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Pharmacological Action Mechanism(s) of Vasodilator Effect of Calcitonin Gene-related Peptide in Rat Basilar Arteries

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ABSTRACT

In the present study, we observed changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) as measured with the fluorescent Ca^{2+} indicator fura-2 in association with force development of the rat basilar arteries during activation by K^+ depolarizing solution and U46619, a thromboxane analogue, in the absence and the presence of calcitonin-gene related peptide (CGRP). CGRP (30 and 100 nM) caused a concentration-dependent inhibition of U46619-induced contraction with decrease in $[Ca^{2+}]_i$, whereas it did not exert any effect on the K^+ (90 mM)-induced contraction and increase in $[Ca^{2+}]_i$. Further, $[Ca^{2+}]_i$-force relationships were determined by plotting the ratio of F_{340}/F_{380} ($[Ca^{2+}]_i$) as a function of the force induced by U46619, and the results were compared with those obtained in the presence of CGRP. The curves obtained in the presence of CGRP (30 and 100 nM) were significantly moved to downward without right shift of the curves suggesting that CGRP inhibited the U46619-induced contraction only by mediation of reduction in $[Ca^{2+}]_i$ without any change in the sensitivity of contractile apparatus to Ca^{2+}. The CGRP-induced attenuation of $[Ca^{2+}]_i$ and force development was significantly inhibited under pretreatment with CGRP (8~37) fragment (100 nM), a CGRP1 receptor antagonist. Both the reduced contraction and reduction in $[Ca^{2+}]_i$ caused by CGRP were fully reversed by pretreatment with charybdotoxin (100 nM) and iberiotoxin (100 nM), large conductance Ca^{2+}-activated K^+ channel blockers, but not by apamin (300 nM), a small conductance Ca^{2+}-activated K^+ channel blocker, and glibenclamide (1 μM), an ATP-sensitive K^+ channel blocker.

In conclusion, it is suggested that the CGRP1 receptor, upon activation by CGRP, are coupled to opening of Ca^{2+}-activated K^+ channel and cause to decrease in $[Ca^{2+}]_i$, thereby leading to vasodilation of the rat basilar artery. However, it is not defined that the mechanism underlying vasodilation whether the K^+ channel blockers, charybdotoxin and iberiotoxin directly block the CGRP receptors and that CGRP-evoked relaxation is dependent on the cyclic AMP or K^+ channel opening or both actions.

Key Words: Basilar artery, Calcitonin gene-related peptide, K^+ channels, Intracellular Ca^{2+}, Vasorelaxation

INTRODUCTION

Although the tone of the blood vessels is mainly regulated by vascular adrenergic nerves, a number of studies have demonstrated that many resistance vessels including cerebral arteries possess non-adrenergic, non-cholinergic (NANC) nerves. Calcitonin gene-related peptide

(CGRP), a potent vasodilator neuropeptide (Brain *et al.*, 1985; Kawasaki *et al.*, 1988), acts as a neurotrsansmitter in the NANC vasodilator nerves (Kawasaki *et al.*, 1988; Fujimori *et al.*, 1989) and is widely distributed in perivascular nerves throughout the vascular system (Mulderry *et al.*, 1985; Kawasaki *et al.*, 1990; Del Bianco *et al.*, 1991).

Particularly, CGRP exerts a potent vasodilating effect on the cerebral artery in vitro (Edvinsson, 1985; Saito *et al.*, 1989) and on the pial artery in situ (McCulloch *et al.*, 1986; Wei *et al.*, 1992). In addition, CGRP-immunoreactive nerve fibers are demonstrated in all major cerebral arteries including posterior cerebral artery (Edvinsson *et al.*, 1987). Recently, we reported an implication of CGRP in the autoregulatory vasodilation of cerebral vascular beds (Hong *et al.*, 1994; 1996). Vasodilator effect of CGRP in cerebral vessels has been reported to be mediated in part via activation of ATP-sensitive K^+ channels (Edwards *et al.*, 1991; Nelson *et al.*, 1990b) and an increase in cAMP levels (Edvinsson 1985; Hong *et al.*, 1996; Kitazono *et al.*, 1993). Thus, CGRP is proposed as a candidate neurotransmitter involved in neurogenic vasodilation of the cerebral vascular bed (Bevan and Brayden, 1987; Saito *et al.*, 1989). Most recently, we have observed the CGRP-induced vasodilation in rat pial arterioles which is significantly inhibited by not only glibenclamide, an ATP-sensitive K^+ channel blocker (Nelson *et al.*, 1990b), but also charybdotoxin, a large conductance Ca^{2+}-activated K^+ channel blocker (Hong *et al.*, 1996).

