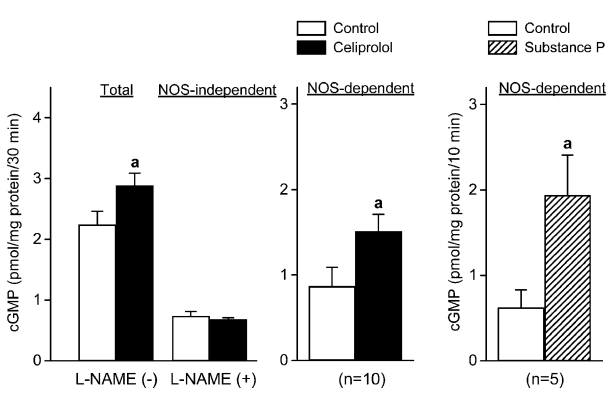


Fig. 8. NO synthase-dependent cyclic GMP production in coronary arteries stimulated by celiprolol ($3 \times 10^{-4}$ M for 30 min, left and center figure) and substance P ($10^{-9}$ M for 10 min, right) measured in incubation medium containing guanylate cyclase from the rat cerebellum, GTP ($5 \times 10^{-4}$ M) and IBMX ($10^{-5}$ M). Significantly different from control (open column), $^aP < 0.01$ (paired *t*-test). *n* denotes the number of arteries from different pigs. Vertical bars represent S.E.

increased the production of cyclic GMP, and L-NAME suppressed this effect. Substance P, used as a known endothelium-derived NO-releasing substance, also increased the formation of cyclic GMP sensitive to the NO synthase inhibitor. Similar effects of these compounds were also observed without IBMX in the incubation media (data not shown). Cyclic GMP production resistant to L-NAME may be due to natriuretic peptides, especially C-type natriuretic peptide in the endothelium, in the presence of IBMX and NO synthesized by NO synthase before the inhibition by L-NAME is established, since this inhibitor was included in the incubation medium from the start of experiments for NO release.

## 4. Discussion

In porcine coronary artery rings contracted with prostaglandin $F_{2\alpha}$, celiprolol in concentrations up to $10^{-4}$ M produced an endothelium-dependent relaxation which was abolished or suppressed by treatment with L-NAME, carboxy PTIO, an NO scavenger, and ODQ, an inhibitor of soluble guanylate cyclase (Garthwaite et al., 1995) but was unaffected by D-NAME and indomethacin. The inhibitory effect of L-NAME was reversed by L-arginine. Removal of $Ca^{2+}$ from the bathing medium abolished the celiprolol-induced relaxation. Mechanisms underlying NO synthesis that do not involve the influx of $Ca^{2+}$ into endothelial cells in the relaxation (Ayajiki et al., 1996; Fulton et al., 1999) can be ruled out as being important in the relaxation. Celiprolol increased the content of cyclic GMP in the coronary arteries. The formation of cyclic GMP in medium containing GTP and a crude soluble guanylate cyclase preparation from the rat cerebellum was increased only when the arteries with the endothelium were stimulated with celiprolol. This indicates that there is an increased release of NO from endothelial cells in response to this compound. Similar results were obtained when the arteries were treated with IBMX, a phosphodiesterase inhibitor. In addition to this finding, the inability of celiprolol to enhance the actions of sodium nitroprusside, an NO donor, suggests that the inhibition of cyclic GMP phosphodiesterase does not participate in the vasodilator response. Celiprolol reportedly liberates NO from the endothelium in the isolated, perfused rat kidney (Kakoki et al., 1999) and from the ischemic heart of anesthetized dogs (Asanuma et al., personal communication). Therefore, it is concluded that celiprolol acts on the endothelium and liberates NO synthesized from L-arginine by constitutive NO synthase, resulting in the increased formation of cyclic GMP in smooth muscle and in relaxation.

