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Effects of Calcium Antagonists on Contractions of Chorionic Arteries in Normal and Preeclampsia Placenta

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This study was undertaken to observe the effects of organic or inorganic calcium antagonists and to investigate the involvement of cyclic nucleotides in regulating the vascular tone in the chorionic artery from normal or preeclamptic placenta. KCl and prostaglandin (PG) $F_{2\alpha}$ produced marked and constant contractions in chorionic arterial preparations of both normal and preeclamptic placentas. Nifedipine (NIF), verapamil (VER) and diltiazem (DIL) reduced the tension that had been produced by KCl and $PGF_{2\alpha}$ in a concentration-dependent fashion in both preparations, and the potency order of the three agents was NIF>VER>DIL. In preeclamptic arteries, however, the magnitudes of vasodilatations induced by NIF and DIL were much smaller than those in normal chorionic arteries. Mg^{2+} and Cd^{2+} also relaxed the tension induced by KCl and $PGF_{2\alpha}$. In preeclamptic chorionic artery, the vasodilatation induced by Mg^{2+} was significantly potentiated, while that by Cd^{2+} was not. Removing endothelium did not alter cyclic GMP content in both preparations. In both preparations contracted by $PGF_{2\alpha}$, nitroprusside markedly increased cyclic GMP content, but neither cyclic GMP nor cyclic AMP content was affected by acetylcholine, NIF, isoproterenol, or Mg^{2+}. The above results suggest that neither cyclic AMP nor cyclic GMP is involved in regulating the vascular tone of chorionic artery and that sensitivity of the artery in preeclampsia to the inhibitory action of calcium antagonist might be different from that in normal placenta.

Key Words : *Preeclampsia, Chorionic artery, Calcium channel antagonist, Endothelium, Cyclic GMP, Cyclic AMP*

INTRODUCTION

Though it is well known that the tone of systemic blood vessels are controlled by sympathetic nervous system, the regulatory mechanism of chorionic artery has yet to be clarified. It is reported that the blood vessels of the placenta and umbilical cord are not subjected to the regulation of sympathetic nervous system (Walker and McLean, 1971; Reilly and Russel, 1977) and that they sensitively respond to 5- hydroxytryptamine (5-HT), but neither to norepinephrine (NE) nor to isoproterenol (ISP). 5-HT, with its plasma concentration surging in the last trimester of pregnancy, has been implicated in the closure of umbilical vessels upon delivery (Jones and Rowsell, 1973) and in the pathomechanism of preeclampsia (Montenegro et al., 1985).

It has also been reported that impairment of uteroplacental circulation with subsequent increase in blood pressure, which is one of the most impor-

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tant features in preeclampsia (Lunell et al., 1982; Romero et al., 1988), is caused by imbalance of prostacyclin and thromboxane A_2, mediators regulating vascular tone (Walsh, 1985; Parisi and Walsh, 1989). Therefore, directly acting vasodilators like magnesium (Mg^{2+}) and some organic calcium antagonists, which have been reported to selectively dilate uteroplacental vessels, have been advocated in managing preeclampsia (Walters and Redmann, 1984; Altura et al., 1987). Mg^{2+} relaxes placental vessels by increasing prostacyclin production as well as by blocking calcium influx (Watson et al., 1986; Nadler et al., 1987). However, few reports are available on the roles of the calcium channel in preeclamptic placental vessels as compared with those from normal gravida.

This study was undertaken to attain clues on the pathomechanism of preeclampsia by observing the involvement of cyclic nucleotides in regulating the vascular tone of the chorionic artery and the effects of organic and inorganic calcium antagonists on the normal or preeclamptic chorionic artery.

MATERIALS AND METHODS

The whole placentas obtained from Department of Obstetrics and Gynecology, Chonnam University Hospital were transferred to the laboratory immersed in ice-cold physiological salts solution (PSS) immediately after delivery.

The criteria for preeclampsia were (1) hypertension (systolic pressure > 140 ㎜Hg, diastolic pressure > 90 ㎜Hg) with proteinuria (>100 ㎎/㎗), or (2) hypertension accompanied by systemic edema. The chorionic artery was excised and trimmed off fat and connective tissues. The artery was then cut into rings of 5 ㎜ in length. To prepare the denuded preparations, rings were gently rubbed 2 to 3 times with a metal rod inserted into the lumen of the ring. In this study, the chorionic arteries from normal gravidas were abbriviated as 'normal artery', and those from preeclamptic patients were as 'PreE artery'.