On the other hand, changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) play a key role in determining the tension development of vascular smooth muscles (Kamm and Stull, 1985). Recent developments in optical techniques with fluorescent-indicator dyes have made of direct determination of ionic changes in intact cells feasible (Grynkiewicz *et al.*, 1985).

In the present study, we simultaneously determined the effects of CGRP on $[Ca^{2+}]_i$ levels and tension development induced by U46619, a thromboxane analogue, in the intact muscle strips of rat basilar arteries. Further, we identified the type of K^+ channels implicated in the vasodilatory action of CGRP by using various K^+ channel blocker such as glibenclamide, apamin, charybdotoxin and iberiotoxin.

METHODS

Measurement of tension

Rats (Sprague-Dawley, 250~300 g) were anesthetized with ether and sacrificed by bleeding from carotid arteries. The brain were removed and placed in ice-cold physiological salt solution (PSS), and the basilar artery was dissected free from the brain surface. The basilar arteries (240~420 μm in outer diameter) were dissected from the surface of cerebral cortex in PSS. The PSS was composed of (mM) 130 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 1.18 NaH_2PO_4, 1.6 $CaCl_2$, 14.9 $NaHCO_3$ and dextrose 5 and aerated with 95% O_2-5% CO_2 and maintained at 37℃ (pH 7.4). Segments of the distal end of the basilar artery (approximately 2 mm in length) were mounted on 40 μm tungsten wires and placed in small vessel myograph (J. P. Tradings, Aarhus, Denmark) maintained at 37℃. Tissues were allowed to equilibrate for 1 hour, during which time PSS was changed every 20 minutes. The maximal responses were determined by addition of 90 mM KCl. Contractile responses were expressed as percentages of the contraction induced by KCl (90 mM) PSS.

Measurement of intacellular Ca^{2+}

For measurement of $[Ca^{2+}]_i$ basilar arterial rings were incubated in PSS containing 10 μM fura-2 acetoxylmethyl ester (fura-2/AM, Molecular Probes) for 3 to 5 hr at room temperature. To help dissolve the fura-2/AM in PSS, the noncytotoxic detergent pluronic F-127 (0.1% w/v) was included in the loading PSS. After the fura-2/AM loading, strips were rinsed with the normal PSS for approximately one hour and then used for experiments. After washing the rings, they were mounted between two tungsten hooks, which were mounted to a force displacement transducer of small vessel myograph (J.P. Trading). The rings were mounted horizontally in a temperature-controlled tissue bath placed on the stage of an inverted microscope (Nikon, TMD-8). The tissue bath was perfused at a rate

of 4 ml/min with PSS warmed at 37℃. The rings mounted were equilibrated under about 5 mN of resting tension for more than 30 min. Changes in fluorescence of the fura-2-Ca^{2+} complxes in the strips were measured by using a Spex Fluorog-2-Spectrofluorometer equipped with a dual wave length excitation device (Model CM 1TII1, Spex Industries, Edison, New Jersey, U.S.A.) connected to an inverted fluoromicroscope (TMD-8, Nikon, Tokyo, Japan). The fluorescence image was obtained by focussing on the medial smooth muscle layer with a Nikon CF UV (Fluor) objective lens (×10). The light emitted from the muscle strip was collected alternatively with 340 nm and 380 nm light, and then was collected by a photomultiplier through a 500-nm filter. The signals were fed into a microcomputer for calculation of the ratio of the fluorescence intensities at 340 nm excitation (F340) to that at 380 nm (F_{380}). Autofluorescence signals were determined by quenching fura-2 signals with 10 mM $MnCl_2$ and these were subtracted from the corresponding values of F_{340} and F_{380} obtained under conditions of fura-2 loading to derive a recalculated ratio. Each experiment was commenced with perfusion with 90 mM KCl-PSS for 3 min. At the end of experiment, the strips were treated with 50 μM ionomycin. Changes in the ratio were expressed as percentages of the differences between basal values and those obtained with 90 mM KCl-PSS.

