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# Modification of Endothelium on Contractile Response of Brain Vessels to Contracting Agents

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## ABSTRACT

To delineate the mechanisms of vasoconstriction and vasodilation in cerebral arteries the effects of some vasoconstrictors and calcium antagonists on the basilar artery (BA) and arterial circle of Willis (WC) were examined and also the role of endothelium in the action of these drugs was investigated in pigs, cats and rabbits. In pig cerebral arteries, dose-dependent contractile responses were elicited by KCl, histamine, 5-hydroxytryptamine (5-HT) and angiotensin, but norepinephrine (NE), phenylephrine (PE) and epinephrine (EP) elicited dose-dependent contractions only under pretreatment with propranolol $10^{-6}$ M. The magnitudes of maximal contractile effects of these drugs were different from each other, and 5-HT was the largest and angiotensin the smallest. Some calcium antagonists dose-dependently inhibited KCl (35 mM)-induced contraction and the order of potency in inhibiting the contraction was nifedipine >> diltiazem > flunarizine > oxybutynin > isosorbide dinitrate (ISDN) > glyceryl trinitrate. 5-HT ($10^{-5}$ M)-induced contraction was dose-dependently inhibited by nifedipine but slightly inhibited by diltiazem and ISDN. In rings with intact endothelium, KCl (35 mM)-induced contraction was not affected by acetylcholine (ACh) but $PGF_{2\alpha}$ ($10^{-5}$M)-induced contraction was dose-dependently relaxed by ACh and adenosine. This endothelium-dependent relaxation was not affected by nifedipine ($10^{-6}$M)-pretreatment but markedly inhibited by methylene blue (50 $\mu$M)-pretreatment. In the porcine arterial rings without endothelium, ACh had no effect or even contracted the $PGF_{2\alpha}$-induced contraction. However, the dose-dependent relaxing effect of ACh appeared when the deendothelized porcine ring and rabbit thoracic aorta with intact endothelium were simultaneously suspended into a bath and this relaxing effect was also inhibited by methylene blue-pretreatment. In cat cerebral arteries, 5-HT and NE elicited dose-dependent contractile responses and ACh also produced dose-dependent contraction regardless of the existence of endothelium. ACh-induced contraction was most prominent. 5-HT ($10^{-5}$M)-induced contraction was not relaxed but contracted additionally by ACh even in the intact endothelial ring. In rabbit cerebral arteries, 5-HT and NE elicited dose-dependent contractile responses and 5-HT-induced contraction was more prominent. In the intact endothelial preparations, 5-HT ($10^{-5}$ M)-induced contraction was markedly relaxed by the addition of ACh($10^{-5}$M) and this endothelium-dependent relaxing effect was inhibited by atropine ($10^{-7}$M)-pretreatment but not affected by diltiazem ($10^{-6}$M)-pretreatment. These results suggest that ACh elicits endothelium-dependent relaxing effect mediated by muscarinic receptors in cerebral arteries of pig and rabbit, and that ACh acts as vasoconstrictor in cat cerebral artery.

**Key Words**: Brain artery, Endothelium, Vasoconstrictor, Calcium antagonist, Pig

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## INTRODUCTION

It is well-known that the appropriate functional maintenance of central nervous system is primarily dependent on the supply of oxygen and glucose via cerebral blood flow, which is mainly controlled by the diameter of cerebral arteries and arterioles. The possible mechanisms of constriction and dilatation of cerebral blood vessels have been investigated by many researchers. Some morphological studies have demonstrated that there are two types of nerve endings in the adventitial layer of cerebral arteries: granular vesicle-containing nerves and agranular vesicle-containing nerves. The former is of sympathetic origin mediating vasoconstriction, and the latter is of non-sympathetic origin mediating vasodilatation (Iwayama *et al.*, 1970; Owman *et al.*, 1974; Lee, *et al.*, 1980; Lee, 1981). Studies in vitro about function of cerebral arteries are very difficult because the vascular wall is very thin and the diameter is too small to make the ring preparation. Therefore the mechanisms of vasoconstriction and vasodilatation of brain arteries in controlling cerebral blood flow still remain controversial (Heistad and Marcus, 1978; Purves, 1978; Saito and Lee, 1985).

Since Furchgott and Zawazki (1980) had first reported that the relaxing effect of acetycholine (ACh) is caused by the releases of endothelium-derived relaxing factor (EDRF) from the observation that ACh relaxed the isolated rabbit thoracic aortic rings with intact endothelium precontracted by norepinephrine (NE) but did not relax the rings without endothelium. The endothelium-dependent vasodilatation has been observed in many vessels of several species (Furchgott 1983). Substances known as vasodilator were reclassified into endothelium-dependent and endothelium-independent vasodilators. The former includes A23187, ACh, ATP, bradykinin, histamine and hydralazine and the latter diazoxide, minoxidil, papaverine, prostacyclin ($PGI_2$), cyclic AMP, cyclic GMP, EDRF, atrial natriuretic factor and glyceryl trinitrate (Peach *et al.*, 1985). It has been reported that $Ca^{2+}$ takes part in the release of EDRF, so the release of EDRF is dependent on extracellular $Ca^{2+}$ (Long and Stone, 1985). In addition, studies about relationship between cardiovascular diseases and endothelial cells revealed that the damge to endothelial cells are implicated to atherosclerosis and certain types of hypertension (Chobanian *et al.*, 1984; Jayakody *et al.*, 1985; Freiman *et al.*, 1986; Sreeharan *et al.*, 1986; Miller *et al.*, 1987).

In clinical sciences, although the pathophysiological mechanisms of cerebral vasospasm are not clarified, mamy experimental data support the hypothesis that cerebral vasospasm may be induced by the activation of releases of spasmogen derived from blood, cerebrospinal fluid or vascular wall (Cook, 1984). It has been reported that either 5-hydroxytryptamine (5-HT) or catecholamines act as a spasmogen in cerebral hemorrhage (Allen *et al.*, 1974; Lobato *et al.*, 1980; Loach and Benedict, 1980), and that glyceryl trinitrate and calcium antagonists inhibit the cerebral vasospasm (Kistler *et al.*, 1979; Allen and Bahr, 1979). To delineate the mechanisms of vasoconstriction and vasodilatation of cerebral arteries, the pharmacological effects of some vasoconstrictors and calcium antagonists on the tension of cerebral arterial rings were examined and also the role of endothelium in cerebral arteries was investigated in pigs, cats and rabbits.

