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Role of Endothelium-derived Relaxing Factor in Cerebral Autoregulation *in vivo*

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ABSTRACT

In anesthetized rats, we examined the possibility that endothelium-derived relaxing factor (EDRF) or nitric oxide (NO) released in response to cholinergic mechanism may contribute to the reflex autoregulation of cerebral blood flow. Suffusion with mock cerebrospinal fluid (CSF), containing acetylcholine (ACh, $10^{-9}\sim10^{-6}$ M) evoked concentration-dependent vasodilatation of the resting pial artery (mean, $19.3\pm1.7\,\mu$m, n=36), which was significantly inhibited not only by Nω-nitro-L-arginine (L-NNA, 10^{-5} M) but also by methylene blue (10^{-6} M) and oxyhemoglobin (10^{-6} M). The muscarinic receptors in the endothelium of pial artery implicated in the release of EDRF were considered to be M_1 and M_3 subtypes. When suffused with mock CSF containing L-arginine it caused a transient vasodilatation, which was strongly inhibited by LY 83583 (10^{-5} M), but not by L-NNA (10^{-5} M). Additionally, both ACh- and L-arginine-induced vasodilation were significantly inhibited by glibenclamide, a specific ATP-sensitive K^+ channel blocker.

On the other hand, changes in pial arterial diameter were plotted as a function of changes in systemic arterial blood pressure. The slopes of regression lines for vasodilation and vasoconstriction were not affected by pretreatment with 10^{-5} M L-NNA, but significantly reduced by 3×10^{-6} M glibenclamide. Thus it is suggested that the reflex vasodilation of rat pial arteries in response to a transient hypotension is not mediated by EDRF (NO).

Key Words: Cerebral autoregulation, Cerebral blood flow, Pial artyeriole, Endothelium-derived relaxing factor

INTRODUCTION

There are a number of anatomical and histochemical evidences for cholinergic innervation of large cerebral artery and pial vessels: parasympathetic fibers originated from the seventh cranial and greater superficial petrosal nerve innervate the internal carotid artery (Chorobsky and Penfield, 1932) and the staining of acetylcholinesterase is observed in the middle cerebral artery of cats (Edvinsson *et al.*, 1972).

Many reports have demonstrated that acetylcholine (ACh) evokes endothelium-dependent relaxation in cerebral arteries of cat (Lee, 1982), rat (Mayhan *et al*, 1987; Faraci, 1990), rabbit (Brayden, 1990) and man (Whalley *et al.*, 1987). Dilatation of pial arterioles to ACh *in vivo* has been reported to occur through endothelium-dependent mechanism (Kontos *et al.*, 1988; Watanabe *et al.*, 1988). A major endothelium-dependent re-

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laxing factor (EDRF) is known as nitric oxide (NO) or a closely related compound derived from L-arginine (Ignarro *et al.*, 1987; Palmer *et al.*, 1988).

Endothelium-dependent NO release has been observed in cerebral arteries (Fujiwara *et al.*, 1986; Watanabe *et al.*, 1988). Otherwise, Brayden (1990) has postulated that cerebral arterial dilatation in response to ACh is determined by the combined effects of membrane hyperpolarization and the action of EDRF (NO). On the other hand, in the vasodilatory response of cerebral autoregulation, EDRF (NO) has been questioned as an underlying mechanism, since cholinergic mechanism is responsible for modulation of cerebral blood flow. Despite a number of data suggesting that an intrinsic cholinergic mechanism produces vasodilation in the intracerebral microvessels, there is no direct information on *in vivo* vasodilator effect of ACh on the pial artery of the cerebrum. Moreover, the involvement of endothelium-derived NO in the cerebral autoregulatory vasodilation is poorly understood.

The goal of this study was (1) to examine effects of ACh on the pial artery on the cerebrum *in vivo* and to test whether dilation in response to ACh and L-arginine is mediated by EDRF (NO) or by other mechanism, and (2) to test whether increase in ACh-induced EDRF are related with the autoregulatory vasodilatory response upon lowering of systemic arterial pressure. For this purpose, we have observed changes in diameter of pial artery under pretreatment with Nω-nitro-L-arginine (L-NNA), an L-arginine analogue and compared to those with glibenclamide, a blocker of ATP-sensitive K^+ channels.

MATERIALS AND METHODS

Preparation of animals

Sprague-Dawley rats (250~300 g) were anesthetized with ether and urethane (1 g/kg, i.p.) and placed on a heating pad to maintain a constant body temperature. After a tracheostomy was performed, each rat was then ventilated with a respirator (Harvard, Model 683) with room air. The left femoral artery was cannulated with PE-50 polyethylene tube for monitoring blood pressure (Statham P23D pressure transducer). Arterial blood sample was collected through the left carotid artery before and after installation of cranial window for blood gas and pH determination (NOVA Biomedicals, STAT Profile 3). The mean arterial blood gas and pH determined during experiments were as follows: pH, 7.37±0.01; $PaCO_2$, 31.3±1.6 mmHg; PaO_2, 98.0±2.2 mmHg). Rectal temperature was monitored continuously and was kept constant (37±0.5℃) with a heating pad.

