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Studies on endothelium-dependent vasorelaxation by hydralazine in porcine coronary artery

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Abstract

Hydralazine relaxed porcine coronary artery strips in a concentration-dependent manner by distinct endothelium-dependent and endothelium-independent actions. With lower doses ($\leq 10^{-6}$ M), the hydralazine-induced relaxation appears to be completely endothelium-dependent and we designed the present study to elucidate the mechanisms of this endothelium-dependent relaxation. Hydralazine (10^{-6} M)-induced endothelium-dependent relaxation was not affected by the presence of 10^{-5} M indomethacin, 10^{-3} M L-N^G-nitro-arginine (L-NOARG) or 10^{-5} M haemoglobin, and was accompanied by accumulation of cGMP but not of cAMP in artery strips. There was a close time-dependent parallel relationship between endothelium-dependent relaxation and accumulation of cGMP induced by hydralazine (10^{-6} M). The endothelium-dependent relaxation and accumulation of cGMP induced by hydralazine showed much slower kinetics than those induced by ionomycin (10^{-7} M). Pretreatment of the strips with actinomycin D (10 μg/ml) significantly inhibited not the endothelium-dependent relaxation and accumulation of cGMP induced by ionomycin (10^{-7} M) but those induced by hydralazine (10^{-6} M). These results suggest that hydralazine induces endothelium-dependent vasorelaxation via the slow accumulation of cGMP in the strips. This does not occur through the release of nitric oxide or prostaglandin I_2 but through immediate transcription and probably expression of a molecule in the endothelium.

Keywords: Hydralazine; Endothelium; Relaxation; cGMP

1. Introduction

The long-lasting hypotensive and antihypertensive properties of hydralazine were first reported in 1950 (Gross et al., 1950), and the drug has since been used for the treatment of hypertension. A direct relaxing action on vascular smooth muscle has generally been accepted as the primary effect of hydralazine (Khayyal et al., 1981). This action is thought to be associated with a change in the calcium balance of smooth muscle cells (Weiss et al., 1981) secondary to the accumulation of cGMP (Schultz et al., 1977) or membrane hyperpolarization (Hermsmeyer et al., 1983). However, it has been demonstrated that the relaxation induced by hydralazine, particularly at low concentrations, is potentiated in the presence of the endothelium (Spokas et al., 1983; Spokas and Folco, 1984; Yen et al., 1989). This suggests that hydralazine induces vasorelaxation in an indirect manner which is endothelium-dependent, in addition to its direct actions on smooth muscle.

It is well known that endothelium-derived relaxing factor (EDRF), nitric oxide (Furchgott and Zawadzki, 1980; Palmer et al., 1987), mediates the endothelium-dependent vasorelaxation induced by various agents which is accompanied by an increase in cGMP level in vascular smooth muscle cells (Furchgott, 1988; Ignarro et al., 1988). However, it has been demonstrated that hydralazine is not a donor of nitric oxide (NO) (Venturini et al., 1993) and that hydralazine-induced vasodilation is insensitive to gossypol, an irreversible inhibitor of the production or the release of EDRF (Förstermann et al., 1989). It has also been demonstrated that changes in cGMP levels do not occur during hydralazine-induced vasorelaxation of endothelium-intact

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preparations (Yen et al., 1989; Fukuda et al., 1994). Thus, although it is considered that hydralazine-induced vasorelaxation is not mediated via the NO/cGMP cascade, the possible existence of a substance or second messenger mediating hydralazine-induced endothelium-dependent vasorelaxation is still unclear. In a preliminary study, we found that hydralazine-induced endothelium-dependent relaxation was also observed in porcine coronary artery strips. Therefore, in the present study, we investigated the mechanism of hydralazine-induced endothelium-dependent relaxation.

2. Materials and methods

2.1. Measurement of tension of arterial strips

Left coronary circumflex arteries were isolated from fresh adult porcine hearts obtained from a local slaughterhouse. Arterial segments were cut into 2×15 mm helical strips, and suspended in 5-ml siliconized glass organ chambers filled with Krebs-Ringer solution with the following composition (mM): 113 NaCl, 4.8 KCl, 2.2. $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgCl_2$, 25 $NaHCO_3$ and 5.5 glucose. The solution was maintained at 37°C and gassed with 5% CO_2/95% O_2. Arterial strips were equilibrated at a passive tension of 1.25 g until the contractile tension induced by 50 mM K^+ Krebs-Ringer solution (K^+ substitution by decreasing Na^+) attained a steady state. Isometric contraction was measured as previously described (Kasuya et al., 1989). For some experiments, the endothelium was removed by gently rubbing the internal surface with a cotton swab. Whether arterial strips possessed an intact endothelium or not was determined by applying ionomycin (10^{-7} M) to tissues precontracted with 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Those showing over 75% relaxation in response to ionomycin were used as endothelium-intact strips. The strips showing consistent contractile response to 10^{-5} M $PGF_{2\alpha}$ before and after the procedure of endothelium denudation and not showing any response to 10^{-7} M ionomycin were used as endothelium-denuded strips. To determine the exact relaxant effect of hydralazine, two strips from the same arterial segment were used for each experiment. The strips were contracted with 10^{-5} M $PGF_{2\alpha}$ and almost attained a plateau state within 30 min. Then, hydralazine was applied to one strip, and the other strip was left as control. The difference in change of tension between hydralazine-applied and control strips was determined and considered as the true relaxant response to hydralazine. Each arterial strip was used for only one experiment.