Drugs

Calcitonin gene-related peptide (CGRP) and CGRP(8～37) (human) were purchased from Peninsula Laboratories, Inc. (Belmont, CA) and was dissolved in 0.1% bovine serum albumin to make a stock solution of 0.1 mM. Apamin (from bee venom), charybdotoxin (Scorpion, Leiurus quinquestriatus var. habraeus) and iberiotoxin (Scorpion, Buthus tamulus) were purchased from Peptide Institute, Inc., and was dissolved in 0.1% bovine serum albumin to make a stock solution of 0.1 mM. U46619 (9,11-dideoxy-11 ,9-epoxymethanoprostaglandin F2), and glibenclamide were purchased from Sigma Chemical Co. (St. Louis, MO). Glibenclamide was sonicated in 0.1 N NaOH and diluted with 5% glucose to make a stock solution of 10 mM. Fura-2/AM was purchased from Molecular Probes (Eugene, OR).

Statistics

Results are expressed as means±SE. Comparisons of tissue responses before and after treatments with antagonists were made with the Student's paired or unpaired t-test and $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of CGRP on changes in $[Ca^{2+}]_i$ and muscle tension by U46619

Fig. 1 shows representative tracings of changes in fluorescence ratio (F_{340}/F_{380}, upper panel) and force development (lower panel) induced by high KCl (90 mM) PSS and followed by 10^{-10} $\sim 3\times10^{-7}$ M U46619, a thromboxane analogue, in the basilar arteries, and figure 2 summarized the data of 5～7 preparations. As shown in Fig. 1 increases in both fluorescence ratio (F_{340}/F_{380}) and force development were parallelly observed by application of high KCl (90 mM)-PSS and $10^{-10}\sim3\times10^{-7}$ M U46619. The increase in $[Ca^{2+}]_i$ reached a maximum level at about 35% (expressed as percentage of the response by 90 mM KCl) at 3×10^{-8} M U46619, but the increase in force reached at maximum level induced by 90 mM KCl. CCRP (30 and 100 nM) attenuated the increase in $[Ca^{2+}]_i$ iuduced by cumulative doses of U46619. Fig. 2C shows the relationships between $[Ca^{2+}]_i$ and tension development induced by U46619 and also shows the effect of CGRP (30 and 100 nM) on stimulation by U46619. CGRP (30 and 100 nM) inhibited the U46619-induced increase in $[Ca^{2+}]_i$ and tension development without changing the threshold $[Ca^{2+}]_i$ for contraction. After washout of CGRP, the effect of U46619 on $[Ca^{2+}]_i$ was almost same as that induced by the first application (data not shown).

Effects of CGRP(8～37) fragment on CGRP-induced responses

The CGRP-induced inhibition of $[Ca^{2+}]_i$ and tension development was significantly inhibited under pretreatment with CGRP(8～37) fragment