Measurement of vessel diameter

Pial microvessels were visualized through an implanted cranial window, as described previously (Hong *et al.*, 1994) (Fig. 1). Briefly described, the head was fixed in prone position with a stereotaxic apparatus (Stoelting) and a square shape crainiotomy (5×5 mm) was made over the right parietal cortex. The dura was opened and the burr hole was covered with warmed mineral oil during operation. Pial precapillary microvessels, ranging in diameter between 15 and 25 μm. were visualized through the implanted cranial window. Cerebral microvessels were allowed to equilibrate for 60 min after installation of cranial window. The window field was suffused with mock cerebrospinal fluid (CSF) at a speed of 0.3 ml/min. The image of pial arterioles was captured with a CCD video camera (Sanyo, VDC 3900) through a stereoscope (Nikon, SMZ-2T), and fed to a television monitor for direct observation and the caliber was measured using a Width Analyzer (Hamamatsu) at 480×magnification. The composition (mM) of the mock CSF was as follows: 125 NaCl, 3.5 KCl, 1.3 $CaCl_2$, 1.1 $MgCl_2$, and 25 $NaHCO_3$. The intracranial pressure was maintained constant at 5~6 mmHg throughout the experiment by adjusting the height of the free end of plastic tubing, which was connected to the outlet of the window. In each rat, only one arteriole under the window was used for observation.

Protocols of *in vivo* experiment

(1) We identified the normal autoregulatory reponse of the pial artery to lowering of arterial blood pressure by bleeding of the blood into the reservior and to its reverse of blood pressure by infusion of the blood under suffusion with mock CSF over the cerebral cortical surface (Hong *et al.*, 1994). If the pial artery on the cerebrum did

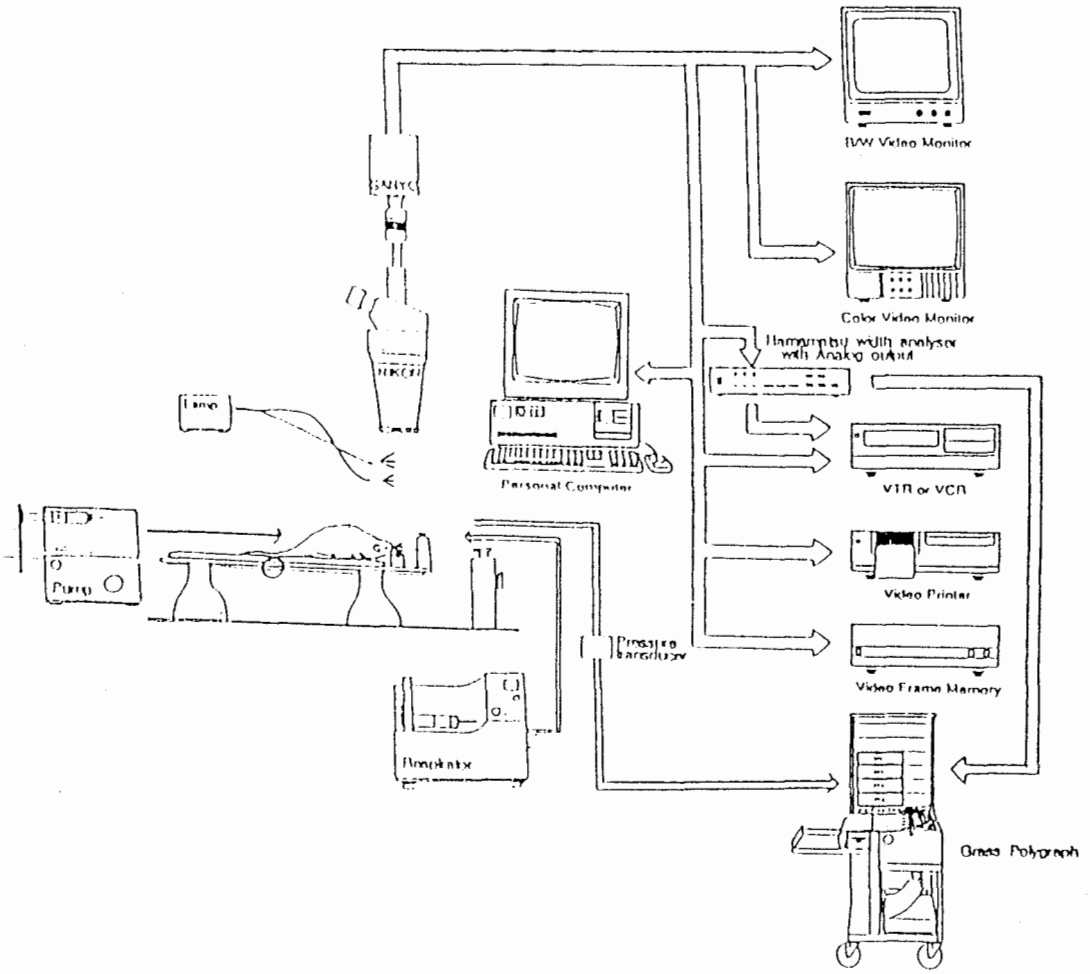

Fig. 1. Schematic diagram for monitoring of the change in diameter of rat pial artery and systemic blood pressure.

not show normal autoregulatory response, it was discarded. (2) We observed the vasodilator responses of the resting pial vessels to ACh or L-arginine suffused with the mock CSF. Each concentration of drug was suffused over the cortical surface for 3 min. (3) The effects of NO inhibitors and glibenclamide on the ACh-induced vasodilation were identified. The changes in vessel diameter were monitored with a Width Analyzer every 4 sec. Finally, the alterations in pial arterial diameter and blood pressure were expressed as percent changes in the baseline diameter for the same animals.