## MATERIALS AND METHODS

**Preparation of cerebral artery**:Pigs of either sex were decapitated as soon as slaughtered and cold (1-4℃) physiological salt solution (PSS) was infused through formen magnum and the head was immersed in cold PSS in a local slaughterhouse and transferred to laboratory within 20 min. The whole brain with intact arteries was rapidly removed. Basilar artery (BA) and arterial circle of Willis (anterior and posterior communicating artery, WC) were isolated under stereoscope and the artery was cut into rings of 4~5 mm length. Cats (B.W.: 2.2-3.3 kg) and rabbits (B.W.: 1.8-2.4 kg) of either sex were anesthesized with intraperitoneal injections of pentobarbital sodium (30 mg/kg) and killed by bleeding from carotid arteries. Other methods are the same as in pigs.

**Recording of mechanical activity**: Ring segments of arteries were mounted in a muscle bath by sliding ring over two parallel stainless steel hook (0.15 mm in diameter). The lower hook was fixed on bottom of the bath and the upper was connected to isometric transducer (Grass FT. 03). The signal from the transducer was displayed on a Grass Instruments model 7D polygraph. The volume of bath was 10 ml and the bath solution was saturated with 95% $O_2$ and 5% $CO_2$, at 37℃ (pH=7.3). The

composition ( mM) of PSS was: 119 NaCl, 4.9 KCl, 1.6 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 11.1 glucose.

During equilibration period of 2 hours, the resting tension was adjusted to 0.5 g(pig) and 0.3 g(cat and rabbit). After this equilibration period, the rings were challenged with 35 mM KCl two times and then main experments were started. Vasoconstrictors were cumulatively administered into bath in order to obtain dose-response curves. In the experiments to observe the relaxing effects of some drugs on the 35 mM KCl-induced contraction, the relaxants were administered cumulatively following the development of steady-state tension by 35 mM KCl. In the experiments in which the effect of calcium antagonists on 5-hydroxytryptamine (5-HT)-induced contraction were observed, the rings were challenged with $10^{-5}$M 5 -HT two times as a control and then the same dose of 5-HT was added to the bath 20 min after pretratment with calcium antagonists. The concentrations of calcium antagonists were increasing step by step and the data were expressed as % of control contraction.

**Experiment observing the effect of EDRF**: The endothelium was removed by gently rubbing with a steel pin inserted into the lumen of the arterial ring. The effects of ACh were compared in the deendothelized rings and in the intact endothelial rings. In order to observe the effect of heterogenous endothelial cells the following procedure was taken. A rabbit thoracic aortic strip (2 cm) with endothelium as the donor of EDRF was simultaneously put in an organ bath in which a deendothelized cerebral ring of pig was mouted. As the control experiment, another rabbit thoracic aortic strip (2 cm) without endothelium was mounted in another bath with a deendothelized cerebral ring of pig.

Drugs used were norepinephrine bitartrate (Sigma), epinephrine bitartrate (Sigma), phenylephrine HCl (Sigma), 5-hydroxytryptamine creatinine sulfate (Sigma), histamine HCl (Sigma), angiotensin II (Sigma), isoproterenol HCl (Sigma), $PGF_{2\alpha}$-tromethamine salt (Upjohn), adenosine (9-$\beta$--D-ribofuranosyladenine, Sigma), acetylcholine bromide (Sigma), propranolol HCl (Sigma), atropine sulfate (Sigma), nifedipine HCl (Sigma), oxybutynin HCl (Marion), isosorbide dinitrate (Ives), flunarizine HCl (Sigma), diltiazem HCl (Sigma) and methylene blue (Merck). The stock solution ($> 10^{-3}$M), of nifedipine and isosorbide dinitrate were dissolved in 95% ethanol and others were dissolved in distilled water. The experiments involving nifedipine, a light sensitive drug, were carried out under dark condition.

## RESULTS

The endothelium was preserved throught the study unless specified otherwise. Whether or not the endothelium was intact was determined by examining the ability of ACh to elicit relaxation as well-documented in peripheral vessels (Furchgott 1983). If ACh relaxed the precontracted rings, the preparations were regarded as intact endothelial rings and if ACh produced additional contraction or no relaxation, they were as deendothelial rings.

### Porcine cerebral arteries

**Basilar artery**: Norepinephrine (NE) and epinephrine (EP) generally produced relaxations in BA rings but caused dose-dependent contractions in the rings pretreated with $10^{-6}$M propranolol, a $\beta$-adrenoceptor antagonist (Fig. 1, 2). Histamine and KCl produced dose-dependent contractions. The $EC_{50s}$ (effective concentration producing 50% of maximal contraction) of NE, EP and histamine were $3.2\times10^{-7}$M, $2.5\times10^{-8}$M, and $1.9\times10^{-7}$M, respectively. KCl-induced contraction was maintained but the contractions induced by the other agonists showed spontaneous decline of tension following a peak amplitude. Following the development of steady-state 35 mM KCl-induced tension, the additions of diltiazem, nifedipine and isosorbide dinitrate (ISDN) produced dose-dependent relaxation (Fig. 5). The concentrations of nifedipine, diltiazem and ISDN required to relax 35 mM KCl-induced contraction to 50%($IC_{50}$) were $5.1\times10^{-9}$M, $3.7\times10^{-7}$M, and $3.8\times10^{-5}$M, respectively.

**Arterial circle of Willis**: In this preparation, NE also could not produce any contractions as in BA. However, EP produced dose-dependent contrac tions without propranolol pretreatment. In proranolol-pretreated rings, dose-dependent contraction was elicited by NE and phenylephrine (PE)(Fig. 1,2), and EP-induced contractions were more potentiated. 5-Hydroxytrypamine (5-HT) or angiotensin also produced a dose-dependent contraction (Fig. 1,3). $EC_{50s}$ of NE, 5-HT, PE and angiotensin were $2.1\times10^{-7}$M, $5.0\times10^{-8}$M, $4.0\times10^{-6}$M, and $1.9\times10^{-8}$M, respectively.