The preparations were fixed with 10% neutral buffered formalin at 4℃ and the sections around 4 ㎛ were obtained. The morphological intactness of the endothelium as well as the smooth muscle was confirmed with light microscopy after hematoxin-eosin staining.

Tension experiments: Ring segments of arteries were mounted in organ bath containing 20 ㎖ PSS, saturated with 95% O_2 and 5% CO_2 kept at 37℃ (pH 7.4) and the changes in tension were recorded on a polygraph.

Radioimmunoassay (RIA) of cyclic AMP and cyclic GMP: The ring preparations were incubated in 20 ㎖ PSS in beaker. After 2-hour equilibration the indicated drugs were added to the bath fluid and then the preparations were frozen and stored at -70℃ until use. Frozen tissues were weighed and homogenized in 1 ㎖ of 10% trichloroacetic acid at 1~4℃ and the homogenate was centrifuged at 2500× g for 30 min at 4℃. The pellet was used for protein assay and the supernatant was extracted 5 times with 4 ㎖ of water-saturated ether. The [^{125}I] RIA kits were used to determine cyclic AMP and cyclic GMP concentrations.

Drugs and Statistics: Composition of normal PSS in mM was 126.9 NaCl, 18.0 $NaHCO_3$, 1.18 KH_2PO_4, 4.7 KCl, 1.6 $CaCl_2$, 1.17 $MgSO_4$, and 5.5 glucose. Ca^{2+}-free PSS was prepared by omitting 1.6 mM $CaCl_2$ and by adding 0.026 mM EDTA. Mg^{+}-free PSS was prepared by omitting 1.17 mM $MgSO_4$ from normal PSS.

Norepinephrine (NE), phenylephrine (PE), acetylcholine (Ach), isoproterenol (ISP), endothelin I (ET-1), prostaglandin (PG) $F_{2\alpha}$, verapamil (VER), nifedipine (NIF) and nitroprusside (NP) were obtained from Sigma (USA), diltiazem (DIL) from Marion(USA), $MgSO_4$ and $CdCl_2$ from Merck (USA), trichloroacetic acid from Fisher Scientific (USA), and [^{125}I] RIA kits from DuPont (USA). All drugs except NIF were dissolved in and diluted with distilled water. NIF was dissolved with 95% ethanol to make 10^{-3} M stock solution, and then diluted with distilled water immediately before use.

Statistical significance ($p<0.05$) was examined by unpaired Student's t-test and two way ANOVA with repeated measure.

RESULTS

Effects of vasoconstrictors and vasodilators

KCl: KCl contracted normal arteries in a dose-dependent fashion and its ED_{50} was 31.7±1.4 mM (mean±SEM, n=8). ED_{80}, the concentration eliciting 80% of the maximal response, was 48.8±1.6 mM, and the active tension produced at this concent-

ration amounted to 2.9±0.1 g (data not shown). This KCl-dose was employed as the precontracted reference in testing the vasodilators. The ED_{50} or ED_{80} as well as maximal contraction of PreE artery did not differ from those of normal artery.

$CaCl_2$: In Ca^{2+}-free PSS containing 48.8 mM KCl, adding 2~10 mM $CaCl_2$ produced vasoconstriction dose-dependently, but higher doses over 10 mM decreased the magnitudes of vasoconstriction (Fig. 1 and Table 1).

α-Adrenoceptor agonists: Neither NE nor PE contracted both arterial preparations (n=5; data not shown).

ET-1: In normal artery, 10^{-8} M ET-1 produced slight contraction as much as 0.6±0.1 g (n=11). However, higher doses produced no greater contraction (data not shown).

$PGF_{2\alpha}$: 10^{-5} M $PGF_{2\alpha}$ produced marked and sustained vasoconstriction in both the normal and the PreE artery. The magnitudes of vasoconstriction in the normal artrery was 3.5±0.4 g (n=28), not differing from those in PreE (3.9±0.4 g, n=25) (Fig. 2). Therefore, the 10^{-5} M $PGF_{2\alpha}$ was employed to investigate the vasodilatory effects of calcium antagonists in further study.