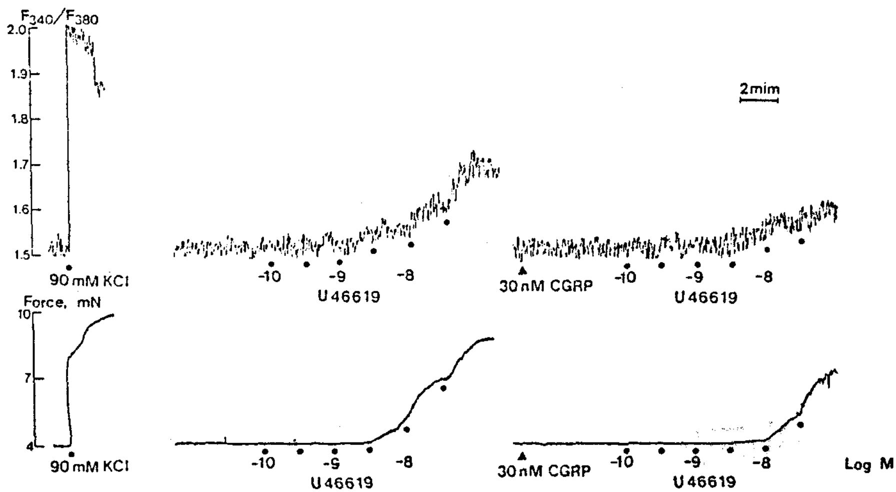

Fig. 1. Representative tracings of changes in fluorescence ratio (upper panel) and force development (lower panel) induced by high K^+ (90 mM) PSS followed by $10^{-10} \sim 3 \times 10^{-7}$ M U46619, a thromboxane analogue. The tension development and increase in $[Ca^{2+}]_i$ by cumulative doses of U46619 were attenuated by pretreatment with 30 nM CGRP.

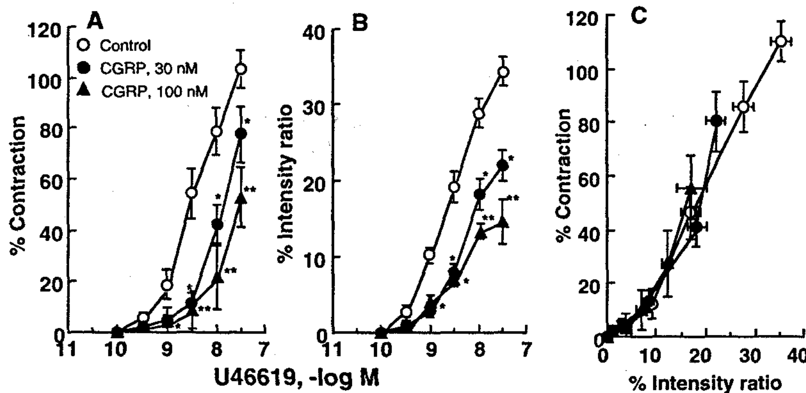

Fig. 2. Effect of CGRP on the force development (A) and fluorescence ratio (B) induced by U46619. (C) Effect of CGRP on $[Ca^{2+}]_i$-force relationship induced by U46619. Each value is expressed as percentage flourescence ratio or force, assuming the values obtained during 90 mM K^+ application to be 100%. CGRP was introduced 10 min prior to application of U46619. Each point represents mean±SEM from 5~8 experiments. *$P<0.05$; **$P<0.01$ vs. Control.

(10^{-7} M) 10 min before application of CGRP. Application of CGRP(8~37) fragment itself exerted little influence on the basal $[Ca^{2+}]_i$ level and tension development (Fig. 3).

Effect of glibenclamide on CGRP-induced responses

Fig. 4 shows the effect of glibenclamide (1 μM), an ATP-sensitive K^+ channel blocker, on the inhibition of 30 nM CGRP on the increases in

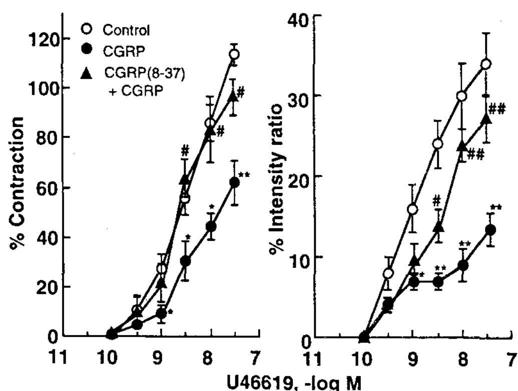

Fig. 3. Effect of CGRP(8~37) fragment on CGRP-induced inhibition of U46619-induced increase in $[Ca^{2+}]_i$ and force development. CGRP(8~37) fragment was introduced 10 min prior to application of CGRP. Each point represents mean±SEM from 4~6 experiments. *P<0.05; **P<0.01 vs. Control. # P<0.05; ##P<0.01 vs. CGRP.