It is surprising that angiotensin, known as the

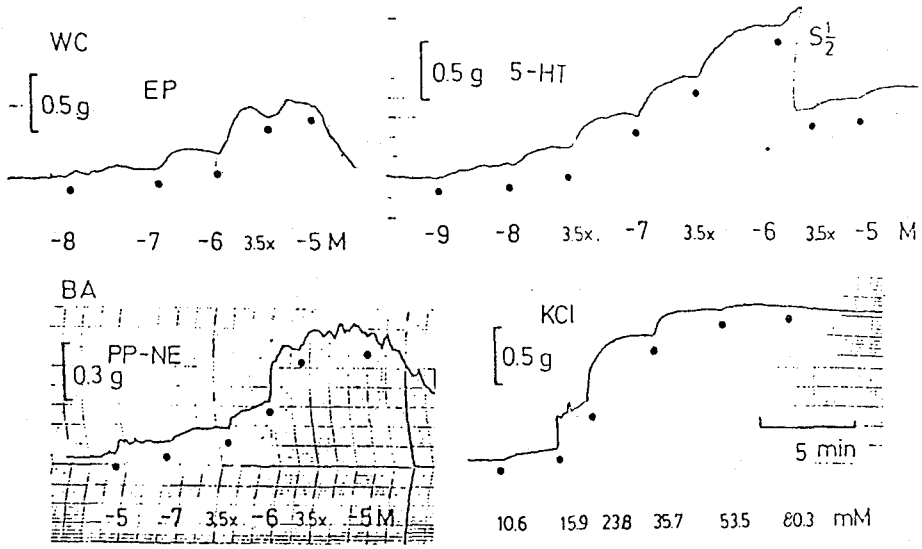


**Fig. 1.** Typical traces showing cumulative dose-dependent contractile responses to some drugs in porcine cerebral arterial rings obtained from arterial circle of Willis (WC upper traces) and basilar artery (BA, lower traces). EP: epinephrine, 5-HT; 5-hydroxytryptamine, PP-NE: norepinephrine was added after pretreatment with propranolol $10^{-6}$ M. At dots, the indicated dose (log M) of each drug was added to the bath. At $S_{\frac{1}{2}}$, amplitude of pen sensitivity was decreased as a half.

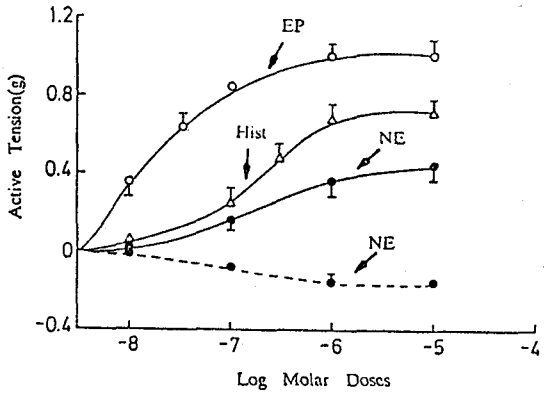


**Fig. 2.** Dose-response curves to epinephrine (EP), histamine (Hist) and norepinephrine (NE) without (dashed line) and with propranolol-pretreatment (solid line) in porcine basilar arterial rings. Each point indicates mean± SEM from 7~15 rings.

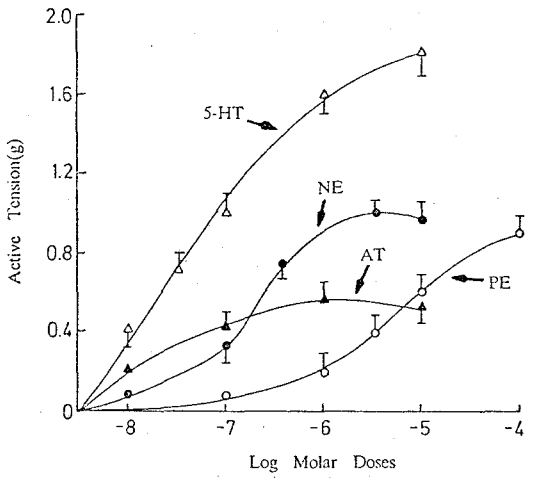


**Fig. 3.** Dose-response curves to 5-hydroxytryptamine (5-HT), norepinephrine (NE), phenylephrine (PE) and angiotensin (AT) in porcine arterial rings obtained from arterial circle of Willis. Each point indicates mean±SEM from 9~17 rings.

most powerful vasoconstrictor in peripheral artery, had the least value of $EC_{50}$ but produced the least maximal contraction among 4 drugs used in this study. And it is interesting that the $EC_{50}$ of NE under propranolol-pretreatment was almost same in both BA and WC. While ACh produced small contractile response in some WC rings, ACh did

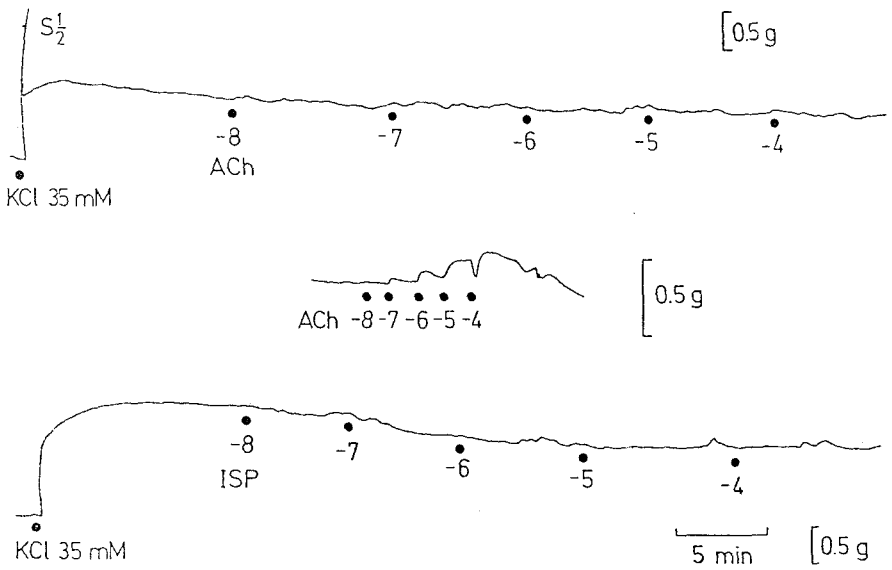


**Fig. 4.** Dose-response curves to 5-hydroxytryptamine (5-TH), norepinephrine (NE), phenylephrine (PE) and angiotensin (AT) in porcine arterial rings obtained from arterial circle of Willis. Each point indicates mean±SEM from 9~17 rings.