Drugs

Acetylcholine chloride (ACh), Nω-nitro-L-arginine (L-NNA), methylene blue and glibenclamide were purchased from Sigma Chemical Co. Pirenzepine dihydrochloride, methoctramine tetrahydrochloride and hexahydro-sila-difenidol hydrochloride (pF-HSiD) were purchased from Research Biochemical International and they were dissolved in distilled water. Oxyhemoglobin was prepared by adding sodium dithionite to 1 mM solution of bovine hemoglobin (Sigma Chemical Co.) in distilled water and then sodium

dithionite was removed by dialysis for 2 hours at 4℃. Glibenclamide was sonicated in 1 ml NaOH (0.1 N) and diluted with 5% glucose to make a stock solution of 10 mM. Ly 83583 was kindly donated from Eli Lilly and Company.

Satatistics

Results are expressed as means±S.E.M. Statistical significance was determined by Student's *t*-test between two groups. Analysis of variance was used for comparisons of the results of vasodilators in the absence and the presence of antagonists. $P < 0.05$ was considered to be statistically significant.

RESULTS

Mean arterial blood pressure of rat used in this experiment was 105.7±6.3 mmHg. In control rat, the basal diameter of pial arteries measured (mean, 19.3±1.7 μm, n=36) remained constant throughtout the experiment unless there was bleeding or an infusion of blood.

Effects of acetylcholine and muscarinic receptor antagonists

Cranial surface was suffused with mock CSF containing ACh (10^{-9}~10^{-6} M) by increasing each concentration every 3 min. ACh evoked concentration-dependent vasodilatation of the resting pial arteries as demonstrated in Fig. 2. The vasodilatory effect of ACh was most prominent at 100 nM level and, thereafter, the vasodilatory effect was decreased.

In the present study, we identified the subtype of muscarine receptors of the endothelium involved in the vasodilatation of the pial artery. Pirenzepine (10^{-7} M), the reference antagonist for M_1 receptor and pF-HSiD (10^{-7} M), the reference antagonist for M_3 receptor showed strong inhibition of ACh-induced relaxation, whereas methoctramine, the reference antagonist for M_2 receptor, was without effect on vasodilation. These results suggest that ACh dilates pial artery

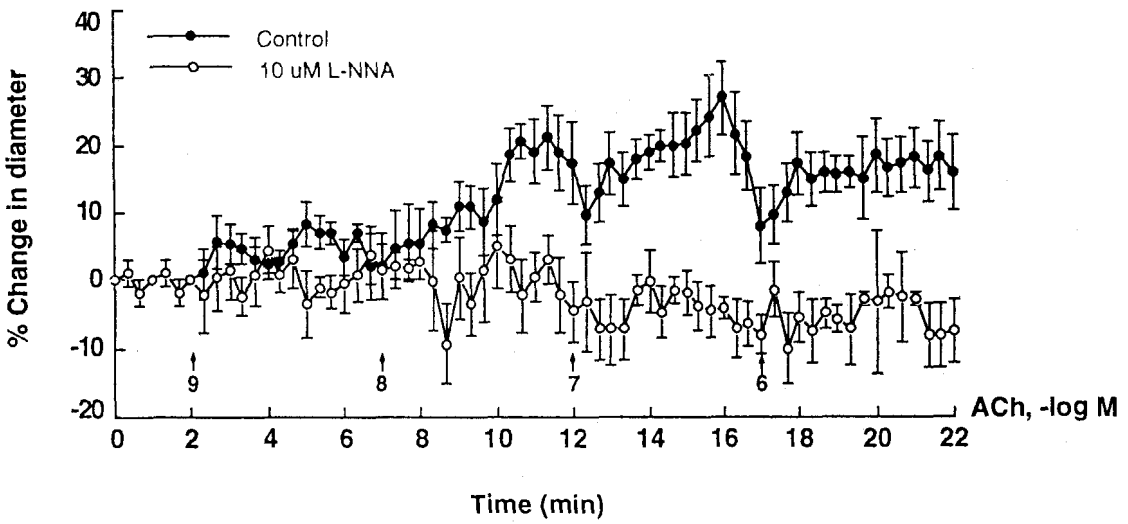

Fig. 2. Line graph showing percent change in diameter of rat pial artery in response to acetylcholine (1~1000 nM) without and with 10 μM N_W-nitro-L-arginien (L-NNA). Control concentration dependent responses were determined by increasing acetylcholine concentrations in the suffusing mock CSF. Sixty minutes later. under suffusion with mock CSF containing 10 μM L-NNA, the same experiment was conducted. L-NNA significantly inhibited the acetylcholine effect when compared between both curved of each concentration ($P<0.05$). Results are expressed as means±S.E.M. from eleven experiments for control and four experiments for L-NNA.

in vivo by activating M_1 and M_3 muscarinic receptor subtypes which located in the endothelium (Fig. 3).

Effects of inhibitors on acetylcholine-induced vasodilation

The maximum dilation induced by 10^{-8}, 10^{-7} and 10^{-6} M ACh was 25.2, 27.9 and 18.5%, respectively. The resting baseline diameter of pial artery was little affected by suffusion of mock CSF containing either L-NNA (10^{-5} M), oxyhemoglobin (10^{-6} M), methylene blue (10^{-6} M) or LY 83583 (10^{-5} M). These results suggest no basal production of an EDRF from pial arteries (Table 1). As demonstrated in Fig. 4. ACh-induced vasodilation was significantly inhibited by methylene blue (10^{-6} M) and oxyhemoglobin (10^{-5} M) regardless of the concentration of ACh used.

