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Effects of the Endothelium on the Contractile Responses to Norepinephrine in Isolated Proximal and Distal Coronary Artery of Pigs

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= ABSTRACT =

Effects of the endothelium on the contractile responses to norepinephrine (NE) were investigated in isolated helical strips of the proximal and distal coronaries artery of pigs. The helical strips were immersed in Tris-buffered Tyrode's solution equilibrated with 100% O_2 at 35℃ and its isometric tension was measured.

NE relaxed the strips precontracted with acetylcholine from both the proximal and distal coronary artery. NE-induced relaxation, which might be induced mainly by β-adrenoceptor function, was dominant in the distal coronary arteries. NE-induced relaxation was converted to a contraction after β-adrenoceptor blockade with propranolol (3×10^{-6}M). α-adrenoceptor-mediated contraction by NE was greater in the proximal coronary artery than the distal coronary artery. Quantitatively, α_1-adrenoceptor-mediated contraction by NE was greater than α_2-adrenoceptor-mediated contraction by NE in both arteries. NE-induced relaxation was decreased by rubbing of endothelium in both arteries. α_1-and α_2-adrenoceptor-mediated contraction by NE were potentiated by rubbing of endothelium in both arteries. Pretreatment with methylene blue, an inhibitor of soluble guanylate cyclase, increased α_1- and α_2-adrenoceptor-mediated contraction by NE in both arteries with endothelium.

From the above results, we suggest that the effect of activation of α-adrenoceptors by NE may be modulated by endothelium in the proximal and distal coronary arteries of pigs. This effect may be mediated via endothelium-derived relaxing factor.

Key Words: α-adrenoceptors, Norepinephrine, Pig coronary artery, Endothelium, Methylene blue

INTRODUCTION

The proximal part of the epicardial arteries is formed from an outgrowth of aortic endothelial mesenchymal cells, while the intramural arteries are derived from the myocardial mesenchyme (Conte & Pellegrini, 1984). Norepinephrine (NE) has a different contractile responses on coronary arteries depending on their location. The relaxing or contracting action of NE has been attributed to segmental variations in the distribution of α- and β-adrenoceptors (Malindzak, 1982).

One of the most important contributions to vascular physiology and pharmacology in the past decade was the discovery in 1980 by Furchgott and Zawadzki that the relaxing effect of acetylcholine on the rabbit aorta is caused indirectly by a substance released from the endothelial cells. The existence of this endothelium-derived relaxing factor (EDRF) was demonstrated in a number of bioassay studies. Endothelial cells are active and regulate vascular

smooth muscle in response to receptor activation or physiological stimuli, e.g. stretch (Rubanyi, 1988). The factors released constitute a variety of chemical substances: nitric oxide (NO) (Palmer et al, 1987), which causes relaxation via stimulation of the soluble guanylate cyclase enzyme (Rapaport & Murad, 1983), a polypeptide, endothelin, which induces contraction of vascular smooth muscle (Yanagisawa et al, 1988) and several arachidonic acid derivatives, such as prostacyclin (Moncada et al, 1976).

NE has previously been shown to induce release of EDRF in epicardial arteries from both dogs and pigs (Cocks & Angus, 1983), but information concerning the action of NE on endothelium in small intramural resistance arteries in the coronary circulation is lacking. Recently, Nyborg (1990) reported that NE could release EDRF in only the proximal part of rat coronary artery. In several blood vessels, the endothelium attenuates the vasoconstriction caused by NE (Carrier & White, 1985), clonidine (Egleme et al, 1984), and phenylephrine (Carrier & White, 1985). Rubanyi and Vanhoutte (1985) found that removal of the endothelium decreased the relaxation due to NE in the dog coronary artery. Recently, Vinet et al (1991) reported in rat aorta that EDRF formation and release was an important factor in the modulation of α-adrenoceptor-mediated vasoconstriction. Though it is possible that the activation of α-adrenoceptors was attenuated by endothelium, these effects were less known in the pig coronary arteries.