In the set of experiments for estimating the effects of haemoglobin and L-N^G-nitro-arginine (L-NOARG) on hydralazine-induced relaxation in endothelium-intact strips, 10^{-5} M haemoglobin or 10^{-3} M L-NOARG was administered 15 min or 60 min, respectively, before 10^{-5} M $PGF_{2\alpha}$ administration. Under these experimental conditions, we first confirmed that the two inhibitors completely inhibited the endothelium-dependent relaxation induced by ionomycin (10^{-7} M). When estimating the effects of indomethacin on hydralazine-induced relaxation in endothelium-intact strips, 10^{-5} M indomethacin was applied 30 min before 10^{-5} M $PGF_{2\alpha}$ administration. Under these experimental conditions, the generation of arachidonate, which mediates endothelium-dependent relaxation (Roger and Peach, 1988), is inhibited. When estimating the effects of actinomycin D on hydralazine-induced relaxation in endothelium-intact strips, 10 μg/ml actinomycin D was applied 30 min before hydralazine application.

2.2. Determination of tissue content of cyclic nucleotides

Porcine coronary artery strips were instantly taken out of the bath at appropriate intervals and immediately frozen in liquid N_2. Frozen strips were homogenized in 1 ml of ice-cold 6% trichloroacetic acid and centrifuged at $2500 \times g$ at 4°C for 15 min. The precipitate was subjected to protein assay using bovine serum albumin as a standard according to the method previously described (Lowry et al., 1951). The supernatant was extracted 3 times with 5 ml of water-saturated ether, and the ether phase was discarded. The water-phase was evaporated to dryness under a stream of N_2 gas at 37°C. Then, the resulting residue was dissolved in 100 ml of 0.05 M sodium acetate buffer, and the level of each of the cyclic nucleotides (cGMP and cAMP) in the solution was estimated by means of radioimmunoassay kits (New England Nuclear) according to the procedure described previously (Furchgott et al., 1984). The cyclic nucleotide content was expressed in pmol/mg protein.

2.3. Preparation of oxyhaemoglobin

Bovine haemoglobin (Sigma, St. Louis, MO, USA) was dissolved in distilled water to give a final concentration of 1 mM. A 10-fold molar excess of sodium hydrosulfite ($Na_2S_2O_4$), a reducing agent, was added to the haemoglobin solution at room temperature. Sodium hydrosulfite was then removed by dialysis against 200 volumes of distilled water at 4°C for 2 h with gentle stirring. The purity of haemoglobin (oxyhaemoglobin) was spectrophotometrically determined, and the solution was frozen in aliquots at −20°C and stored for up to 14 days.

2.4. Drugs

(±)-Isoproterenol hydrochloride, L-N^G-nitro-arginine (L-NOARG), indomethacin, ionomycin calcium salt, actinomycin D and bovine haemoglobin were from Sigma. The injectable solution of nitroglycerin (Millisrol) was from Nihon Kayaku (Tokyo, Japan). $PGF_{2\alpha}$ was from Wako (Osaka, Japan). Hydralazine (Apresoline) was from

Ciba-Geigy (Basel, Switzerland). All drugs were prepared as aqueous solutions except for $PGF_{2\alpha}$ and indomethacin, which were dissolved in ethanol. Hydralazine solution was freshly prepared daily by dissolving the drug in distilled water.

2.5. Statistical analysis

Values are expressed as means ± S.E. Comparisons were made by one-way analysis of variance (ANOVA) followed by Bonferroni's correction or Student's *t*-test for unpaired values. Differences with P values less than 0.05 were considered statistically significant.