ISP and ACh: The potent peripheral vasodilators, ISP and ACh did not affect the normal and PreE artery with or without intact endothelium at all (n=7, Fig. 2).

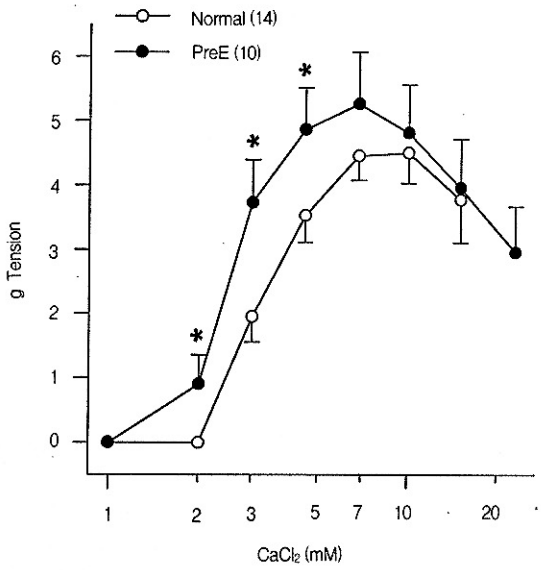
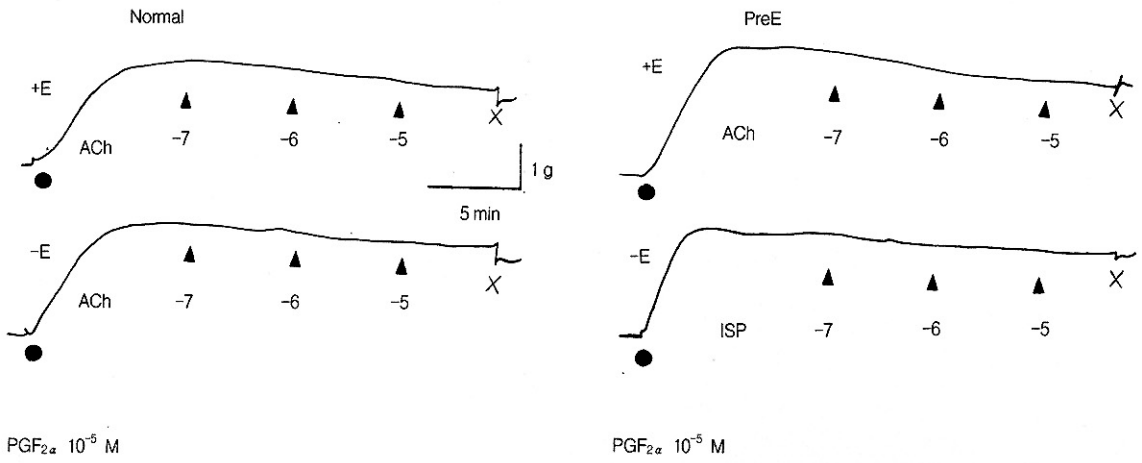


Fig. 1. Concentration-response curves to $CaCl_2$ in normal and preeclamptic (PreE) chorionic arterial rings. All rings were pretreated with calcium free physiologic salt solution. The $CaCl_2$ was added to the bath in a cumulative fashion, i.e., after the equilibrium tension had been reached, the next dose was added. Each point represents the mean±SEM. Numerals in parentheses are the number of experiments. Asterisks indicate the significant differences between normal and PreE arteries (* $p<0.05$).

Table 1. Comparisons of ED_{50} or IC_{50}s between normal and PreE artery.

Agents	PreTx.	Normal		Significance	PreE	
		No.	ED_{50} or IC_{50}		No.	ED_{50} or IC_{50}
$CaCl_2$	-	14	3.5 ± 0.17 mM	**	10	2.6±0.08 mM
NIF	KCl	6	$1.5 \pm 0.16 \times 10^{-7}$ M	**	11	$7.7 \pm 1.4 \times 10^{-7}$ M
VER	KCl	8	$6.8 \pm 1.2 \times 10^{-7}$ M			†
DIL	KCl	13	$4.1 \pm 0.56 \times 10^{-6}$ M	**	10	‡
Mg^{2+}	KCl	12	13.9 ± 2.15 mM	**	13	5.2 ±0.75 mM
Cd^{2+}	KCl	7	0.26 ± 0.03 mM		7	0.31±0.07 mM
NIF	$PGF_{2\alpha}$	7	$1.0 \pm 0.11 \times 10^{-7}$ M	**	5	$3.6 \pm 0.45 \times 10^{-6}$ M
DIL	$PGF_{2\alpha}$	4	$1.7 \pm 0.21 \times 10^{-6}$ M		7	$2.3 \pm 0.34 \times 10^{-6}$ M
Mg^{2+}	$PGF_{2\alpha}$	10	5.2 ± 0.52 mM	**	6	1.3 ±0.26 mM
Cd^{2+}	$PGF_{2\alpha}$	6	0.34 ± 0.05 mM		7	0.45±0.06 mM