In the present experiment, we examined the mechanism of contractile responses to NE and the effect of the endothelium on α-adrenoceptor-mediated contraction by NE of isolated helical strips of the proximal and distal coronary arteries of pigs.

METHODS

Pig hearts were obtained from the slaughter house and transported to the laboratory in ice-cold (4℃) oxygenated Tris-buffered Tyrode's solution. The left anterior descending coronary artery was carefully dissected as the proximal segment (outer diameter: OD 3~4 mm) and its first branch as the distal segment (OD 0.5~1 mm), cleaned of fat and periarterial connective tissue, and recovered for 2 hours at room temperature. For the measurement of mechanical tension, the arteries were cut spirally with a width of approximately 2 mm and a length of 10 mm. After the muscle strips were ligated at each end with thread, muscle strips were suspended in a themostatically controlled (35℃) organ bath (50 ml) under 0.25 g tension. Changes in tension were measured isometrically by means of force transducer (F-60, Narco Biosystem), then amplified and recorded on a physiograph (MK-IV, Narco Biosystem)(Chang et al, 1990).

Tris-buffered Tyrode's solution had the following composition (mM): NaCl 158, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, Tris 5, and Glucose 6. This solution was equilibrated with 100% O_2. The pH was adjusted to 7.4 at 35℃.

The endothelium was removed by gently rubbing 3 to 5 times with filter paper. The effectiveness of this procedure of endothelium removal has been demonstrated by the unresponsiveness to substance P (10^{-8} M) on ACh (10^{-7} M) -induced contraction (Furchgott, 1983; Beny et al, 1986; Cohen et al, 1988).

Cumulative dose-response curves were obtained by stepwise increases in the concentration of norepinephrine; additions were made as soon as a steady response was obtained from the preceding dose. The adrenergic antagonists used were propranolol (β-, unselective), prazosin(α_1-selective), yohimbine (α_2-selective). Concentrations are expressed as the log of the final molar concentration of the drug in the bath.

Drugs used were noradrenaline HCl (Sigma), propranolol (Sigma), prazosin HCl (Sigma), acetylcholine HCl (Sigma), yohimbine (Sigma), methylene blue (Sigma). Drugs were dissolved in distilled water except for prazosin which was

dissoloved in dimethyl sulfoxide.

Statistical significance was assessed by paired T-test. P values of less than 0.05 were considered significant.

RESULTS

The effect of NE on the ACh-induced contraction and effect of endothelium on NE-induced vasorelaxation in the pig coronary artery were studied (Fig. 1). Proximal and distal coronary arterial strips contracted when they were exposed to ACh (10^{-7} M), and the amplitudes of these contractions were used as a reference for each arterial strip. After equilibration of each arterial strip to the presence of ACh (10^{-7} M), addition of NE to the organ bath caused vasorelaxation. Arterial strips with and without endothelium relaxed in response to NE in a dose-dependent manner. The removal of endothelium caused significant attenuation of NE-induced vasorelaxation. Distal coronary artery was more sensitive to relaxation caused by NE than proximal coronary artery.

In order to evaluated the role of α-adrenergic activation by NE, the β-adrenoceptor blocker, propranolol (3×10^{-6} M) was first added to the organ bath (Fig. 2). Contractile force was ex-