3. Results

3.1. Hydralazine-induced relaxation in porcine coronary artery strips

In both endothelium-intact and endothelium-denuded porcine coronary artery strips precontracted with 10^{-5} M $PGF_{2\alpha}$, hydralazine caused slow relaxation in a concentration-dependent manner (Fig. 1a). As shown in Fig. 1b, the maximum response to hydralazine was 62 ± 6% and 24 ± 5% in endothelium-intact strips and endothelium-denuded strips, respectively. In endothelium-intact porcine coronary artery strips, the minimum effective dose and EC_{50} value of hydralazine for relaxation were 10^{-6} M and 1.6×10^{-6} M, respectively, and these values were significantly lower than those of hydralazine in endothelium-denuded porcine coronary artery strips (10^{-5} M and 3.8×10^{-5} M, respectively). The hydralazine (10^{-6} M)-induced relaxation was completely endothelium-dependent. Thus, 10^{-6} M hydralazine was used in the following experiments for investigating the mechanism of hydralazine-induced endothelium-dependent relaxation.

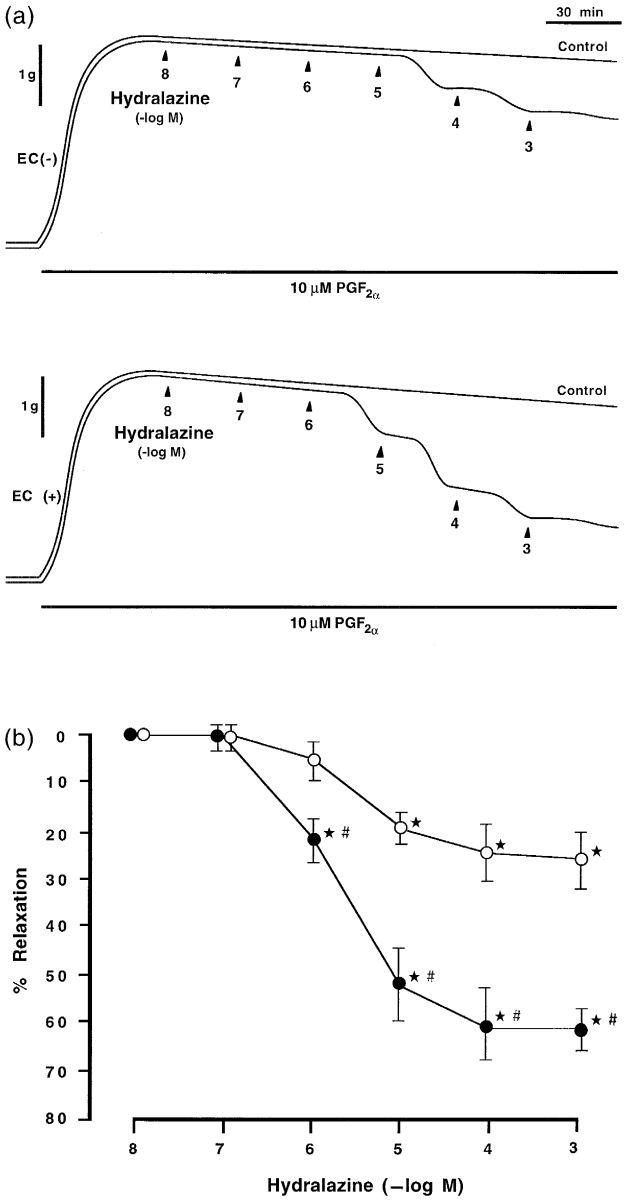

Fig. 1. (a) Typical tracings showing hydralazine-induced relaxation of porcine coronary artery strips. Endothelium-intact and -denuded strips precontracted with $PGF_{2\alpha}$ (10^{-5} M) relaxed in response to cumulative application of hydralazine. Control strips precontracted with $PGF_{2\alpha}$ (10^{-5} M) were left during the application of hydralazine. (b) Dose-response relationships for hydralazine-induced relaxation of porcine coronary artery strips with (●) and without endothelium (○). In this and in subsequent figures, relaxant responses are expressed as percentage of reduction of $PGF_{2\alpha}$ (10^{-5} M)-induced tension. Vertical bars represent ± S.E. ($n = 5$). * Significantly different from control values with $P < 0.05$ (ANOVA with Bonferroni correction). # Significantly different from respective values for endothelium-denuded strips, $P < 0.05$ (Student's *t*-test for unpaired values).

3.2. Effects of indomethacin, L-NOARG and haemoglobin on hydralazine-induced endothelium-dependent relaxation

To determine whether NO or eicosanoids mediate the hydralazine-induced endothelium-dependent relaxation, we investigated the effects of preincubation of endothelium-intact porcine coronary artery strips with indomethacin (10^{-5} M), L-NOARG (10^{-3} M) or haemoglobin (10^{-5} M) on hydralazine-induced relaxation. As shown in Fig. 2, hydralazine (10^{-6} M)-induced relaxation was not significantly affected by the presence of indomethacin, haemoglobin or L-NOARG. The decrease in tension of tissue precontracted with 10^{-5} M $PGF_{2\alpha}$ caused by 10^{-6} M hydralazine was 22 ± 2.1%, 19.1 ± 2.1%, 21 ± 3.2% and 28 ± 5% in controls and in the presence of indomethacin, haemoglobin and L-NOARG, respectively.