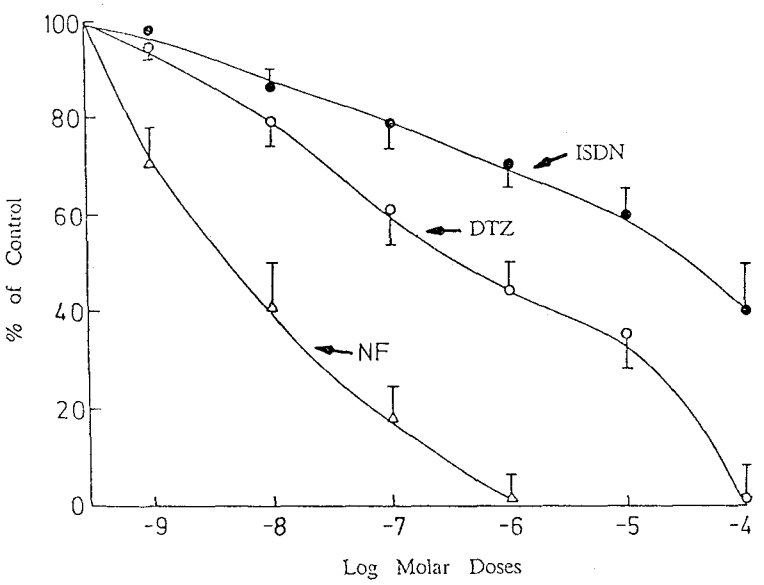


**Fig. 5.** Effects of isosorbide dinitrate (ISDN), diltiazem (DTZ) and nifedipine (NF) on the KCl (35 mM)-induced contraction in the porcine basilar arterial rings. Each point indicates mean± SEM from 9~16 rings.

not produce contraction in BA rings (Fig. 4).

35 mM KCl-induced contraction was dose-dependently relaxed by the addition of flunarizine and oxybutynine, known as calcium antagonists, with $IC_{50s}$ of $6.2\times10^{-6}$M and $1.8\times10^{-5}$M, respectively. However, the contraction was only slightly inhibited by the addition of glyceryl trinitrate (Fig. 6). Isoproterenol (ISP), a $\beta$-adrenoceptor agonist, dose-dependently inhibited the KCl-induced contraction and the maximal concentration ($10^{-4}$M)of ISP inhibited the KCl-induced tension by 40% (Fig. 4). However, ACh did not aftect the KCl-

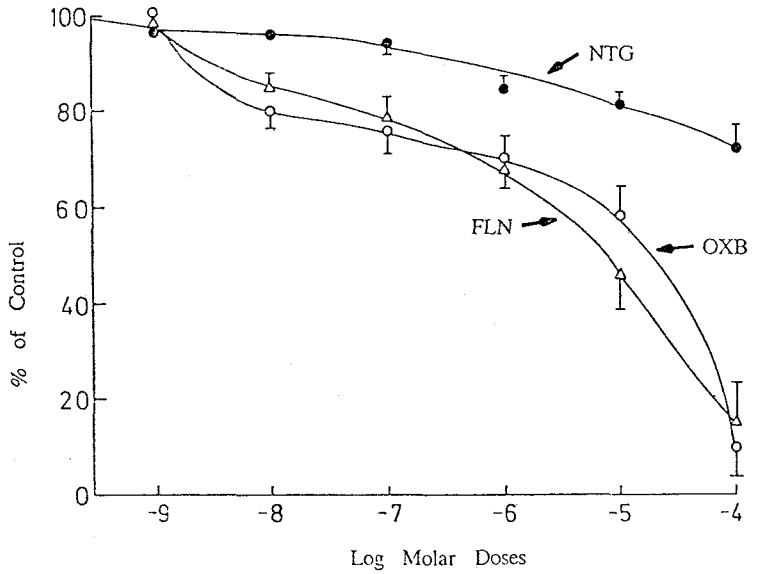


**Fig. 6.** Effects of glyceryl trinitrate (NTG), oxybutynin (OXB) and flunarizine (FLN) on the KCl (35 mM)-induced contraction in th arterial rings obtained from porcine arterial circle of Willis. Each point indicates mean±SEM from 11~15 rings.

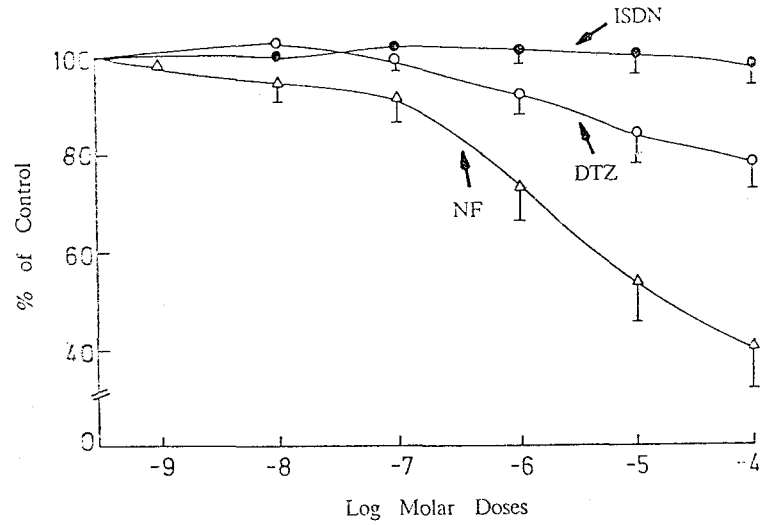


**Fig. 7.** Effects of isosorbide dinitrate (ISDN), diltiazem (DTZ) and nifedipine (NF) on the 5-hydroxytryptamine ($10^{-5}$ M)-induced contraction in the porcine arterial rings obtained from arterial circle of Willis. Each point indicates mean± SEM from 10~14 rings.

induced contraction regardless of endothelium integrity. Although $PGF_{2\alpha}$-induced contraction was dose-dependently relaxed by ACh, ACh did not affect KCl-induced contraction in the same preparation (Fig. 4).

$10^{-5}$M 5-HT-induced contraction was dose-dependently inhibited by the preteratment with nifedipine ($IC_{50}=1.6\times10^{-5}$M), but hardly inhibited by the pretreatment with diltiazem and ISDN (Fig. 7).

**Role of endothelium**: $PGF_{2\alpha}$ ($10^{-5}$M), a potent vasoconstictor of pheripheral artery, produced a prominent and steady-state contraction in cerebral arteries. ACh relaxed the $PGF_{2\alpha}$-induced contraction in intact endothelial rings but had no effect on or even additionally contracted the $PGF_{2\alpha}$-induced contraction in deendothelized ring(Fig. 8, 9). The ACh-induced relaxing effect appeared immediately after the addition of ACh and was in a dose-dependent fashion. Adenosine also had a dose-dependent relaxing effect on $PGF_{2\alpha}$-induced contraction in intact endothelial rings. These