Effect of L-arginine and nitric oxide inhibitors

Cranial surface was suffused with mock CSF containing L-arginine (10^{-6}, 10^{-5} and 10^{-4} M) by

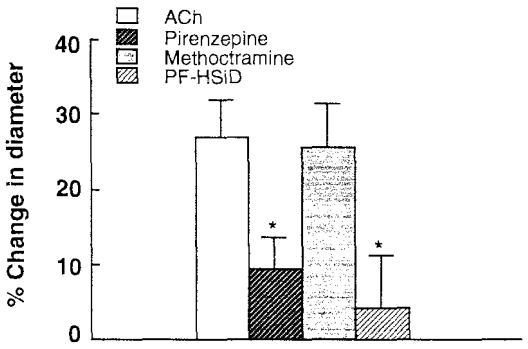

Fig. 3. Effect of specific muscarinic receptor antagonists on the acetylcholine-induced dilatation of rat pial artery. The results represent means±S.E.M. from three experiments. *P<0.05.

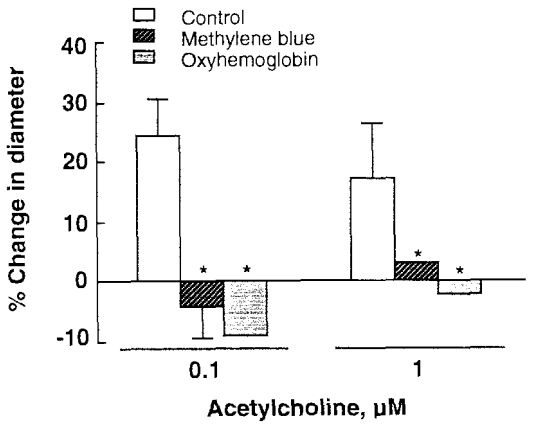

Fig. 4. Inhibitory effects of methylene blue (10^{-6} M) and oxyhemoglobin (10^{-6} M) on the acetylcholine-induced relaxation. The results represent means±S.E.M. from three experiments. *P<0.05.

Table 1. Effects of various antagonists on mean arterial blood pressure (MABP) and basal pial arteriolar diameter

Antagonists(M)	n	MABP (mmHg)	Basal diameter (μm)	Changes in diameter (%)
Glibenclamide (10^{-6})	15	105.5±4.7	19.3±1.6	−1.1±0.8
N_{ω}-nitro-L-arginine (10^{-5})	12	106.7±5.5	18.8±1.5	−2.2±3.1
OxyHb (10^{-6})	3	104.9±8.2	20.5±1.4	−0.1±1.1
Methylene blue (10^{-6})	3	104.1±6.3	20.9±1.9	−3.1±1.1
LY 83583 (10^{-5})	3	107.4±4.2	17.8±3.2	−0.6±1.3

The basal diameter was measured 20 min after application of glibenclamide and N_W-nitro-L-arginine and 5 min after application of methylene blue and LY83583, respectively.

n represents number of experiments.

Data are expressed as mean±S.E.M.

OxyHb: oxyhemoglobin

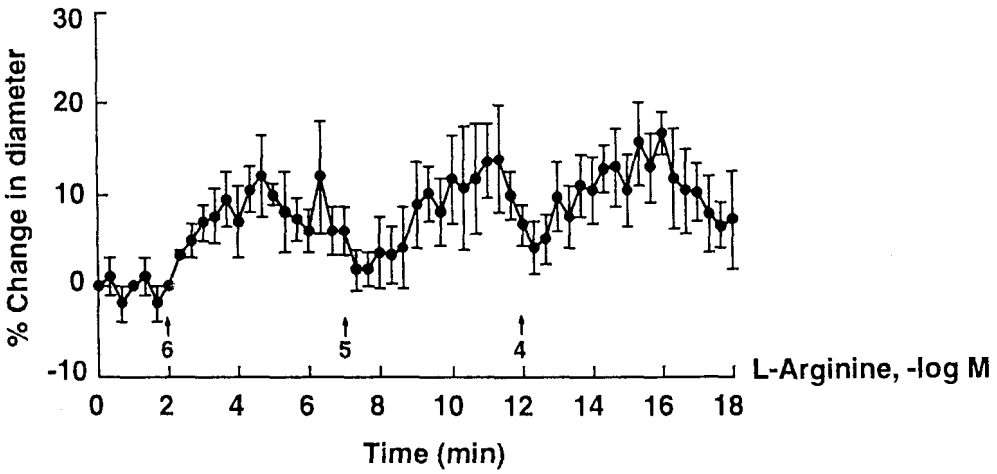

Fig. 5. Line graph showing percent change in diameter of rat pial arteries in response to L-arginine. The responses were determined by increasing L-arginine concentrations (1, 10 and 100 μM) in the suffusing mock CSF. They showed transient vasodilation ($P<0.05$) but not concentration-dependent manner. Results are expressed as means±S.E.M. from ten experiments.

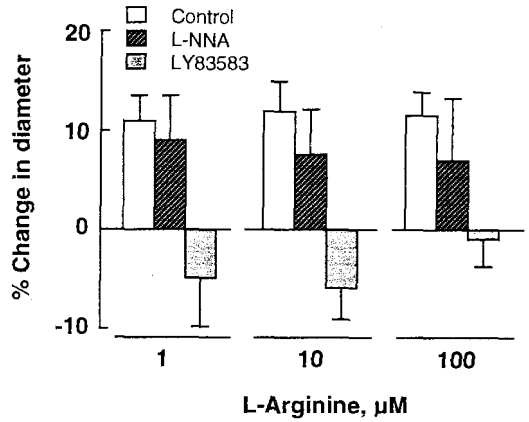

Fig. 6. Effect of N_W-nitro-L-arginine (L-NNA, 10^{-5} M) and LY 83583 (10^{-5} M) on the L-arginine-induced dilatation of rat pial artery. Results are expressed as means±S.E.M. from five to eight experiments.

increasing its concentration every 3 min. L-Arginine caused transient vasodilation of the resting pial artery by each concentration but it did not show a concentration-dependent manner (Fig. 5).