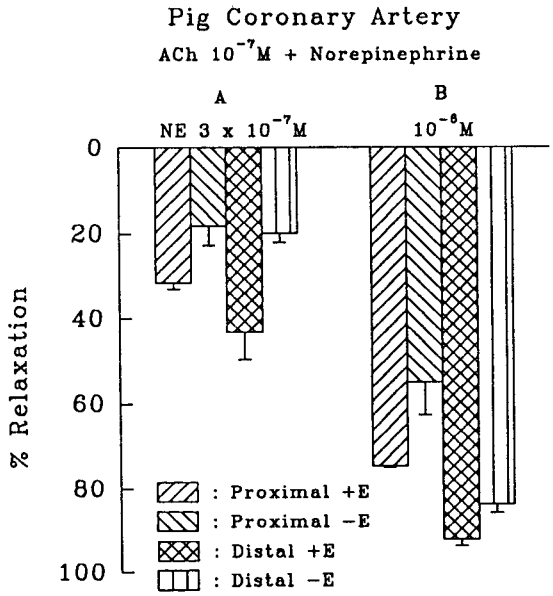

Fig. 1. Effects of norepinephrine (NE) on the ACh-induced contraction in the proximal and distal coronary arteries with intact endothelium (+E) and rubbed endothelium (-E). NE-induced relaxation in the distal coronary arteries was greater than that in the proximal coronary arteries. NE-induced relaxation was attenuated by the rubbing of endothelium in both arteries. A: 3×10^{-7} M NE B: 10^{-7} M NE. Ordinate: Percentage of relaxation to the contraction induced by 10^{-7} M ACh. Each bar shows the mean of percent relaxation (n=6) and each vertical line is S.E..

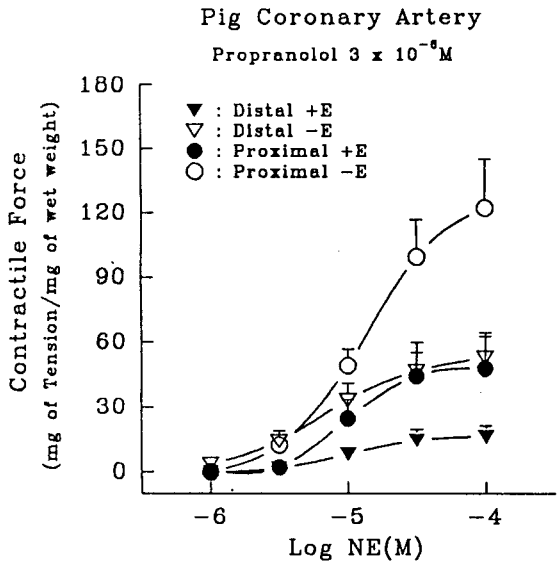

Fig. 2. Effect of endothelium on the contractions induced by norepinephrine (NE) after pretreatment with propranolol in the proximal and distal coronary arteries. NE-induced contraction in the proximal coronary artery was greater than that in the distal coronary artery. NE-induced contraction was potentiated by the rubbing of endothelium in both arteries. Each point shows the mean of contractile force (n=6) and each vertical line is S.E.. Intact endothelium (+E), Rubbed endothelium (-E)

pressed as mg tension per mg of wet weight. In the presence of propranolol, NE caused contraction of arterial strips with or without endothelium in a dose-dependent manner (NE 10^{-6} M~ 10^{-4} M). NE-induced contractions in the arterial strips without endothelium were significantly greater than those in the arterial strips with endothelium (at 10^{-4} M NE, proximal, $p<0.02$; distal, $p<0.05$). Proximal coronary artery was more sensitive to contraction caused by NE than distal coronary artery.

To evaluate the role of the α_1- or α_2-adrenoceptors in the coronary artery, β- and α_2-adrenoceptors or β- and α_1-adrenoceptors were blocked by antagonists and the arterial strip was exposed to NE (Fig. 3, 4). In the presence of propranolol (3×10^{-6} M) and yohimbine (5×10^{-7} M), NE caused vasoconstriction in a dose-dependent manner in both coronary arteries with endothelium. This α_1-adrenoceptor-mediated contraction by NE was significantly enhanced by removal of endothelium (at 10^{-4} M NE, proximal, $p<0.05$; distal, $p<0.01$) (Fig. 3). In the presence of propranolol (3×10^{-6} M) and prazosin (10^{-7} M), however, NE caused less contraction in both coronary arteries with endothelium (at 10^{-4} M NE; proximal 1.7 ± 1.0, distal 3.4 ± 1.2 mg T/mg wet weight). This α_2-adrenoceptor-mediated contraction by NE was significantly enhanced by removal of endothelium (at 10^{-4} M NE, proximal, $p<0.05$; distal, $p<0.01$) (Fig. 4).