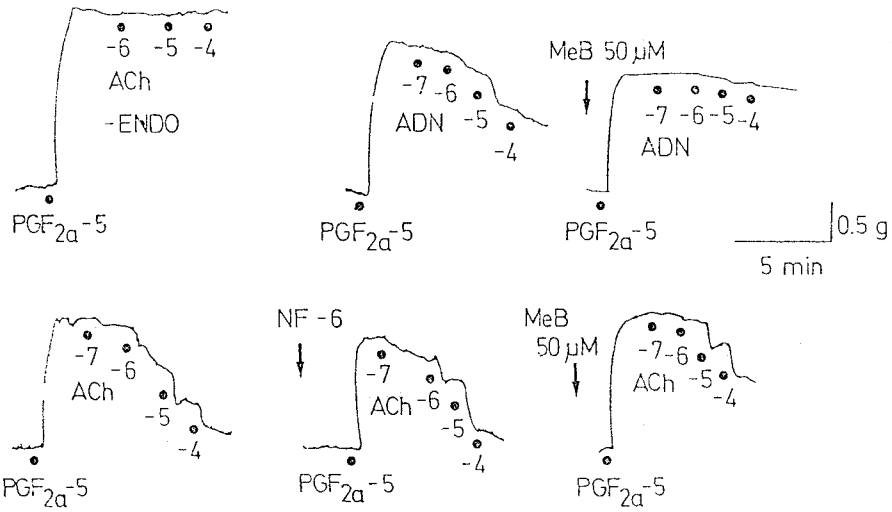


**Fig. 8.** Typical traces showing effects of acetylcholine (ACh) and adenosine (ADN) on the $10^{-5}$M $PGF_{2\alpha}$-induced contraction in basilar (upper) and circle of Willis (lower) rings of porcine cerebral arteries. First trace (−ENDO) was obtained from a ring without endothelium and other traces from rings with intact endothelium. ACh and ADN have dose-dependent relaxant effect attenuated by pretreatment with methylene blue (MeB) and not by nifedipine (NF). Invert arrows mean pretreatment with the indicated drugs. Other legends are the same as in Fig. 1.

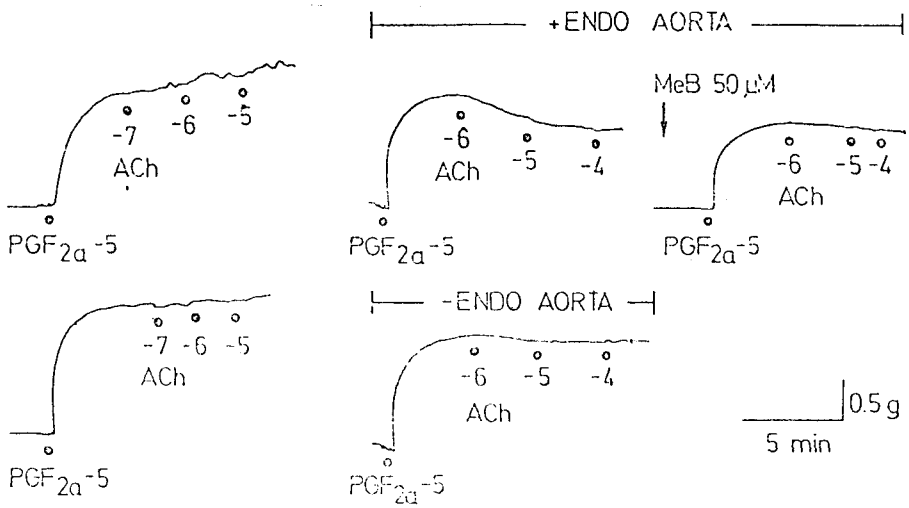


**Fig. 9.** Typical traces showing influences of endothelium on the actylcholine-effect on $PGF_{2\alpha}$-induced contraction in rubbed rings obtained from procine arterial circle of Willis. In these traces, in order to provide endothelium for the rubbed porcine cerebral arterial rings, a part of rabbit thoracic aorta with intact endothelium are suspended in the same organ bath (+ENDO AORTA). Other legends are the same as in Fig. 1 and 8. Other details are in text.

endothelium-dependent relaxations of both drugs were scarcely affected by nifedipine ($10^{-6}$M)-pretreatment but markedly inhibited by the pretreatment with methylene blue (50 $\mu$M) known as an EDRF-releasing blocking agent (Fig. 8).

In order to ascertain that the ACh-induced relaxation was dependent on the release of EDRF, the following procedure was taken under the

assumption that EDRF to be released from the endothelial cells of rabbit aortic strip by ACh was diffused into adjacent porcine cerebral arterial ring without the endothelium and then the porcine arterial ring could be relaxed. A rabbit thoracic aortic strip with endothelium was mounted in the organ bath of the deendothelial cerebral ring in which ACh had no relaxing effect on $PGF_{2\alpha}$-induced contraction. In these preparations, the addition of ACh produced a dose-dependent relaxation, as expected (Fig. 9). This relaxing effect was abolished by pretreatment with methylene blue. However, the addition of ACh did not produce relaxation in the preparation mounted with deendothelized thoracic aortic strip of rabbit.

### Cat cerebral arteries

**Basilar artery**: Dose-dependent contraction was produced in cat basilar rings without propranolol pretreatment by NE, which induced relaxation without propranolol pretreatment in porcine cerebral arteries. ACh, which did not produce contraction in porcine BA, induced marked contractions in a dose-dependent fashion, and 5-HT also produced a dose-dependent contraction (Fig. 10, 11). The $EC_{50s}$ of 5-HT, NE and ACh were $1.7\times10^{-6}$M, $7.2\times10^{-6}$M, and $1.3\times10^{-5}$ M, respectively and the magnitude of maximal contraction was the largest in ACh (Fig. 11).

**Arterial circle of Willis**: NE produced a dose-dependent contraction also in WC rings without propranolol-pretreatment. 5-HT and ACh also produced contractions in a dose-dependent manner (Fig. 10, 12). The $EC_{50s}$ of 5-HT, NE and ACh were $3.1\times10^{-6}$M, $6.4\times10^{-6}$M and $1.8\times10^{-5}$ M, respectively, being similar to $EC_{50}$ each in BA. ACh showed the largest maximal contraction (Fig. 12).

**Role of endothelium**: ACh did not induce relaxation in cat cerebral arterial rings (BA and WC: 27 cases) which took no procedure to remove the endothelium. Following the development of steady-state contraction by 5-HT ($10^{-5}$M), the addition of ACh ($10^{-6}$M) produced additional contraction in absence of any relaxation.

### Rabbit cerebral arteries

**Basilar artery**: Basilar arterial rings were only used in the experiment of rabbit cerebral artery. In rabbit basilar arteries, because of very small diameter of the artery it was difficult to insert a pair of hooks for suspension. Thus arterial rings were apt to be damaged and the responsiveness was very poor . When the 35 mM KCl-induced contraction was less than 0.1g in this experiment the ring was excluded from data analysis. NE produced a dose-dependent contraction regardless of propranolol pretreatment and 5-HT also induced contraction (Fig. 13). The $EC_{50s}$ of NE and 5-HT were $2.8\times10^{-6}$M and $1.2\times10^{-7}$M, respectively.