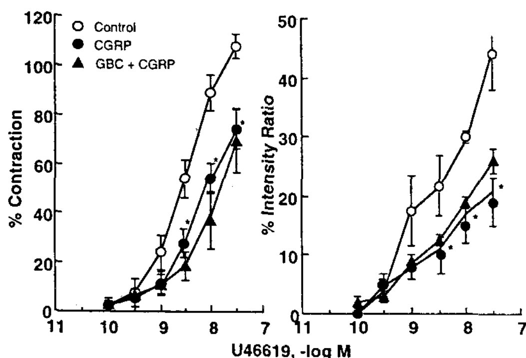

Fig. 4. Effect of glibenclamide (GBC, 1 μM) on CGRP-induced attenuation of force development and fluorescence ratio by U46619. Arterial preparations were incubated with glibenclamide for 10 min before or during application of CGRP. Each point represents mean±SEM from 4~5 experiments. *P<0.05; **P<0.01 vs. Control.

$[Ca^{2+}]_i$ and tension development by U46619 in the basilar arterial preparations. In the present study, 1 μM glibenclamide was applied 10 min piror to application of CGRP. Pretreatment

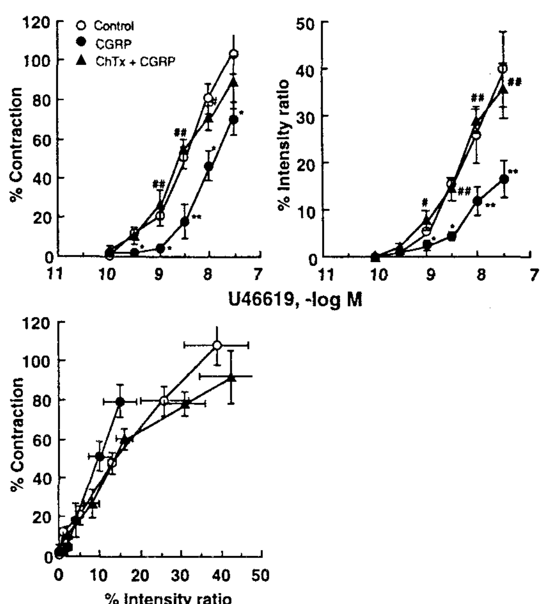

Fig. 5. Effect of charybdotoxin (ChTx, 100 nM) on CGRP-induced attenuation of force development (A) and fluorescence ratio (B) by U46619. (C) Effcet of ChTx on CGRP-induced atenuation of $[Ca^{2+}]_i$-force relationship induced by U46619. Vascular preparations were incubated with ChTx for 10 min before or during application of CGRP. Each point represents mean±SEM from experiments. *P <0.05; **P<0.01 vs. Control. # P<0.05; ##P< 0.01 vs. CGRP.

with glibenclamide failed to inhibit CGRP-induced attenuation of both $[Ca^{2+}]_i$ and force development.

Effects of charybdotoxin and iberiotoxin on CGRP-induced responses

Fig. 5 and 6 show the effect of charybdotoxin and iberiotoxin (100 nM, each), the large conductance Ca^{2+}-activated K^+ channel blockers, on the inhibition of 30 nM CGRP on the increases in $[Ca^{2+}]_i$ and tension development by U46619 in the basilar arterial preparations. When 100 nM charybdotoxin was applied 10 min piror to application of CGRP, the ability of CGRP to reduce the increase in $[Ca^{2+}]_i$ and the tension development by U46619 was completely abolished. Furthermore, iberiotoxin