Asterisks indicate significant differences between normal and PreE arteries (** $p<0.01$). 'PreTx.': Pretreated vasoconstrictors to evaluate the vasodilatory effects of calcium channel antagonists. '†': VER-induced vasodilatation in PreE artery was not examined. '‡': IC_{50} of DIL-induced vasodilatation could not be calculated.



$PGF_{2\alpha}$ 10^{-5} M $PGF_{2\alpha}$ 10^{-5} M

Fig. 2. Typical traces showing effects of acetylcholine (ACh) and isoproterenol (ISP) on $PGF_{2\alpha}$-induced contractions in chorionic arterial rings with (+E) and without (-E) endothelium. Left two traces were obtained from a normal (Nor) placenta and right from a preeclamptic (PreE) placenta. Drug concentrations are 10^{-x} M.

Effects of calcium antagonists on the contraction induced by KCl and PGF2α

In this study, NIF, VER, and DIL were used as organic calcium channel blockers, whereas Mg^{2+} ($MgSO_4$) and Cd^{2+} ($CdCl_2$) were employed as inorganic calcium antagonists.

Calcium antagonists in KCl-contraction: All of the three organic antagonists relaxed the 48.8 mM KCl-induced tension in a dose-dependent fashion, but their dilatory potencies were different. In normal arteries, the ranks of IC_{50}s (inhibitory concentrations of 50%) of these antagonists were NIF>VER>DIL, indicating that NIF was the most potent among them (Fig. 3, Table 1). In the PreE artery, however, the maximal dilatation and dilatory potency induced by either NIF or DIL were significantly attenuated than in normal artery, i.e., IC_{50} of NIF in the PreE artery was five-fold greater than that in the normal artery, and IC_{50} of DIL could not be calculated because the magnitude of the maximal dilatation did not reach 50% (Fig. 4).

The inorganic calcium antagonists, Cd^{2+} and Mg^{2+}, relaxed the KCl-contraction of the normal and PreE arteries in a dose-dependent fashion. Cd^{2+}-induced relaxing responses did not significantly differ in both arteries, whereas Mg^{2+} reduced the tension more sensitively in the PreE artery than in the normal artery (Table 1, Fig. 5).

Calcium antagonists in $PGF_{2\alpha}$-contraction: NIF and DIL relaxed the $PGF_{2\alpha}$-contraction of both arteries in a dose-dependent manner. NIF-induced relaxation was much greater in the normal artery than in the

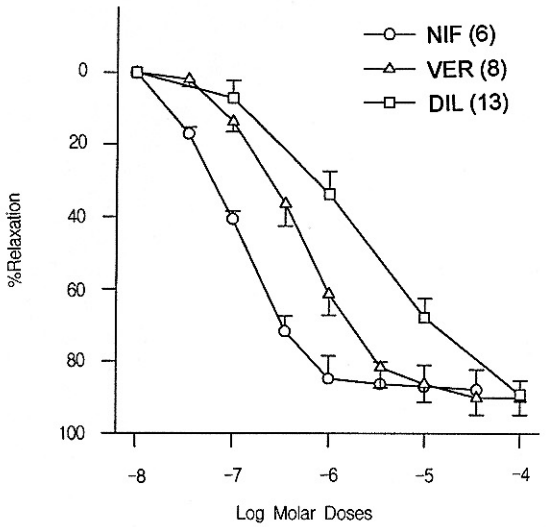


Fig. 3. Inhibitory effects of nifedipine (NIF), verapamil (VER) and diltiazem (DIL) on the KCl 48.8 mM-induced contractions in normal placental chorionic arteries. Other legends are in Fig. 1.