**Role of endothelium**: Only in 13 out of 33 rabbit cerebral rings which did not take procedure

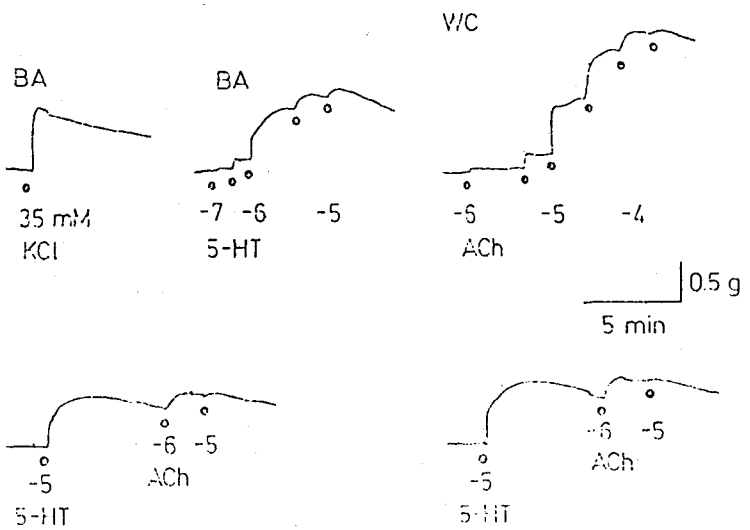


**Fig. 10.** Traces represent the contractile responses to 5-hydroxytryptamine (5-HT) and acetylcholine (ACh) and the contractile effect potentiated by acetylcholine on the 5-HT-induced contractions in cat cerebral arterial rings. BA: rings from basilar artery, WC: rings from arterial circle of Willis. Other legends are the same as in Fig. 1.

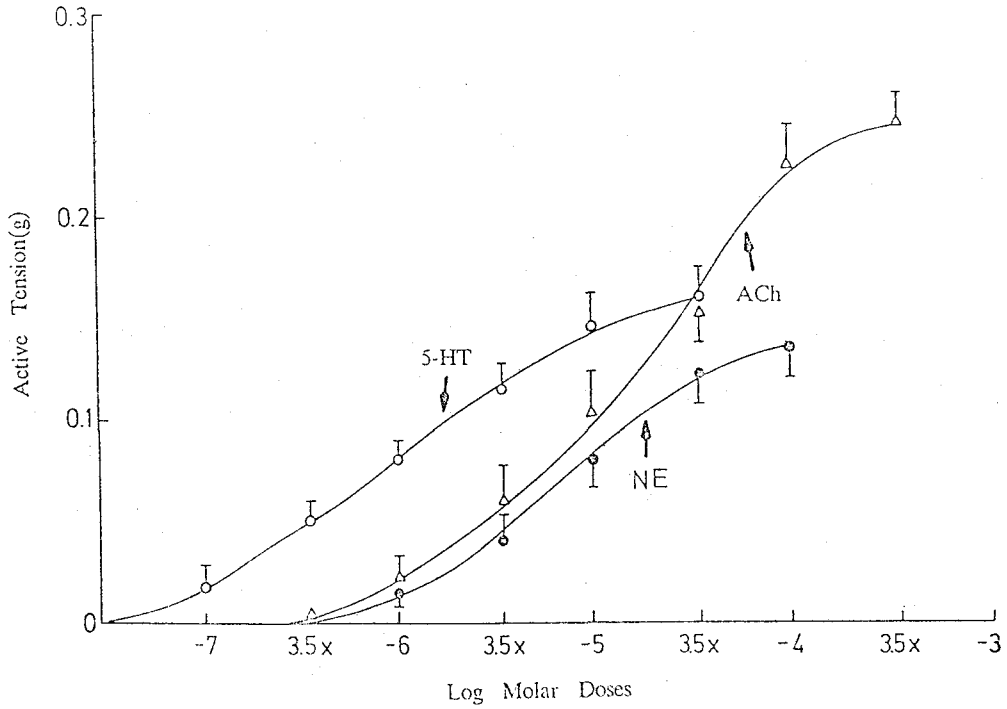


**Fig. 11.** Dose-response curves to acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and norepinephrine (NE) in the cat basilar arterial rings. Each point represents the mean±SEM from 13~19 rings.

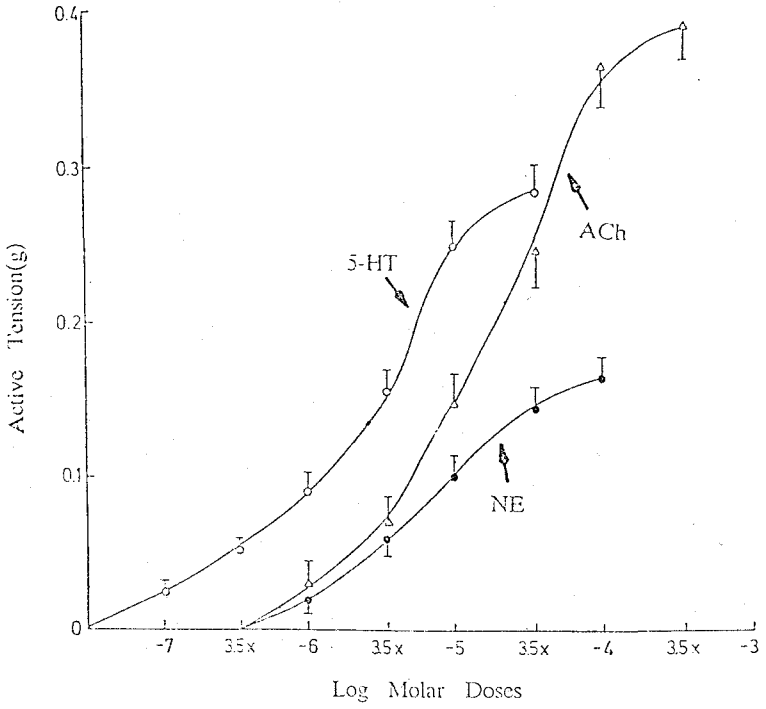


**Fig. 12.** Dose-response curves to acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and norepinephrine (NE) in the cat cerebral arterial rings from circle of Willis. Each point represents the mean±SEM from 13~18 rings.