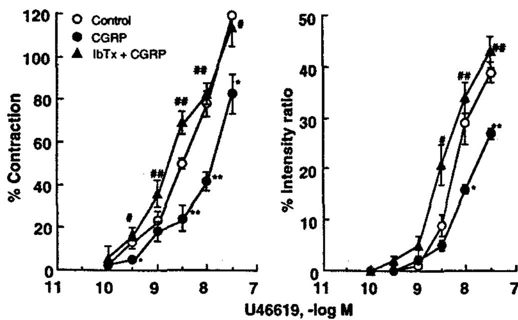

Fig. 6. Effect of iberiotoxin (IbTx, 100 nM) on CGRP-induced attenuation of force development and fluorescence ratio by U46619. Vascular preparations were incubated with IbTx for 10 min before or during application of CGRP. Each point represents mean±SEM from 5~7 experiments. *$P<0.05$; **$P<0.01$ vs. Control. #$P<0.05$; ##$P<0.01$ vs. CGRP.

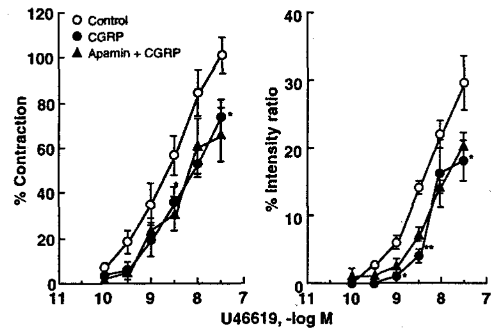

Fig. 7. Effect of apamin (0.3 μM) on CGRP-induced attenuation of force development and fluorescence ratio by U46619. Vascular preparations were incubated with apamin for 10 min before or during application of CGRP. Each point represents mean±SEM from 5~7 experiments. *$P<0.05$; **$P<0.01$ vs. Control.

also inhibited the effect of CGRP on U46619-induced increases in $[Ca^{2+}]_i$ and tension development.

Effect of apamin on CGRP-induced responses

We examined the effect of apamin, a blocker of small conductance Ca^{2+}-activated K^+ channel, on the responses to CGRP. However, the inhibitory effect of CGRP on the U46619-induced increases in $[Ca^{2+}]_i$ and force development was little affected by pretreatment with apamin (Fig. 7)

DISCUSSION

Of all the peptides that have been identified in the perivascular nerves, CGRP appears to be the most potent vasodilator of the cerebral vasculature both in vitro and in situ. Furthermore, CGRP has been shown to be involved in transmural nerve stimulation-induced vasodilation of cerebral arteries in vitro (Saito *et al.*, 1989). A growing body of evidence have demonstrated that CGRP exerts an extremely potent vasodilating effect on the cerebral artery in vitro (Edvinsson, 1985; Saito *et al.*, 1989) and on the pial artery in situ (McCulloch *et al.*, 1986; Wei *et al.*, 1992). In the cerebral vessels, CGRP-immunoreactive nerve fibers are demonstrated in all major arteries and cortical arterioles including human and rat posterior cerebral artery (Edvinsson *et al.*, 1987). Therefore, CGRP may be a candidate neurotransmitter involved in nonsympathetic neurogenic vasodilation of the cerebral vascular bed (Bevan and Brayden, 1987).

In the present study, we employed U46619, a thromboxane A_2 mimetic analogue, for inducing vasoconstriction. Thromboxane A_2 is a strong vasoconstrictor as well as a potent inducer of platelet aggregation, which is thought to be involved in ischemic ststes (Halushka and Lefer, 1987). The vasoconstrictor mechanism of U46619 has been proposed to involve its abilities to increase transmembrane Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels (Toda, 1982), Ca^{2+} release from intracellular stores and in addition, to increase the Ca^{2+}-sensitivity of the contractile proteins (Yamagishi *et al.*, 1992). The effects of U46619 producing inositol 1,4,5-trisphosphate (IP_3) production and Ca^{2+} release as well as transmembrane Ca^{2+} influx have been documented to be inhibited by the membrane hyperpolarizing action of K^+ chan-