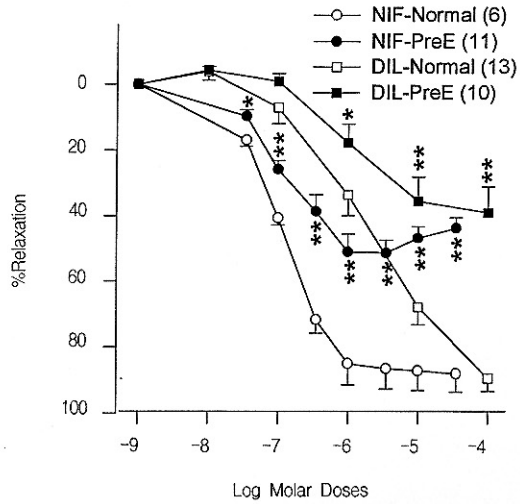


Fig. 4. Inhibitory effects of nifedipine (NIF) and diltiazem (DIL) on the KCl 48.8 mM-induced contractions in chorionic arterial rings obtained from normal and preeclamptic (PreE) placentas. Asterisks indicate the significant differences between normal and PreE arteries (* $p<0.05$; ** $p<0.01$). Other legends are in Fig. 1.

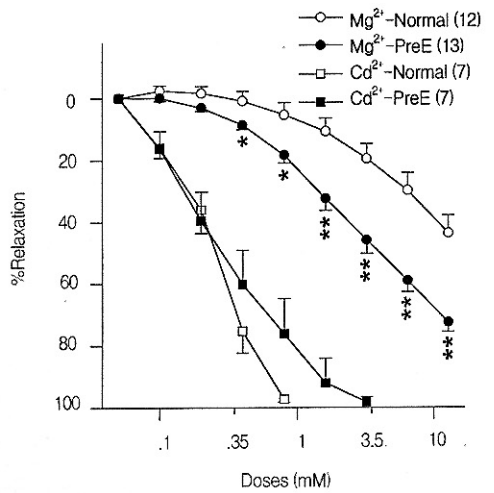


Fig. 5. Inhibitory effects of Mg^{2+} and Ca^{2+} on the KCl-induced tension in normal and preeclamptic chorionic arterial rings. Other legends are in Fig. 4.

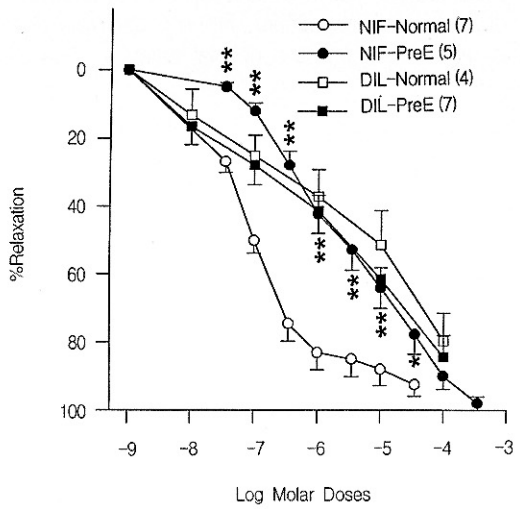


Fig. 6. Inhibitory effects of nifedipine (NIF) and diltiazem (DIL) on 10^{-5} M $PGF_{2\alpha}$-induced tension in normal and preeclamptic chorionic arterial rings. Other legends are in Fig. 4.

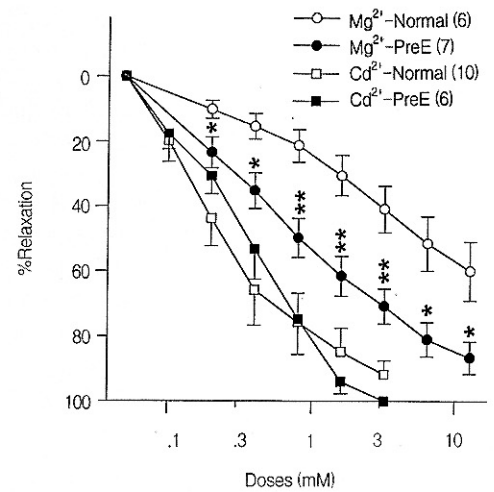


Fig. 7. Inhibitory effects of Mg^{2+} and Cd^{2+} on 10^{-5} M $PGF_{2\alpha}$-induced contraction in normal and preeclamptic chorionic arterial rings. Other legends are in Fig. 4.