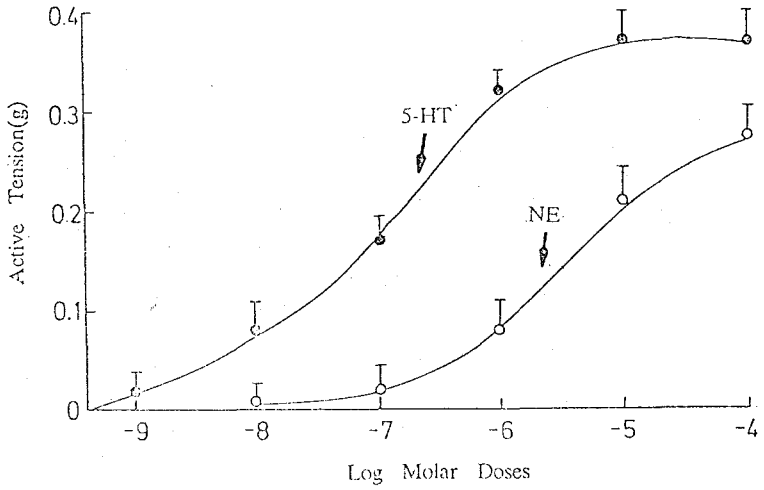


**Fig. 13.** Dose-response curves to 5-hydroxytryptamine (5-HT) and norepinephrine (NE) in the rabbit basilar arterial rings. Each point represents the mean± SEM from 17~22 rings.

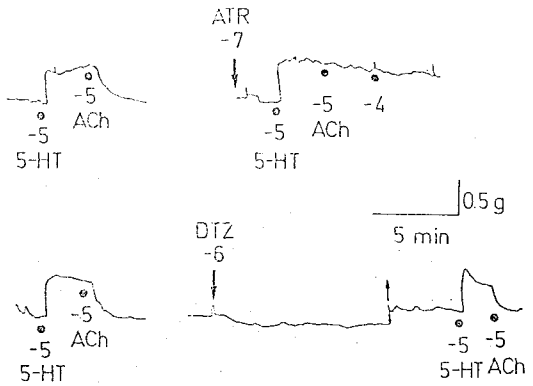


**Fig. 14.** Traces represent the influences of pretreatments with atropine (ATR) and diltiazem (DTZ) on the relaxing effect of acetylcholine (ACh) on the 5-hydroxytryptamine (5-HT)-induced contraction in rabbit basilar arterial rings with intact endothelium. At upward arrow, the resting tension was readjusted to the basal level. Other legends are the same as in Fig. 8.

removing endothelium, ACh ($10^{-5}$M) relaxed markedly 5-HT ($10^{-5}$M)-induced contraction. In the remaing 20 and the 17 rings with removed endothelium, ACh showed a tendency to contract. The endothelium dependent relaxation of ACh was abolished by $10^{-7}$M atropine-pretreatment, whereas it was hardly affected by $10^{-6}$M diltiazem-pretreatment (Fig. 14). Diltiazem per se slightly reduced basal tension. After this reduction of basal tension was mechanically readjusted, the addition of 5-HT elicited contraction. The phasic contraction of 5-HT was similar to that of the control but the steady-state tension, i.e. tonic tension, was not maintained in the presense of diliazem. In this state, the addition of $10^{-5}$M ACh induced relaxation similar to the control relaxation (Fig. 14).

## DISCUSSION

In this experiment, the effects of some vasoconstrictors, $\alpha$-adrenoceptor agonists (PE and NE), $\alpha$-and $\beta$-adrenoceptor equipotent agonist (EP) and others (5-HT, histamine, angiotensin II and KCl) on the circular muscle of porcine cerebral arteries were very different from their effects on peripheral arteries. NE and EP are potent peripheral vasoconstrictors. However, NE produced relaxation in porcine cerebral arteries whereas EP caused contraction in arteral circle of Willis. Under pretreatment with propranolol, a $\beta$-adrenoceptor antagonist, however, NE, EP and PE induced dose-dependent vasoconstrictions. These results suggest that $\beta$-adrenoceptors are more sensitive than $\alpha$-adrenoceptors to sympathomimetics in smooth muscles of porcine cerebral arteries of resting tension. While angiotensin, one of the most potent vasoconstrictors in peripheral arteries, had the lowest $EC_{50}$, it showed the least efficacy among vasoconstrictors used in this study. Taking into account the observations that angiotensin has no direct effect on the cerebral vessels (Strandgaard *et al.*, 1975; Olesen, 1972), that this drug produces vasoconstriction smaller

than that induced by NE (Reynier-Rebuffel, 1982), and that angiotensin produces relaxation via release of $PGI_2$ in dog cerebral arteries (Toda and Miyazaki, 1981, 1984), it was inferred that angiotensin has both vasoconstricting and vasodilatory effects on cerebral vessels. The sum of these effects finally showed a the small contraction in porcine cerebral arteries differeing from effects of peripheral arteries.

It is well known that KCl-induced contraction is derived from the calcium influx via potential-operated calcium channels ($POC_s$) and that 5-HT-induced contraction is derived from both the calcium influx via receptor-operated calcium channels ($ROC_s$) and the intracellular calcium release (Schwartz and Taira, 1983; Dubé *et al.*, 1985). In this study high concentration of KCl produced a steady-state contraction. The KCl-induced tension was dose-dependently relaxed by calcium antagonists. Nifedipine, a derivative of dihydropyridne, showed a marked inhibition to the contraction whereas glyceryl trinitrate and ISDN, compounds of organic nitrate, showed only a weak inhibition. While 5-HT-induced contraction was dose-dependently relaxed by nifedipine, it was seldom affected by diltiazem and ISDN. These data suggest that KCl produces contraction by calcium influx via $POC_s$ and 5-HT produce contraction by calcium influx via $ROC_s$, at least in part, in porcine cerebral arteries, and that organic nitrates act as a vasodilator of low-potency in the cerebral arteries, differing from the previous reports in which organic nitrates act as inhibitors of calcium influx as well as those of intracellular calcium release in coronary arteies (Baik and Yoon, 1987; Gross *et al.*, 1981).

In pig cerebral arteries, only KCl produced a steady-state contraction, whereas contractions by the other vasoconstrictors used in this experiment showed spontaneous decline of tension. However, $PGF_{2\alpha}$, known as a vasoconstrictor in dog cerbral arteries (Toda and Miyazaki, 1984), also induced a marked and stady-state contraction in this study. So $PGF_{2\alpha}$ was used as the vasoconstrictor in the experiments observing the role of endothelial cells. In intact endothelial rings, $10^{-5}$M $PGF_{2\alpha}$-induced contractions were dose-dependently relaxed by the addition of ACh and adenosine but in the deendothelized rings, ACh had no effect on or even contracted further the $PGF_{2\alpha}$-induced contractions. This endothelium-dependent relaxent effects were not affected by the pretreatment with $10^{-6}$M nifedipine, the concentration of which could abolish 35 mM KCl-induced contraction. Nifedipine is one of potent calcium antagonists which inhibit calcium influx through calcium channels in cell membrane (Schwartz and Taira, 1983). This result is not agreement with the reports that calcium influx takes part in the release of EDRF in rabbit thoracic aorta (Singer and Peach, 1982; Long and Stone, 1985). However, pretreatment with methylene blue, a inhibitor of the release of EDRF (Murakami *et al.*, 1987; Nagase *et al.*, 1987; Nishiye *et al.*, 1988), markedly inhibited the ACh-induced endothelium-dependent relaxation.