On the other hand, upon pretreatment with LY 83583 (10^{-5} M) the L-arginine-induced vasodilatation was strongly inhibited and rathr it was converted to constriction. However, L-arginine effect was little affected by L-NNA (10^{-5} M, Fig. 6).

Effect of glibenclamide

Suffusion of cranial surface with mock CSF containing 10^{-6} M glibenclamide for 20 min did not elicit any change in the resting baseline diameter of pial artery (Table 1). Not only ACh but also L-arginine-induced vasodilation was significantly inhibited as shown in Fig. 7.

Effects of L-NNA and glibenclamide on cerebral autoregulation

As demonstrated in Fig. 8, on lowering mean arterial blood pressure by bleeding, the diameter of pial artery increased correspondingly, and on reverse of the arterial blood pressure by infusion, the diameter decreased. Changes in pial arterial diameter were plotted as a function of changes in mean arterial blood pressure and the results were analyzed with the slopes of regression lines (Fig. 9). Mean slopes of regression lines for vasodilation phase (0.78) and vasoconstriction phase (vehicle group. −0.89) were not significantly altered by pretreatment with 10^{-5} M L-NNA, However, their slopes were markedly reduced to 0.11 for

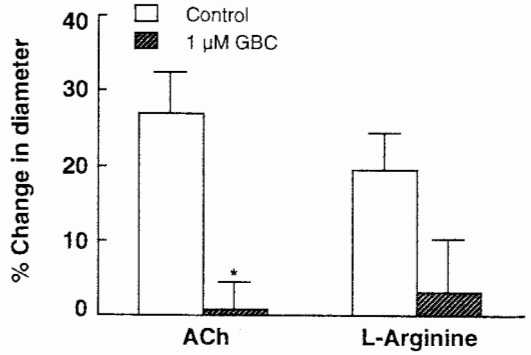

Fig. 7. Inhibitory effects of glibenclamide on the acetylcholine(ACh)- and L-arginine-induced relaxation. The results represent means±S.E.M. from four experiments. *, $P<0.05$.

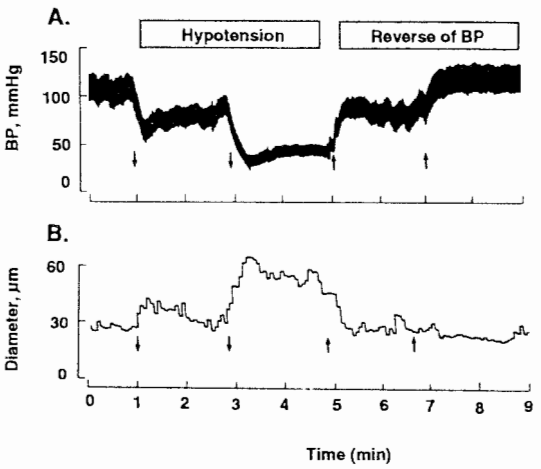

Fig. 8. Tracings of systemic arterial blood pressure (BP, A) and change in pial arterial diameter fo rat (B). Downward arrows represent the bleeding phase and upward arrows the infusion of the blood of reservoir.

vasodilation ($p<0.05$) and 0.18 for vasoconstriction phase ($p<0.05$) by pretreatment with 3×10^{-6} M glibenclamide.

DISCUSSION

In the present study we measured changes in pial artery diameter instead of measurement of cerebral blood flow based on experimental results that the responses of pial artery parallel changes in cerebral blood flow under many circumstances (Rosenblum *et al.*, 1990).

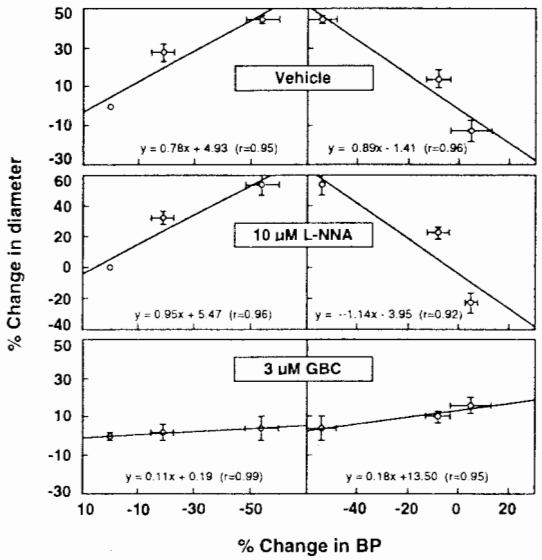

Fig. 9. Changes in pial arterial diameter were plotted as a function of changes in systemic arterial blood pressure under suffusion with mock CSF containing N_W-nitro-L-arginine (10^{-5} M) and glibencl-amide (3×10^{-6} M), in comparison to the vehicle effect. The results represent means ±S.E.M. from five experiments, respectively.