β-Adrenoceptor agonists and some β-adrenoceptor antagonists are reported to elicit relaxation mediated by endothelium-derived NO (Rubanyi and Vanhoutte, 1985; Gray and Marshall, 1992; Ferro et al., 1999; Gao et al., 1991; Cockcroft et al., 1995). In the present study, the $EC_{50}$ value of the β-adrenoceptor-mediated relaxation produced by isoproterenol in the arteries exposed to celiprolol, a $\beta_1$-adrenoceptor antagonist, and repeatedly rinsed was about 100 times higher than that obtained under control conditions, which suggests that the β-adrenoceptor-blocking action was not removed by repeated rinsing. Nevertheless, the concentration–response curve of celiprolol was reproducible in repeated trials. Treatment with butoxamine, a $\beta_2$-adrenoceptor antagonist, failed to reduce the response to celiprolol. Recently, evidence that $\beta_3$-adrenoceptors are involved in the endothelium-dependent relaxation of the rat aorta and carotid artery (Trochu et al., 1999; MacDonald et al., 1999) has been reported. However, cyanopindolol, a $\beta_3$-adrenoceptor antagonist, did not inhibit the relaxation induced by celiprolol. Isoproterenol-induced relaxations were not reduced by endothelium denudation in porcine arteries. The findings so far obtained allow us to conclude that the relaxation induced by celiprolol is not associated with $\beta_1$-, $\beta_2$- and $\beta_3$-adrenoceptor blockade nor with agonistic actions on β-adrenoceptors. Celiprolol does not seem to liberate endothelium-derived NO-releasing substances, since the response was not influenced by treatment with atropine and capsaicin.

The addition of superoxide dismutase elicits relaxation of the rat aorta only when the endothelium is preserved (Shinozaki et al., 2000). On the basis of measurement of superoxide anions released from the endothelium (Shinozaki et al., 1999), the induced relaxation is considered to be due to scavenging of superoxide, which produces artery contraction. In the present study, porcine coronary arteries with an intact endothelium contracted in response to L-NAME, and endothelium denudation abolished the response. It is postulated that there is a basal release of endothelial NO in the arteries used. The superoxide dismutase-induced relaxation was endothelium dependent. In order to exclude the possible superoxide-scavenging action of celiprolol, we evaluated the concentration–response curve for celiprolol before and during treatment with superoxide dismutase. However, there was no significant difference between the curves, suggesting that the superoxide dismutase-like action is not involved in the endothelium-dependent, NO-mediated relaxation in response to celiprolol. The inability of superoxide dismutase to potentiate the endothelium-dependent relaxation has been reported elsewhere (Angus and Cocks, 1989). Endogenous $Cu^{2+}$, $Zn^{2+}$-superoxide dismutase in the vicinity of vascular smooth muscle appears to protect endothelium-derived NO from the inhibitory effect of superoxide (Laight et al., 1998).

Evidence has accumulated that the basal release of NO from the endothelium plays an important role not only in vascular smooth muscle but also in platelets and low-density lipoprotein. Peak plasma concentrations of celiprolol in dogs and monkeys average 4.1 and $1.3 \times 10^{-5}$ M, respectively, when hypotensive doses are orally administered (Sugihara et al., 1989). These concentrations are

almost identical to those causing the release of NO from isolated porcine coronary arteries. Tolvanen et al. (1996) have demonstrated that endothelium-dependent relaxations caused by acetylcholine are greater in mesenteric artery rings isolated from spontaneously hypertensive rats pretreated with a depressor dose of celiprolol than in those from the non-treated rats. An increased production of NO is expected to augment the therapeutic action of β-adrenoceptor-blocking agents on hypertension, promote prophylaxis of attacks of myocardial infarction by interference with thrombosis, and prevent atherosclerosis by causing decreased smooth muscle proliferation and low-density lipoprotein oxidation. It is reported that acetylcholine stimulates the release of NO from the endothelium in canine corpus cavernosum penis (Okamura et al., 1999). Endothelial NO may compensate the effect of neurogenic NO responsible for an elevation of cavernous pressure in patients with impaired cavernous nerve function. According to Cleophas et al. (1996), celiprolol is better than atenolol, which possesses no vasodilator action, in terms of patient compliance and sexual function in men.

The present study provided evidence that celiprolol acts on sites other than β-adrenoceptors in endothelial cells, liberates NO to activate soluble guanylate cyclase in smooth muscle and increases the production of cyclic GMP responsible for porcine coronary artery relaxation. Endogenous NO synthesized locally at its site of action may be more beneficial in the prevention and treatment of vascular dysfunction than NO donors that liberate NO from molecules uniformly distributed in the circulation.

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