PreE, i.e., the IC_{50} of the former is 1/36 of that of the latter. But the relaxing response to DIL did not differ between both arteries (Table 1, Fig. 6).

Cd^{2+} and Mg^{2+} relaxed the $PGF_{2\alpha}$-contraction in a dose-dependent fashion as they did in the KCl-contracted preparations. The relaxing response to

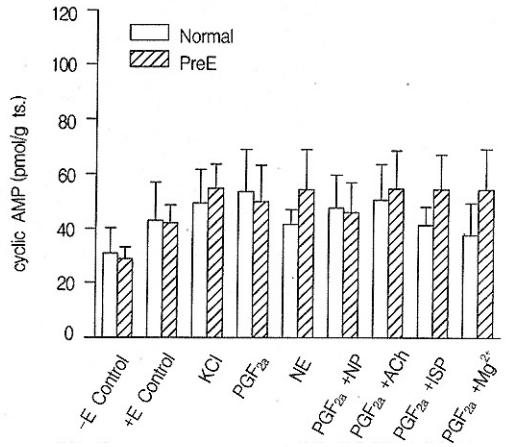


Fig. 8. Contents of cyclic AMP in chorionic arterial tissues of normal and preeclamptic placentas incubated with the indicated vasoactive agents for 5 to 20 min. All tissues excepts the -E control are endothelium-intact preparations. Each column represents mean±SEM from 4 rings. -E: endothelium-removed preparation; +E: endothelium-intact preparation; $PGF_{2\alpha}$: 10^{-5} M prostaglandin $F_{2\alpha}$; NE: 10^{-5} M norepinephrine; NP: 10^{-6} M nitroprusside; ACh: 10^{-5} M acetylcholine; ISP: 10^{-6} M isoproterenol.

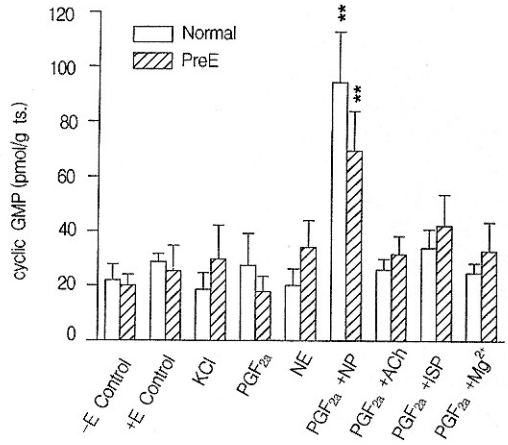


Fig. 9. Contents of cyclic GMP in chorionic arterial tissues of normal and preeclampsia placentae incubated with the indicated vasoactive agents for 5 to 20 min. Asterisks indicate significant difference from control ($p<0.01$). Other legends are the same as those of Fig. 8.

Mg^{2+} was more sensitive in the normal than in the PreE artery, but the response to Cd^{2+} did not differ in both preparations (Table 1, Fig. 7).

Cyclic AMP and cyclic GMP Contents

Removing the endothelium did not affect either of the cyclic nucleotides contents in the normal and PreE arterial preparations. The preparations were exposed to the indicated drugs for the period that maximal responses were observed in the tension experiments. Each incubation condition was as follows: 48.8 mM KCl for 10 min, 10^{-5} M $PGF_{2\alpha}$ for 10 min, 10^{-6} M NE for 5 min, 10^{-5} M $PGF_{2\alpha}$ for 10 min + 10^{-6} M NP for 5 min, 10^{-5} M $PGF_{2\alpha}$ for 10 min + 10^{-6} M ACh for 5 min, and 10^{-5} M $PGF_{2\alpha}$ for 10 min + ISP for 5 min, 10^{-5} M $PGF_{2\alpha}$ for 10 min + 5.2 mM Mg^{2+} for 20 min. In all conditions except 10^{-5} M $PGF_{2\alpha}$ for 10 min + 10^{-6} M NP for 5 min incubation, no significant changes in the cyclic AMP and cyclic GMP contents were observed in both arterial preparations. The cyclic GMP contents in the preparations incubated with $PGF_{2\alpha}$ + NP were significantly increased in the normal and PreE artery (Fig. 8 and 9).