Our major findings are: First, both ACh and L-arginine caused increases in the resting diameter of pial artery on the rat cerebrum. Second, methylene blue, oxyhemoglobin and LY 83583, soluble guanalate cyclase inhibitors and NO scavenger, exerted strong inhibition of both ACh- and L-arginine-induced vasodilation. L-NNA, NO synthase inhibitor, also selectively inhibited the ACh- but not the L-arginine-induced relaxation. Third, the autoregulatory vasodilator response to hypo-tension was not affected by L-NNA, but by glibenclamide. These findings suggest that the vasodilatory responses of cerebral pial arteries to ACh are dependent on formation of NO from L-arginine, nevertheless, the autoregulatory vasodilatory response is probably not dependent on EDRF (NO).

In the cerebral circulation, experimental observations suggest that NO mediates the dilations of cerebral blood vessels to ACh (Faraci, 1990; Rosenblum *et al.*, 1990). NO or a closely related compound is at least one type of EDRF that is synthetized from L-arginine and relaxes vascular smooth muscle via stimulation of guanylate cyclase (Ignarro, 1990; Moncada *et al.*, 1991).

In the present study, the resting baseline diameter of pial artery was little affected by suffusion of mock CSF containing either glibenclamide, L-NNA, oxyhemoglobin, methylene blue or LY 83583, suggesting that basal production of an EDRF does not contribute to resting tone of pial arteries. If basal release of an EDRF was present in the resting state, application of the above inhibitors would be anticipated to decrease the baseline diameter. These speculations were consistent with some experminet *in vivo* (Kontos *et al.*, 1988; Marshall *et al.*, 1988; Watanabe *et al.*, 1988), in which they could not detect basal release of a relaxing factor from pial arterioles. Otherwise, Faraci (1990) demonstrated Nω-monomethyl-L-arginine-induced constriction of the rat basilar artery, which was antagonized by L-arginine, in the *in vivo* experiment. Thus it was considered that these endothelium-dependent mechanisms might differ between the small artery (pial artery) and the large one (basilar artery), as Faraci (1991) indicated that the rat basilar artery in 10-fold more sensitive than pial artery to L-arginine analogue.

Responses to ACh are attennated by pretreatment with soluble guanylate cyclase inhibitor, methylene blue (Martin *et al.*, 1985), LY 83583 (Schmidt *et al.*, 1985) and oxyhemoglobin (Nishiye *et al.*, 1989), and L-NNA, NO synthase inhibitor (Moncada *et al.*, 1989), suggesting that agonist-induced NO production participates in the vasodilation to ACh. More interestingly, ACh-induced vasodilatin was decreased by treatment with glibenclamide, an ATP-sensitive K^+ channel blocker (Schmid-Antomarichi *et al.*, 1987). In the pial artery on the cerebrum *in vivo* the fact that NO is present in cerebral endothelium (Poeggel *et al.*, 1992), and ACh is an effective dilator (Marshall *et al.*, 1988; Rosenblum *et al.*, 1986), and dilation to ACh occurs through endothelium-dependent mechanisms (Kontos *et al.*, 1988; Watanabe *et al.*, 1988) indicates a role for EDRF (NO) in modulation of cerebral vascular tone. Further, Brayden (1990) has demonstrated that, in the rabbit middle cerebral artery, ACh-induced vasodilator response is dependent on both hyperpolarization and cyclic GMP pathway since it is greatly reduced when K^+ channel blocker and methylene blue are combined. With these results, our findings support a role for endothelium-dependent hyperpolarization induced by glibenclamide-sensitive K^+ channel activation (Brayden *et al.*, 1990; Standen *et al.*, 1989) either alone or in combination mechanism of ACh-induced vasodilation is by release of NO.

In the present study we have observed that L-arginine causes an increase in the pial artery diameter, which is transient and not concentration-dependent. L-Arginine-induced vasodilation was attenuated not only by LY 83583, an inhibitor of soluble guanylate cyclase (Schmidt *et al.*, 1985) and formation of superoxide anion (Mülsch *et al.*, 1988), but also by glibenclamide as ACh-induced vasodilation was inhibited.

Our results provide two characteristic considerations: one is that basal release of EDRF (NO) from L-arginine may not be possible as aforementioned, since L-arginine-induced vasodilation reflects availability of L-arginine being limiting for production of NO in rat pial artery (Gold *et al.*, 1989). Second consideration is that L-arginine is directly utilized to produce NO-cyclic GMP pathway by cerebral endothelium and both ACh- and L-arginine-induced releaxation that NO released from endothelium contribute to membrane hyperpolarization in association with activation of ATP-sensitive K^+ channel. This kind of finding has been reported by Tare *et al.*, (1990) and Garland and McPherson (1992). Rand and Garland (1992) showed that NO produced by muscarinic stimulation is involved in the smooth muscle hyperpolarization and relaxation of the rabbit basilar artery. Nevertheless, it is not clear how much significantly the hyperpolarization as a separate factor contributes to dilatation of the pial artery independent of NO. Further *in vivo* study remains to be clarified (Brayden, 1990; Nishiye *et al.*, 1989). The present *in vivo* experiment could not explain the involvement of glibenclamide-sensitive K^+ channels electrophysiologically.

Our main finding is that while NO released from the endothelium either by ACh or L-argi-

nine contributes basal vasodilator one to cerebral pial arteries, it does not play a modulator role in cerebrovascular autoregulatory dilatation in anesthetized rats because vasodilator response remains intact after pretreatment with L-NNA. These findings are consistent with the results demonstrated by Wang *et al.* (1992) and Saito *et al.* (1993). Thomas *et al.* (1992), otherwise, have reported that NO plays a role in determining the upper limit of autoregulation by influencing cerebrovascular tone. However, our experiment did not conduct to test its effect on the state of hypertension.