In the preparation that a rabbit thoracic aorti strip with intact endothelium was mounted in the same organ bath along the porcine cerebral ring which had not shown an endothelium-dependent relaxation, the addition of ACh produced a dose-dependent relaxing effect on $PGF_{2\alpha}$-induced tension. This endothelium-dependent relaxation was also inhibited by pretreatment with methylene blue. In the other hand, the preparation mounted with deendothelized aortic strips was not produce relaxed by addition of ACh. These data indicate that ACh releases EDRF from the endothelial cell of porcine cerebral arteries as in peripheral arteries and the EDRF also released from arteries of different species or different sites could relax the porcine cerebral arteries.

In cat cerebral arteries, NE and 5-HT produced dose-dependent contractile responses and ACh also produced dose-dependent contractions in rings with or without endothelium. Even in intact endothelial rings, following development of steady-state contraction by 5-HT, the addition of ACh produced additional contraction instead of relaxation. ACh produced more prominent contraction than NE and 5-HT did, regardless whether the endothelium exists or not.

Some authors reported that ACh increased cerebral blood flow in cat in vivo (D'Alecy, 1977; Vasquez and Purves, 1977), while the others observed that ACh induced relaxation at lower concentration but contraction at higher concentration (Lee *et al.*, 1980; Lee, 1982). However, $10^{-6}$M ACh, the concentration which relaxes most peripheral arteries, produced additional increase in tension of 5-HT-contracted arteries. Furthermore, ACh had more prominent contractile efficacy than 5-HT and NE in this study. These data suggest that ACh act mainly as a vasoconstrictor rather than a vasodilator in cat cerebral arteries.

In rabbit cerebral arteries, 5-HT and NE produced contractions in a dose dependent man-

ner, but ACh could not elicit contractile response, differing from the data obtained from cat cerebral arteries. In intact endothelial rings ACh relaxed the 5-HT-induced contraction to the basal tension and the relaxing effect was inhibited by atropine-pretreatment but seldom affected by diltiazem-pretreatment. Diltiazem reduced the basal tension and inhibited tonic contraction by 5-HT but did not affect the ACh-induced relaxation. This inhibitory effect of diltiazem on 5-HT-induced tonic tension is consistent with the fact that calcium antagonists inhibit some vasoconstrictor-induced tonic contraction, which is elicited by calcium influx (Dubê *et al.*, 1985). However, the data that the calcium antagonist did not inhibit the ACh-induced relaxation are consistent with the results obtained from porcine cerebral arteries. It suggests that the mechanism participating in the release of EDRF in cerebral arteries is different from that in peripheral arteries in which the release of EDRF is extracellular calcium-dependent (Singer and Peach, 1982; Long and Stone, 1985).

Conclusively, it is inferred that the responses to some vasoconstrictors in cerebral arteries are different from those in peripheral arteries and that ACh has vasoconstrictor effect in cat cerebral arteries. Endothelial cells are suggested to play an important role in vascular compliance of rabbit and pig cerebral arteries.

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## ═국문초록═

# 혈관 수축제의 뇌혈관 수축반응에 대한 혈관근 내피세포의 역할

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돼지, 가묘 및 가토의 뇌동맥에서 기저동맥(basilar artery, BA)과 circle of Willis동맥(WC)의 동맥환을 만들어 혈관수축제와 *calcium* 길항제의 효과 그리고 내피세포의 역할은 검토하였다. 돼지 BA와 WC에서 norepinephrine (NE), phenylephrine (PE)및 epinephrine (EP)은 propranolol $10^{-6}$M 전처리하에서 그리고 KCl, histamine, 5-hydroxytryptamine (5-HT) 및 angiotensin은 모두 용량의존성 수축반응을 일으켰다. 그 최대수축력은 angiotensin에서 가장 적었고, 5-HT에서 가장 강력하였다. KCl 35 mM 수축반응은 calcium 길항제로 용량의존성으로 억제되었고, 그 효력은 nifedipine >> diltiazem > flunarizine > oxybutynin > isosorbide dinitrate (ISDN) > glyceryl trinitrate의 순서였다. 5-HT $10^{-5}$M의 수축반응은 nifedipine으로는 용량의존성으로 억제되었으나 diltiazem과 ISDN으로는 경미한 억제만을 일으켰다. 내피세포동맥환에서 KCl 35 mM의 수축반응은 acetylcholine (ACh)으로 거의 영향받지 않았으나 $PGF_{2\alpha}$ $10^{-5}$M의 수축반응은 ACh과 adenosine으로 용량의존성으로 억제되었고, 이 내피세포 의존성억제는 nifedipine $10^{-6}$M로는 영향받지 않으나 methylene blue 50 $\mu$M로는 현저히 억제되었다. 내피제거동맥환에의 $PGF_{2\alpha}$의 수축반응은 ACh의 영향이 없거나 더욱 수축되었다. 내피세포를 제거하지 않은 가토흉부대동맥편을 bath에 함께 넣어주면 ACh의 용량의존성 이완반응이 나타났고 이 이완반응도 methylene blue로 억제되었다. 가묘 BA와 WC에서 5-HT와 NE는 용량의존성 수축반응을 일으켰고 ACh도 내피세포 존재유무와 관계없이 강력한 용량의존성 수축반응을 일으켰으며 그 최대수축력은 ACh에서 가장 강력하였다. 내피세포동맥환에서 5-HT $10^{-5}$M의 수축반응은 ACh에 의하여 이완반응없이 더욱 수축되었다. 가토 BA에서 5-HT와 NE는 용량의존성 수축반응을 일으켰고 5-HT의 수축반응이 더욱 현저하였다. 내피세포동맥환에서 5-HT $10^{-5}$M의 수축반응은 ACh $10^{-5}$ M로 현저하게 이완되었고 이 내피세포의존성 이완반응은 atropine $10^{-7}$M로는 억제되었으나 diltiazem $10^{-6}$M로는 거의 억제되지 않았다.

이상의 실험성적은 돼지와 가토의 뇌동맥에서 ACh은 내피세포-의존성 이완반응을 일으키며 그 이완반응은 muscarinic receptor를 통해 나타났고, 가묘뇌동맥에서 ACh은 혈관수축제의 역할을 갖고 있음을 시사하고 있다.