3.3. Effect of hydralazine on cyclic nucleotide levels in porcine coronary artery

To determine whether hydralazine-induced endothelium-dependent relaxation is associated with a rise of

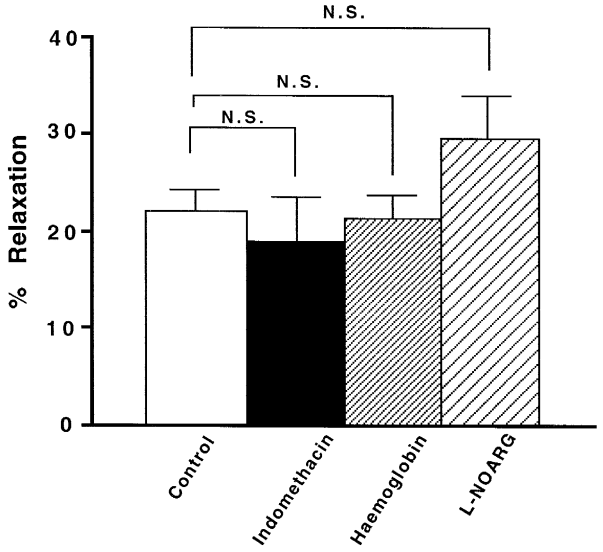

Fig. 2. Effects of indomethacin, haemoglobin and L-NOARG on endothelium-dependent relaxation induced by hydralazine. Endothelium-intact porcine coronary artery strips were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M), and the effects of pretreatment for 30 min with indomethacin (10^{-5} M), for 10 min with haemoglobin (10^{-5} M) and for 1 h with L-NOARG (10^{-3} M) on hydralazine (10^{-6} M)-induced relaxation were determined. Vertical bars represent ±S.E. ($n = 4$–5). N.S., not significantly different from control values (Student's *t*-test for unpaired values).

cAMP or cGMP in smooth muscle cells, we investigated the effect of hydralazine on tissue levels of cAMP and cGMP during relaxation induced by hydralazine. As shown in Fig. 3a, 10^{-6} M hydralazine did not affect cAMP levels in porcine coronary artery strips, whereas 3×10^{-6} M isoproterenol, an endothelium-independent relaxant, induced 1.8- and 1.6-fold increases in cAMP levels in porcine coronary artery strips with and without endothelium, respectively. The level of cAMP in endothelium-intact porcine coronary artery strips was unchanged during the period after exposure to 10^{-6} M hydralazine (5, 10, 20 and 30 min) (Fig. 3b). 10^{-6} M hydralazine induced a 1.5-fold increase in cGMP level in endothelium-intact porcine coronary artery strips (Fig. 4a). Ionomycin (10^{-7} M), an endothelium-dependent relaxant, also induced a 3.5-fold increase in cGMP level in endothelium-intact

Table 1
Effects of actinomycin D on vasorelaxation induced by hydralazine

	Control	Actinomycin D
Hydralazine (1 μM)	23±4%	5±5% [a]
Ionomycin (0.1 μM)	80±5%	82±3%

Endothelium-intact porcine coronary artery strips were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M). Then, hydralazine (10^{-6} M) or ionomycin (10^{-7} M) was applied to the strips. Pretreatment of strips for 30 min with actinomycin D (10 μg/ml) was conducted before application of the drugs. Relaxant responses are expressed as percentages of reduction of $PGF_{2\alpha}$ (10^{-5} M)-induced tension. Each value represents mean±S.E. ($n = 5$–8). [a] Significantly different from respective control values with $P < 0.05$ (Student's *t*-test for unpaired values).