On the other hand, in the present study muscarinic receptors mediating endothelium-dependent vasodilation in rat pial arteries were considered to be M_1 and M_3 subtypes. Muscarinic receptors were differentiated into subtypes according to their selective inhibitions for specific antagonists, pirenzepine (highest affinity for M_1 and intermediate for M_3), methoctramine (M_2), and pF-HSiD (M_3) (Lambrecht *et al.*, 1988; Hulme *et al.*, 1990). In the present study with rat pial arteries, muscarinic receptors mediating endothelium-dependent vasodilation appeared to be of the M_1 and M_3 subtypes, but not the M_2 subtype. According to Garcia-Villaon *et al.* (1991), endothelial cells of rabbit pial arteries contain receptors of the M_3 subtype which mediate release of EDRF in agreement with our results. The involvement of M_1 receptor subtype in rat pial arteries is explained as a fact that pirenzepine has an intermediate affinity for M_3 even though it has higher affinity for M_1 subtype (Hulme *et al.*, 1990) and M_1 receptors mediate phosphoinositide hydrolysis in bovine pial vessels (Garcia-Villalon *et al.*, 1991).

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REFERENCES

Brayden JE: *Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. Am J Physiol 259 (Heart Circ Physiol 28): H668-H673, 1990*

Chorobsky J and Penfield W: *Cerebral vasodilator nerves and their pathway from the medulla oblongata. Arch Neurol Psychiat 28: 1257-1289, 1932*

Edvinsson L, Neilsen KC, Owman C and Sporrong B: *Cholinergic mechanisms in pial vessels. Z Zellforsch Mikros Anat 134: 311-325, 1972*

Faraci FM, Mayhan WG, Heistad DD: *Responses of rat basilar artery to acetylcholine and platelet products in vivo. Stroke 22: 56-60, 1991*

Faraci FM: *Role of nitric oxide in regulation of basilar artery tone in vivo. Am J Physiol 259 (Heart Cric Physiol 28): HI1216-HI1221, 1990*

Fujiwara S, Kassel NF, Sasaki T, Nakagomi T and Lehman RM: *Selective hemoglobin inhibition of endothelium-dependent vasodilation of rabbit basilar artery. J Nedurosurg 64: 445-452, 1986*

Gold ME, Bush PA and Ignarro LJ: *Depletion of L-arginine causes reversible tolerance to endothelium-dependent relaxation. Biochem Biophys Res Commun 164: 714-721, 1989*

Garcia-Villalon AL, Klause DN, Ehlert FS and Duckles SP: *Heterogeneity of muscarinic receptor subtypes in cerebal blood vessels. J Pharmacol Exp Ther 258: 304-310, 1991*

Garland CJ and MePherson GA: *Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. Br J Pharmacol 105: 429-435, 1992*

Hong KW, Pyo KM, Lee WS, Yu SS and Rhim BY: *Pharmacological evidence that calcitonin gene-related peptide is implicated in cerebral autoregulation. Am J Physiol 266(Heart Circ Physiol 35): H11-H16, 1994.*

Hulme EC, Birdsall NJ and Buckely NJ: *Muscarinic receptor subtypes. Ann Rev Pharmacol Toxicol 30: 633-673, 1990*

Ignarro LJ, Buga GM, Wood KS, Byrns RE and Chaudhuri G: *Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 84: 92265-9269, 1987*

Ignarro LJ: *Biosynthesis and metabolism of endothelium-derived nitric oxide. Ann Rev Pharmacol Toxicol 30: 535-560, 1990*

Kontos HA, Wei EP and Marshall JJ: *In vivo bioassay of endothelium-derived relaxing factor. Am J Physiol 255 (Heart Circ Physiol 24). H1259-H1262, 1988*

Lambrecht G, Feifel R, Forth B, Strohman C, Tacker R and Mutschler E: *p-Fluoro-hexahydro-sila-difenidol: The first M_2 beta-selective muscarinic antagonist. Eur J Pharmacol 152: 193-194, 1988*

Lee TJF: *Cholinergic mechanism in the large cat cerebral artery. Circ Res 50: 870-879, 1982*

Marshall JJ, Wei EP and Kontos HA: *Independent blockade of cerebral vasodilatation from acetylcholine and nitric oxide. Am J Physiol 255 (Heart Circ Physiol 24): H847-H854, 1988*

Martin W, Villani GM, Jothianandan D and Furchgott RF: *Selective blockade of endothelium-derived and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. J Pharmacol Exp Ther 232: 708-716, 1985*

Mayhan WG: *Impairment of endothelium-dependent dilatation of basilar artery during chronic hypertension. Am J Physiol 257 (Heart Circ Physiol 28): H1455-H1462, 1990*

Moncada S, Palmer RMJ and Higgs EA: *Biosynthesis of nitric oxide from L-arginine: a pathway for the regulation of cell function and communication. Biochem Pharmacol 38: 1709-1715, 1989*

Moncada S, Palmer RMJ and Higgs EA: *Nitric oxide: Physiology pathophysiology, and pharmacology. Pharmacol Rev 43: 109-142, 1991*

Mülsch A, Bassenge E and Busse R: *Nitric oxide synthesis in endothelial cytosol: Evidence for a calcium-dependent and a calcium-independent mechanism Naunyn-Schmiedeberg's Arch Pharmacol 340: 767-770, 1988*