DISCUSSION

Altura et al. (1972) and Maigaard et al. (1986) reported that the human chorionic artery did not respond to NE but it was sensitively contracted by 5-HT or prostaglandins. In this study, it was observed that the normal and PreE arteries did not respond to NE, nor to PE, and that ISP did not affect the $PGF_{2\alpha}$-induced contraction as well as the tissue cyclic AMP contents. These results are in agreement with the previous reports that umbilicoplacental vessels are not influenced by the autonomic nervous system (Reilly and Russell, 1977) and that they did not respond to α-adrenoceptor agonists (Reviriego et al., 1990) and a β-agonist, ISP (Dyer, 1970)

The contraction induced by $PGF_{2\alpha}$ in the normal and PreE artery with or without intact endolthelium was not affected by Ach, which elicited the endothelium-dependent vasodilatation (Furchgott, 1983). Besides, neither Ach nor removing endothelium did alter the cyclic GMP contents, which were, in contrast, markedly increased by the NP-treatment. ET-1

is well-known as a powerful vasoconstrictor abundant in vascular endothelium (Sanchez-Ferrer and Marin, 1990). However, the maximal tension induced by ET-1 was about six-fold smaller than that by $PGF_{2\alpha}$. From the above results we propose a hypothesis that the endothelium in human chorionic artery is stays out in regulating the vascular tone.

The PreE artery was much more susceptible to $CaCl_2$ than the normal. And the rank of inhibiting potencies of the calcium antagonists in both of the arterial preparations precontracted by KCl or $PGF_{2\alpha}$ was NIF>DIL>>Cd^{2+}>Mg^{2+} on the base of molar IC_{50}. However, the inhibiting potencies of these agents in the normal artery did not even partly correspond to the potencies in PreE artery. The vasodilatory effect of NIF was more significantly attenuated in the PreE artery than in the normal. However, the effect of Mg^{2+} was more signifcantly potentiated in the PreE than in the normal artery. It is well known that KCl increases calcium influx through the voltage-dependent calcium channel (VDC) (Schwartz and Taira, 1983; Dube et al., 1985) and that $PGF_{2\alpha}$ increases intracellular calcium concentration by calcium influx via the receptor-operated calcium channel (ROC) (Godfraind and Miller, 1982) as well as by release of calcium from intracellular stores (Mikkelson and Anderson, 1978; Usune et al., 1989). NIF and DIL block the VDC (Cauvin et al., 1983), and Mg^{2+} and Cd^{2+} also inhibit the VDC by binding to cell membrane (Toda, 1973; Levine and Coburn, 1984; Nasu, 1984). Therefore, it is suggested that the contraction induced by either KCl or $PGF_{2\alpha}$ and the inhibitory effect of the calcium antagonists on the contraction are closely related to the calcium influx via the VDC and that the characteristics of calcium channels in chorionic artery could be changed in the process of preeclampsia.

However, despite the similarity of these four calcium antagonists in vasodilatory mechanisms, it is difficult to distinctly explain the reason why Mg^{2+}-induced vasodilatation is potentiated but the NIF-induced one is decreased in the PreE artery. Recently, VDC was subdivided into several types such as L, T, N, and P (Spedding and Paoletti, 1992), and NIF, DIL and Cd^{2+} is reported to be sensitive to L-type calcium channel (Hume, 1985; Yatani et al., 1988). In contrast, the vasodilatory mechanism of Mg^{2+} is still controversial, i.e., the vasodilatation was reported to result from the reduction of calcium influx through VDC and the inhibition of the actions of intracellular calcium (Saida, 1982; Karaki et al., 1983), and from the regulation of the Na^{+}-Ca^{2+} exchanger (Altura and Altura, 1974). Watson et al. (1986) and Nadler et al. (1987) also suggested that Mg^{2+} increases the production of prostacyclin in endothelium, whereas Skajaa et al. (1990) reported that neither prostanoids nor endothelium is related to Mg^{2+}-induced vasorelaxation.

Conclusively, our present observations as well as other reports cited above infer that neither endothelium nor cyclic nucleotides is involved in regulating the vascular tone of the chorionic artery and suggest that either the sensitivity of L-type calcium channels is in part blunted or a certain mechanism involving Mg^{2+}-induced relaxation is sensitized in the process of preeclampsia.

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