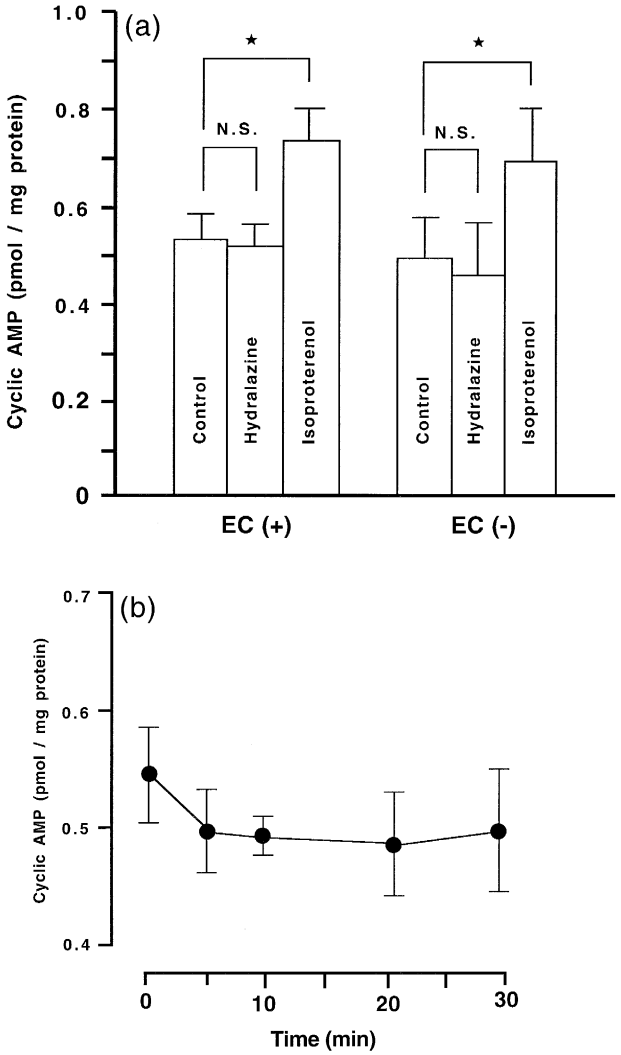

Fig. 3. (a) The level of cAMP in porcine coronary artery strips treated with hydralazine and isoproterenol. Porcine coronary artery strips with and without endothelium were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M). Then, strips exposed to hydralazine (10^{-6} M) were frozen in liquid N_2 after 30 min and those exposed to isoproterenol (3×10^{-6} M) were frozen in liquid N_2 after 2 min. Vertical bars represent ±S.E. ($n = 6$). * Significantly different from control value with $P < 0.05$; N.S., not significantly different (Student's *t*-test for unpaired values). (b) Effect of duration of exposure to hydralazine on cAMP content of endothelium-intact porcine coronary artery strips. Endothelium-intact porcine coronary artery strips were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M). Then, strips exposed to hydralazine (10^{-6} M) were frozen in liquid N_2 after 5, 10, 20 and 30 min. Vertical bars represent ±S.E. ($n = 6$).

porcine coronary artery strips. Nitroglycerin (3×10^{-5} M), an endothelium-independent relaxant, induced 1.6- and 3-fold increases in cGMP levels in porcine coronary artery strips with and without endothelium, respectively. The time-dependent relationship between the rise of cGMP level and the relaxation induced by ionomycin and hydralazine was next investigated. As shown in Fig. 4b, the cGMP level in endothelium-intact porcine coronary artery strips stimulated with 10^{-7} M ionomycin rose rapidly, reached a peak at 5 min and then declined to the control

level at 25 min. Although the strips began to relax within 30 s and showed maximum relaxation at 5 min, in close parallel with the rise of cGMP level, the relaxation thereafter remained nearly at the maximum level in spite of a decline in cGMP to the control level. The cGMP level in endothelium-intact porcine coronary artery strips stimu-