In order to clarify that the difference of contractile force by NE between arterial strips with endothelium and without endothelium is due to release of EDRF, arterial strips were prein-

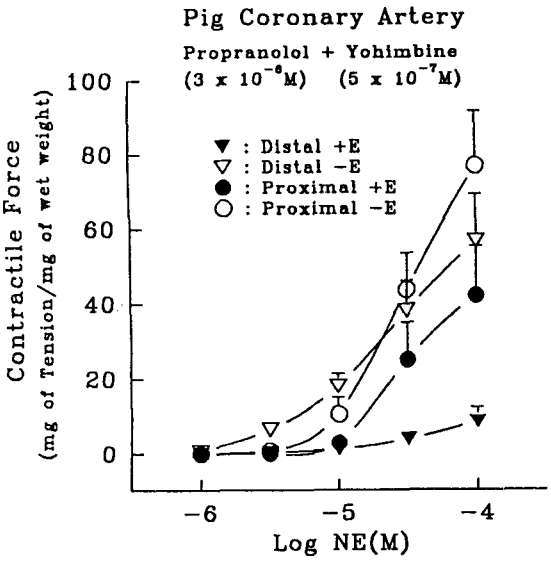

Fig. 3. Effect of endothelium on the contractions induced by norepinephrine (NE) after pretreatment with propranolol and yohimbine in the proximal and distal coronary arteries. NE-induced contraction in the proximal coronary artery was greater than that in the distal coronary artery. NE-induced contraction was potentiated by the rubbing of endothelium in both arteries. Each point shows the mean of contractile force (n=6) and each vertical line is S.E.. Intact endothelium (+E), Rubbed endothelium (-E)

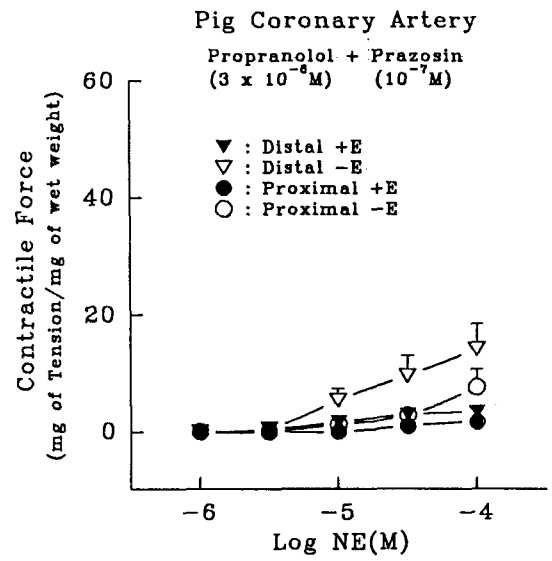

Fig. 4. Effect of endothelium on the contractions induced by norepinephrine (NE) after pretreatment with propranolol and prazosin in the proximal and distal coronary arteries. NE-induced contraction was potentiated by the rubbing of endothelium in both arteries. Each point shows the mean of contractile force (n=6) and each vertical line is S.E.. Intact endothelium (+E), Rubbed endothelium (-E)