Nishiye E, Nakao K, Itoh T and Kuriyama H: *Factors inducing endothelium-dependent relaxation in the guinea pig basilar artery as estimated from the actions of hemoglobin. Br J Pharmacol 96: 645-655, 1989*

Palmer RMJ, Ferrige AG and Moncada S: *Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature (Lond) 327: 524-526, 1987*

Poeggel G, Muller M, Seidel I, Rechardt L and Bernstein HG: *Histochemistry of guanalate cyclase, phosphodiesterase, and NADPH-diaphorase(nitric oxide synthase) in rat brain vasculature. J Cardiovas Pharmacol 20 (Suppl 12): S76-S79, 1992*

Rand VE and Garland CJ: *Endothelium-dependent relaxation to acetylcholine in the rabbit basilar artery: importance of membrane hyperpolarization. Br J Pharmacol 106: 143-150, 1992*

Rosenblum WI: *Endothelial-dependent relaxation demonstrated in vivo in cerebral arterioles. Stroke 17: 494-497, 1986*

Rosenblum WI, Nishimura H and Nelson GH: *Endothelium-dependent L-arginine- and L-NMMA-sensitive mechanisms regulate tone of brain microvessels. Am J Physiol 259 (Heart Circ Physiol 28): H1396-H1401, 1990*

Saito S, Wilson DA, Hanley DF and Traystman RJ: *Nitric oxide synthase does not contribute to cerebral autoregulatory phenomenon in anesthetized dogs. J Autonomic Nervous System 49: S73-S76, 1994*

Schmidt MJ, Sawyer BD, Truex LL, Marshall WS and Fleisch JH: *LY 83583, and agent that lowers intracellular levels of cyclic guanosine 3'5'-mino-phosphate. J Pharmacol Exp Ther 232: 764-769, 1985*

Schmid-Antomarchi H, De Whillie J, Fosset M and Lazdunski M: *The antidiabetic sulfonylurea glibenclamide is a potent blocker of the ATP-modulated K^+ channel in insulin-secreting cells. Biochem Biophys Res Commun 146: 21-25, 1987*

Standen NB, Quale JM, Davies NW, Brayden JE, Huang Y and Nelson MT: *Hyperpolarizing vasodilatiors activate ATP-sensitive K^+ channels in arterial smooth muscle. Science (Wash DC) 245: 177-180, 1989*

Tare M, Parkington HC, Coleman HA, Neild TO and Dusting GJ: *Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. Nature 346: 69-71, 1990*

Thomas CL, Kelly, PAT, Ritchil, IM, Sharkey, J and Arbuthnottm GM: *Cerebrovascular autoregulation to hypertension induced in inhibition of nitric oxide synthesis. Br J Pharmacol 108: 129p, 1993*

Wang Q, Paulson OB and Lassen NA: *Is autoregulation of cerebral blood flow in rats influenced by nitro-L-arginine, a blocker of the synthesis of nitric oxide? Acta Physiol Scand 145: 297-198, 1992*

Watanabe M, Rosenblum W and Nelson GH: *In vivo effect of methylene blue on endothelium-dependent and endothelium-independent dilations of brain microvessels in mice. Circ Res 62: 86-92, 1988*

Whalley ET, Amure YO and Lye RH: *Analysis of the mechanism of action of bradykinin on human basilar artery in vitro. Naunyn-Schmeideberg's Arch Pharmacol 335: 433-437, 1987*

=국문초록=

뇌혈류 자가조절에 대한 Endothelium-derived Relaxing Factor의 역할

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본 연구에서는 콜린성 기전에 반응하여 분비되는 내피 의존성 이완물질(endothelium-derived relaxing factor, EDRF)나 nitric oxide (NO)가 마취 흰쥐의 뇌혈류 자가조절기전에 관여할 가능성을 관찰하였다. Acetylcholine ($10^{-9}-10^{-6}$ M)을 포함한 mock 뇌척수액(CSF)을 관류시 뇌연막동맥은 농도에 의존하여 이완반응을 나타내었고(평균; $19.3\pm1.7\mu$m, n=36), 이러한 이완반응은 Nω-nitro-L-arginine(L-NNA, 10^{-5} M)에 의해서 억제되었을 뿐 아니라 methylene blue (10^{-6} M)나 oxyhemoglobin (10^{-6} M)에 의하여도 억제되었다. 한편 이러한 acetylcholine에 의한 뇌연막동맥의 이완반응을 매개하는 무스카린 수용체는 무스카린 수용체 길항제의 봉쇄효과를 관찰한 실험에서 M_1과 M_3 아형으로 생각되었다. L-Arginine을 함유한 mock CSF로 관류시 일어나는 일시적인 혈관이완반응은 NY 83583 (10^{-5} M)에 강력히 억제되었으나 L-NNA (10^{-5} M)에 의해서는 억제되지 아니하였다. 한편 acetylcholine과 L-arginine에 의한 혈관이완반응은 ATP-sensitive K^+ 통로 봉쇄제인 glibenclamide에 의해 유의하게 봉쇄되었다. 나아가 뇌연막동맥의 직경 변화를 동맥압의 변화에 대하여 검정한 결과 혈과이완과 혈관수축의 회귀 직선의 경사도는 10^{-5} M L-NNA의 전처치에 의하여 영향을 받지 아니하였으나, 3×10^{-6} M glibenclamide에 의해 유의하게 감소되었다. 이러한 결과로 보아 혈압하강에 대해 쥐의 뇌연막동맥에 나타나는 혈관이완반응은 EDRF(NO)에 의해 매개되지 않는다고 사료된다.