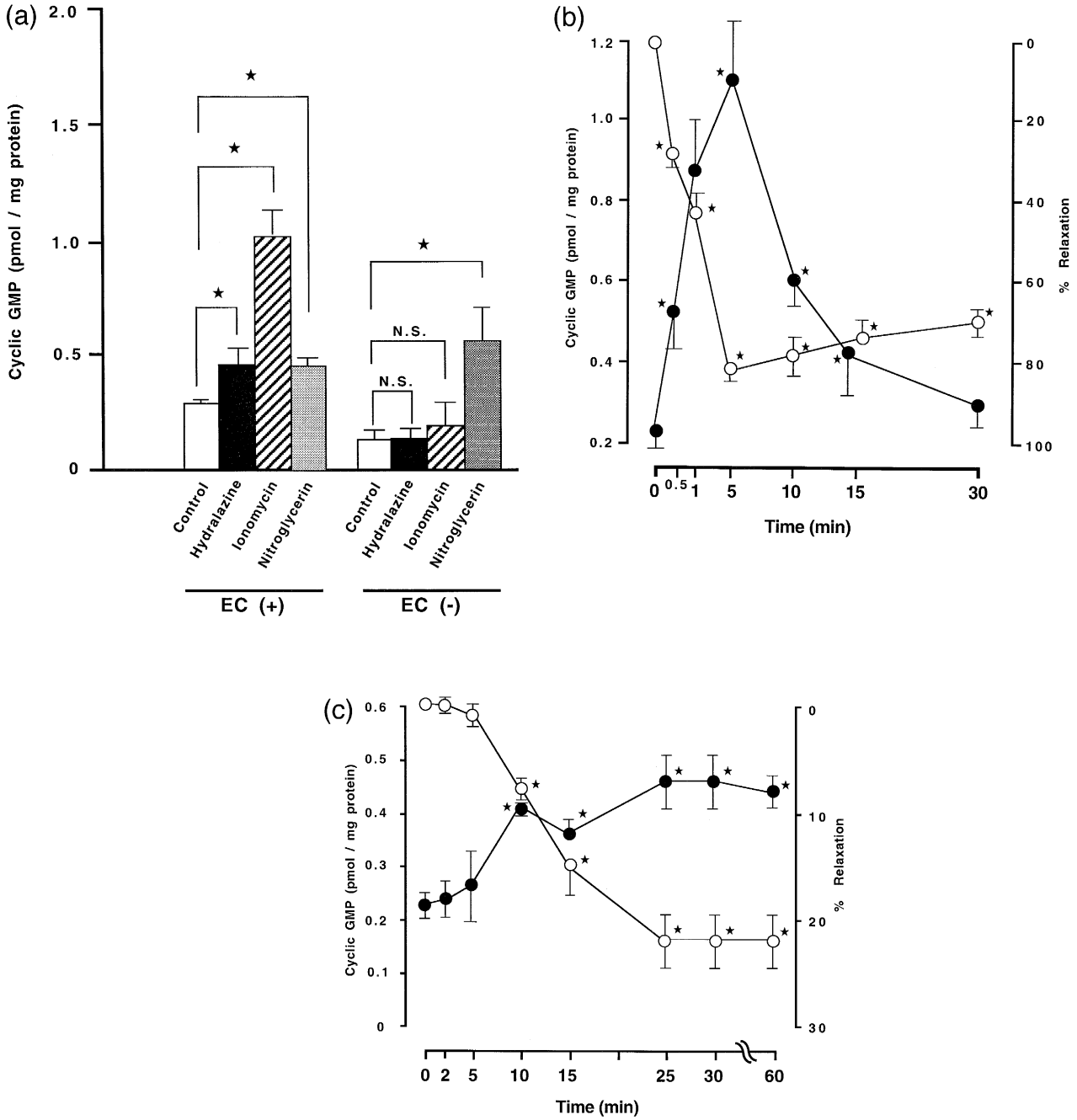

Fig. 4. (a) The level of cGMP in porcine coronary artery strips treated with hydralazine, ionomycin and nitroglycerin. Porcine coronary artery strips with and without endothelium were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M). Then, strips exposed to hydralazine (10^{-6} M) were frozen in liquid N_2 after 30 min and those exposed to ionomycin (10^{-7} M) and nitroglycerin (3×10^{-5} M) were frozen in liquid N_2 after 2 min. Vertical bars represent $\pm$S.E. ($n = 4$–8). * Significantly different from control values with $P < 0.05$; N.S., not significantly different (Student's *t*-test for unpaired values). (b) Time-course of relaxation and cGMP level of endothelium-intact porcine coronary artery strips following application of ionomycin. Endothelium-intact porcine coronary artery strips were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M). Then, strips exposed to ionomycin (10^{-7} M) were frozen in liquid N_2 after 0.5, 1, 5, 10, 15 and 25 min. Relaxation (○) and cGMP levels (●) were determined in the same strips. Vertical bars represent $\pm$S.E. ($n = 6$). * Significantly different from control value with $P < 0.05$ (ANOVA with Bonferroni correction). (c) Time course of relaxation and cGMP level of endothelium-intact porcine coronary artery strips following application of hydralazine. Endothelium-intact porcine coronary artery strips were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M). Then, strips exposed to hydralazine (10^{-6} M) were frozen in liquid N_2 after 5, 10, 15, 25, 30 and 60 min. Relaxation (○) and cGMP levels (●) were determined in the same strips. Vertical bars represent $\pm$S.E. ($n = 6$). * Significantly different from control value with $P < 0.05$ (ANOVA with Bonferroni correction).