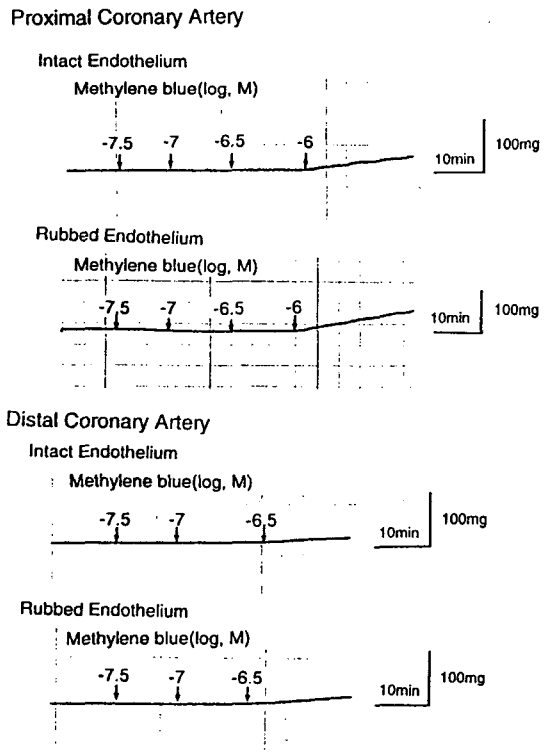

Fig. 5. *Contractile response induced by methylene blue (MB) in the pig coronary arteries. The optimal concentration that inhibits the action of EDRF but does not affect contraction was determined dose-dependently. In the proximal coronary arteries, the contraction began at the concentration of 10^{-6} M MB. In the distal coronary arteries, the contraction began at the concentration of 3×10^{-7} M MB. Therefore the optimal concentration of MB was determined in the proximal and distal coronary arteries as 3×10^{-7} M MB and 10^{-7} M MB, respectively*

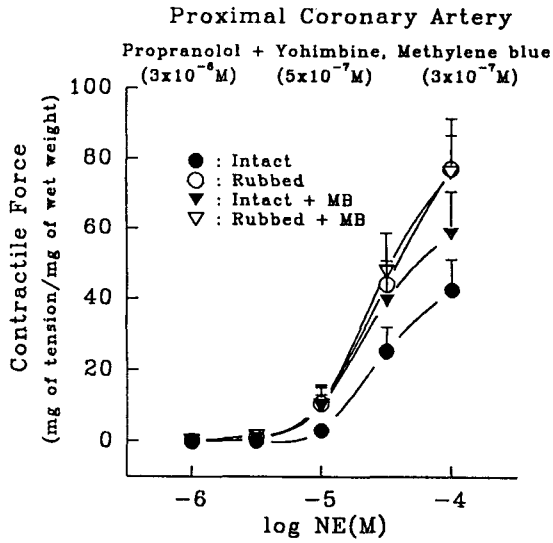

Fig. 6. *Effect of methylene blue (MB) on the NE-induced contraction after pretreatment with propranolol and yohimbine in the proximal coronary arteries with intact endothelium (Intact) and rubbed endothelium (Rubbed). NE-induced contraction in the strips with intact endothelium was significantly increased by the pretreatment with methylene blue. Each point shows the mean of contractile force (n= 6) and each vertical line is S.E..*

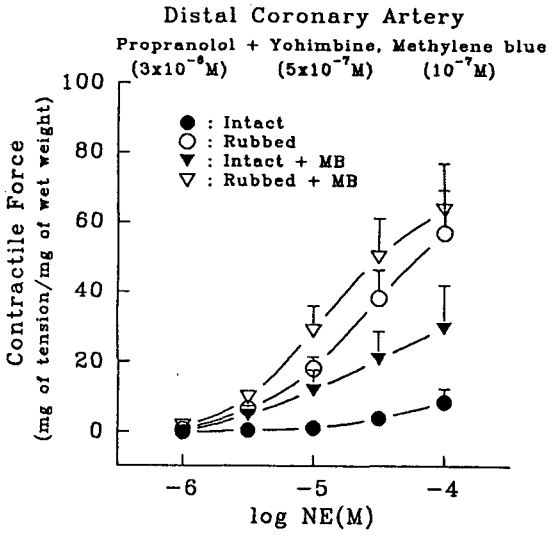

Fig. 7. *Effect of methylene blue (MB) on the NE-induced contraction after the pretreatment with propranolol and yohimbine in the distal coronary arteries with intact endothelium (Intact) and rubbed endothelium (Rubbed), NE-induced contraction in the strips with intact endothelium was significantly increased by pretreatment with methylene blue. Each point shows the mean of contractile force (n=6) and each vertical line is S.E..*