Table 2
Effects of actinomycin D on increases in cGMP induced by hydralazine

	Actinomycin D	cGMP (pmol/mg protein)
Control	–	0.10 ± 0.03
Control	+	0.13 ± 0.01
Ionomycin (0.1 μM)	–	0.39 ± 0.08 [a]
Ionomycin (0.1 μM)	+	0.41 ± 0.03 [a]
Hydralazine (1 μM)	–	0.20 ± 0.01 [a]
Hydralazine (1 μM)	+	0.14 ± 0.01

Endothelium-intact porcine coronary artery strips were submaximally contracted with $PGF_{2\alpha}$ (10^{-5} M). Then, strips exposed to hydralazine (10^{-6} M) were frozen in liquid N_2 after 30 min and those exposed to ionomycin (10^{-7} M) were frozen in liquid N_2 after 2 min. Pretreatment of strips for 30 min with actinomycin D (10 μg/ml) was conducted before application of the drugs. Each value represents mean ± S.E. (n = 6–8). [a] Significantly different from control values for the strips without actinomycin D pretreatment with $P < 0.05$ (ANOVA with Bonferroni correction).

lated with 10^{-6} M hydralazine rose slowly, reached a plateau at 25 min, and then remained at this level for at least 60 min. The time-dependent hydralazine (10^{-6} M)-induced relaxation of the strips was in proportion to the rise of cGMP level in the strips (Fig. 4c).

3.4. Effect of actinomycin D on hydralazine-induced relaxation and cGMP accumulation

To determine whether transcription of a certain molecule is involved in the hydralazine-induced endothelium-dependent relaxation and accumulation of cGMP, the effect of actinomycin D on hydralazine-induced relaxation and accumulation of cGMP was investigated. As shown in Table 1, pretreatment of endothelium-intact porcine coronary artery strips with actinomycin D (10 μg/ml) significantly reduced the hydralazine (10^{-6} M)-induced relaxation, but did not affect the ionomycin (10^{-7} M)-induced relaxation. Preatreatment of the endothelium-intact strips with actinomycin D (10 μg/ml) had no effect on the basal level of cGMP and the ionomycin (10^{-7} M)-induced increase in cGMP, but selectively blocked the increase in cGMP level induced by hydralazine (10^{-6} M) (Table 2).

4. Discussion

In the present study, hydralazine induced slow vasorelaxation by distinct endothelium-dependent and endothelium-independent actions in porcine coronary artery strips. When determining the exact relaxant action of hydralazine, we considered two possible factors that could influence the results, the gradual decline in tension of the strips precontracted by $PGF_{2\alpha}$ during experiments carried out over a long time and the depressive effect of removal of the endothelium on the bioactivity of vascular smooth muscle cells. In order to avoid interference by the first factor, as described in Section 2, two strips from the same arterial segment were used for hydralazine assay and control, respectively, and then the difference in change of tension between them was considered as the true relaxant response to hydralazine. Next, we confirmed that the amplitude of the contraction elicited by $PGF_{2\alpha}$ was not affected by removal of the endothelium, despite the fact that the ionomycin-induced relaxation was completely abolished by removal of the endothelium. Thus, the differences in the sensitivity and responses to hydralazine between endothelium-intact and -denuded preparations can be considered to be the true endothelium-dependent phenomenon. Hydralazine at 10^{-6} M induced relaxation in porcine coronary artery strips, a response which was completely endothelium-dependent. This is consistent with the notion that the endothelial component of the hydralazine response represents the major contribution to the net relaxant effect on vascular smooth muscle, particularly at lower concentrations (Spokas et al., 1983; Spokas and Folco, 1984; Yen et al., 1989). It is noteworthy that at concentrations attained in the plasma of patients receiving hydralazine for the treatment of hypertension ($10^{-7} \sim 10^{-6}$ M) (Israili and Dayton, 1977), the antihypertensive response induced by hydralazine is possibly mediated by the endothelial component. Thus, we designed the present study to elucidate the mechanism of hydralazine (10^{-6} M)-induced endothelium-dependent relaxation using porcine coronary artery strips.

Hydralazine-induced endothelium-dependent relaxation was not affected by pretreatment with indomethacin (Fig. 2), indicating that cyclooxygenase-related eicosanoids including prostaglandin I_2 (PGI_2) (Moncada et al., 1976; Gryglewski et al., 1976; Roger and Peach, 1988) do not contribute to the effect of hydralazine. This is consistent with the previous observation that arachidonate metabolites do not contribute to hydralazine-induced vasorelaxation (Spokas et al., 1983). In addition, NO also does not contribute to hydralazine-induced endothelium-dependent relaxation for the following reasons: neither L-NOARG, an inhibitor of NO generation from L-arginine (Moore et al., 1990), nor haemoglobin, a chelator of NO (Martin et al., 1985), affected hydralazine-induced endothelium-dependent relaxation (Fig. 2). These results suggest that cyclooxygenase-related eicosanoids and NO are not involved in the hydralazine-induced endothelium-dependent relaxation.