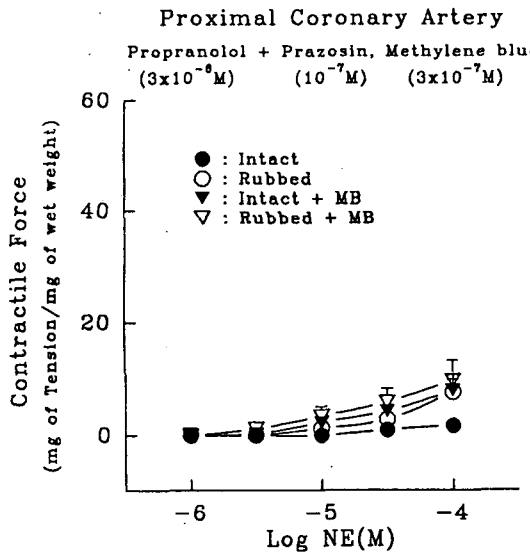

Fig. 8. Effect of methylene blue (MB) on the NE-induced contraction after pretreatment with propranolol and prazosin in the proximal coronary arteries with intact endothelium (Intact) and rubbed endothelium (Rubbed). NE-induced contraction in the strips with intact endothelium was significantly increased by pretreatment with methylene blue. Each point shows the mean of contractile force (n=6) and each vertical line is S.E..

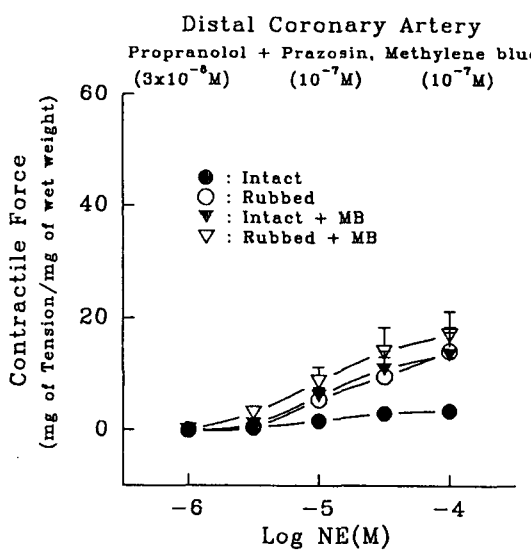

Fig. 9. Effect of methylene blue (MB) on the NE-induced contraction after the pretreatment with propranolol and prazosin in the distal coronary arteries with intact endothelium (Intact) and rubbed endothelium (Rubbed). NE-induced contraction in the strips with intact endothelium was significantly increased by the pretreatment with methylene blue. Each point shows the mean of contractile force (n=6) and each vertical line is S.E..

cubated with methylene blue (MB) for 15 minutes. MB is a well known inhibitor of EDRF (Martin et al, 1985). Exposure of arterial strips to a high concentration of MB changed the basal tone under resting conditions (Fig. 5). Therefore, arterial strip was preincubated with MB at concentration which did not affect the basal tone. In the presence of propranolol (3×10^{-6} M) and yohimbine (5×10^{-7} M), NE-induced contractions in the proximal and distal coronary arterial strips with endothelium were significantly enhanced by preincubation with MB (at 10^{-4} M NE, proximal, $p<0.05$; distal, $p<0.05$). However, the maximal amplitude of these contractions did not reach to the maximal level of contraction in arterial strips without endothelium (Fig. 6, 7). In the presence of propranolol (3×10^{-6} M) and prazosin (10^{-7} M), NE-induced contraction in the proximal and distal coronary arterial strips with endothelium was also significantly increased by preincubation with MB (at 10^{-4} M NE, proximal, $p<0.01$; distal, $p<0.05$) and these contractions reached the maximal level of contraction in arterial strips without endothelium (Fig. 8, 9).

DISCUSSION

The characterization of the adrenoceptor subtype population of coronary vasculature is important for the explanation of the mode of action of drugs on cardiac function. It is well known that norepinephrine (NE) has a different action on coronary arteries depending on the species and their location (Malindzak, 1982). The relaxing or contracting action of NE has

been attributed to segmental variations in the distribution of α- and β-adrenoceptors. With the recent discovery of endothelium-derived substances it seemed appropriate to re-examine the effects of norepinephrine on the coronary circulation.