Considerable data from various laboratories have shown that the increases in cAMP and cGMP levels correlate well with the relaxation of vascular smooth muscle in response to various agents (Bülbring and Tomita, 1987; Murad, 1986). We then investigated whether hydralazine-induced endothelium-dependent relaxation is associated with a rise in cAMP or cGMP level in porcine coronary artery strips. Hydralazine did not affect the level of cAMP in the strips during the period after exposure to hydralazine (Fig. 3a,b). In contrast, hydralazine increased the cGMP level especially in endothelium-intact porcine coronary artery strips

(Fig. 4a). This hydralazine-induced increase in cGMP may be brought about specifically in vascular smooth muscle cells, because hydralazine (10^{-6} M) did not affect the cGMP level in cultured endothelial cells (data not shown). Moreover, there was a close time-dependent parallel relationship between relaxation and accumulation of cGMP induced by hydralazine (Fig. 4c). These findings suggest that the endothelium-dependent vasorelaxant effect of hydralazine may be mediated via the increase in smooth muscle cGMP. It is worth noting that the endothelium-dependent relaxation and accumulation of cGMP induced by hydralazine were much slower than those induced by ionomycin (Fig. 4b,c). The vasorelaxation induced by ionomycin in the present study was mediated through the NO/cGMP cascade, because L-NOARG and haemoglobin, which completely inhibited ionomycin-induced endothelium-dependent relaxation, also inhibited the accumulation of cGMP induced by ionomycin (data not shown). It has been previously demonstrated that the kinetics of vasorelaxation and accumulation of cGMP in tissues, mediated by NO derived from the endothelium, after stimulation by various agents as well as nitro-compounds are generally fast (Keith et al., 1982; Kelm and Schrader, 1990; Martin et al., 1985; Spokas et al., 1983), which is similar to the case of ionomycin in the present study. In contrast, the endothelium-intact strips treated with hydralazine (10^{-6} M) showed a slow onset of relaxation and a long-lasting accumulation of cGMP. Furthermore, hydralazine-induced accumulation of cGMP was not affected by the presence of L-NOARG and haemoglobin (data not shown). Thus, it is confirmed that the hydralazine-induced endothelium-dependent relaxation in porcine coronary artery is not mediated through the NO/cGMP cascade.

It has been clearly demonstrated that phenylhydrazine and related chemical substances such as hydralazine activate soluble guanylate cyclase and elevate tissue levels of cGMP via heme-dependent but NO-independent mechanisms (Ignarro et al., 1984; Ignarro, 1989). However, the lack of effect of hydralazine on tissue levels of cGMP in the endothelium-denuded strips (Fig. 4a) suggests that other mechanism may be involved in hydralazine-induced endothelium-dependent relaxation. The question arises as to how hydralazine induces endothelium-dependent accumulation of cGMP. Previous studies have revealed that hydralazine interacts with DNA and acts as a potential carcinogen (Williams et al., 1980) and that hydralazine inhibits DNA methylation and may induce gene expression in cloned T cell lines (Cornacchia et al., 1988). It has been also demonstrated that the mRNA level of lysyl hydroxylase, an essential enzyme in collagen biosynthesis, is induced by hydralazine (Yeowell et al., 1992). Thus, we hypothesized that hydralazine-induced endothelium-dependent relaxation might be mediated by the biosynthesis of some organic molecule. To investigate this possibility, we studied the effect of actinomycin D, a specific inhibitor of DNA transcription, on hydralazine-induced relaxation.

Pretreatment of endothelium-intact porcine coronary artery strips with actinomycin D significantly reduced hydralazine-induced relaxation, but did not affect ionomycin-induced relaxation (Table 1). Furthermore, actinomycin D pretreatment significantly inhibited the hydralazine-induced endothelium-dependent accumulation of cGMP (Table 2). This effect of actinomycin D is not due to damage of the endothelium, because the accumulation of cGMP induced by ionomycin was not affected by actinomycin D pretreatment. Moreover, the effect of actinomycin D was specifically expressed in the endothelium, because the relaxation induced by the higher dose of hydralazine (10^{-5} M) in endothelium-denuded porcine coronary artery strips was not affected by actinomycin D pretreatment (data not shown). These results suggest that the hydralazine-induced endothelium-dependent relaxation in porcine coronary artery strips is associated with the immediate transcription of some molecule in endothelial cells. In the present study, however, it was not determined which signaling pathway in hydralazine-induced endothelium-dependent relaxation is mediated by the actinomycin D-sensitive molecule biosynthesized in the endothelium. The possibility that a known or unknown vasorelaxant factor is immediately synthesized after stimulation of the endothelium with hydralazine is of particular interest. Further study is needed to elucidate this possibility.

In conclusion, hydralazine induces endothelium-dependent vasorelaxation via slow and long-lasting accumulation of cGMP in coronary artery strips, which is not mediated through the release of nitric oxide or PGI_2 but through the immediate transcription and probably expression of a molecule in the endothelium.

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