In the present study, NE induced a concentration-dependent relaxation in both proximal and distal coronary arteries. The amplitudes of NE-induced relaxation in distal coronary arteries were greater than proximal coronary arteries. The removal of endothelium attenuated the vasorelaxation response to NE in both coronary arteries. In both arteries, however, NE-induced relaxation was converted to contraction after β-adrenoceptor blockade and this contraction was enhanced by removal of endothelium. NE-induced contration in the proximal coronary arteries was greater than distal coronary arteries. This finding is also apparant for dog (Cohen et al. 1983) and human coronary arteries (Godfraind & Miller, 1983). It indicates that both α- and β-adrenoceptors are present and the role of β-adrenoceptors is dominant in both arteries. However, the distribution of α-adrenoceptors showed a gradual decrease from proximal coronary arteries to distal coronary arteries. α-adrenoceptor-mediated contraction is opposed both by β-adrenoceptor-mediated relaxation and by endothelium-dependent vasodilatation. This may be explained by coronary circulation morphogenesis during which the proximal part of the epicardial arteries is formed from an outgrowth of aortic endothelial mesenchymal cells while the intramural arteries are derived from the myocardial mesemchyme (Conte & Pellegrini, 1984).

The effect of endothelium removal appears to be important for the action of NE after blocking β- and α_1-adrenoceptors (i.e., α_2-receptor stimulation) as well as after blocking β- and α_2-adrenoceptors, because this α_2-adrenoceptor stimulation induced less contraction in the strips with endothelium and induced significant contraction after removal of endothelium (Fig. 4). The results obtained in the present work suggested the importance of a functional endothelium in the modulation of α-adrenoceptor-mediated vasoconstriction by NE. The presence of an intact endothelium appears to exert an inhibitory effect upon the ability of the vessels to contract in response to α-adrenergic stimulation. The modulatory mechanism of endothelium on α-adrenoceptor-mediated contractions is unknown. but EDRF seems to be one of the factors involved in this process. It has been postulated that α-adrenergic stimulation could activate an endothelial receptor-mediated release of EDRF that could conteract the agonist-induced vasoconstriction; the removal of the endothelial layer would eliminate this mechanism and enhance the constrictor effect.

The precursor of EDRF appears to be L-arginine, which is transformed enzymatically by nitric oxide synthase to citrulline, yielding NO (Moncada et al, 1988). EDRF is released both toward the underlying vascular smooth muscle and toward the vascular lumen (Busse et al, 1985). EDRF induces relaxation of vascular smooth muscle by activating soluble guanylate cyclase and thus increasing the production of cyclic GMP (Cherry et al, 1982; Rapoport et al, 1983; Vanhoutte et al, 1986; Furchgott & Zawadzki, 1989).

Methylene blue and hemoglobin inhibit EDRF-mediated relaxation, and also inhibit the relaxation induced by vasodilator substances acting through the releasing or formation of nitric oxide (NO). In fact, methylene blue is an inhibitor of guanylate cyclase (Ignarro et al, 1985; Martin et al, 1985). In the present study, in order to see whether that the difference of contractile force between arterial strips with endothelium and without endothelium is due to release of EDRF. NE was added to arterial strips with or without endothelium before and after pretreatment with methylene blue. Methylene blue significantly increased α_1- and α_2-adrenoceptor-mediated contractions in the strips with endothelium, but did not significantly increase the same contractions in the strips without endothelium in the proximal and distal coronary arteries. These finding suggest that endothelial factors, especially EDRF, modulate α-

adrenergic activation by NE in pig coronary arteries.

α_1-adrenoceptor-mediated contraction by NE in the strips with endothelium was increased by pretreatment with methylene blue but these contractions did not reach the level without endothelium (Fig 6, 7). This finding suggest that other factors in addition to EDRF can modulate α-adrenoceptor activation, such as endothelium-derived hyperpolarizing factor (EDHF) (Feletou & Vanhoutte, 1988; Komori et al, 1989), or prostaglandin derivatives (Moncada & Vane, 1979).

From the above results, it is suggested that the effect of activation of α-adrenoceptors by NE may be modulated by endothelium, and endothelium-derived relaxing factor in the proximal and distal coronary arteries of pigs.

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