nel opener, cromakalim (Yamagishi *et al.*, 1992a; 1992b). The major findings of this study in the rat basilar artery are that 1) the CGRP caused vasorelaxation and decrease in $[Ca^{2+}]_i$, 2) both vasorelaxation and decrease in $[Ca^{2+}]_i$ in response to CGRP were markedly inhibited by charybdotoxin and iberiotoxin, large conductance Ca^{2+}-activated K^+ channel blockers (Gimenez-Gallogo *et al.*, 1988; Galvez *et al.*, 1990), 3) However, they were affected neither by glibenclamide, an ATP-sensitive K^+ channel blocker (Schmid-Antomarchi *et al.*, 1987) nor by apamin, a small conductance Ca^{2+}-activated K^+ channel blocker (Blatz and Magleby, 1986). 4) CGRP-induced attenuation of force development and decrease in $[Ca^{2+}]_i$ was antagonized by a COOH-terminal fragment CGRP(8～37), a CGRP1 receptor antagonist (Chiba *et al.*, 1989; Dennis *et al.*, 1990).

Charybdotoxin, a component of the venom of the scorpion Leirus quinquestriatus, is a 37 amino acid peptide with three disulphide bridge (Sugg *et al.*, 1990). It was first introduced as a selective blocker of large conductance Ca^{2+}-activated K^+ channel in skeletal muscle (Miller *et al.*, 1985). Apamin is a bee venom polypeptide of 18 amino acids with two disulphide bridges, which has been shown to block a Ca^{2+}-activated K^+ channel of small conductance (Blatz and Magleby, 1986).

CGRP(8～37), which lacks agonist activity, has been shown to block CGRP-induced activation of adenylate cyclase in rat liver membranes (Chiba *et al.*, 1989) and to compete for CGRP-binding sites with nanomolar affinity in a number of rat and guinea pig peripheral and central tissues (Dennis *et al.*, 1990). Therefore, it was proposed that CGRP receptors senstitive to the antagonist activity of CGRP(8～37) be designated as CGRP1 receptors, while those insensitibve to CGRP(8～37) be designated CGRP2 receptors (Dennis *et al.*, 1990). In the present study, CGRP(8～37) antagonized both the relaxant effect of CGRP as well as CGRP-induced decrease in $[Ca^{2+}]_i$. Therefore, these results indicate that the CGRP receptors which are present in the rat basilar artery and mediate vasodilation appear to be of the CGRP1 subtype.

The inhibitory effect of CGRP on $[Ca^{2+}]_i$-tension relationship showed that the inhibitory effect is mainly due to the decrease in $[Ca^{2+}]_i$, although this was not the case with high K^+-induced contraction. In rat basilar arteries, CGRP may decrease $[Ca^{2+}]_i$ by the following two mechanisms: 1) direct inhibition of voltage dependent Ca^{2+} channels, and 2) indirect inhibition of Ca^{2+} channels following the activation K^+ channels. The first possibility may be less likely since CGRP did not reduce the high K^+ induced increases in $[Ca^{2+}]_i$. Therefore, a probable action site of CGRP is K^+ channels. Since the relationships between $[Ca^{2+}]_i$ and tension development are not changed by CGRP, it is not likely that the response to CGRP is mediated by modulation of Ca^{2+} sensitivity of contractile proteins.

In the present in vitro studies with rat basilar artery, CGRP caused a concentration-dependent vasorelaxation in accordance with a decrease in $[Ca^{2+}]_i$. Nevertheless, these effects were significantly inhibited by charybdotoxin and iberiotoxin, but not by glibenclamide as contrasted to the above results with the pial arteries (Hong *et al.*, 1996). The decrease in $[Ca^{2+}]_i$ by CGRP is considered to be ascribed to the indirect inhibition of voltage-dependent Ca^{2+} channels in association with opening of the K^+ channels and hyperpolarization of the smooth muscle cell membrane (Yamagishi *et al.*, 1992a; 1992b). This suggestion is derived from some reports that the vasodilator effect of CGRP is mediated in part via activation of ATP-sensitive K^+ channels (Nelson *et al.*, 1990)

Recently, a lot of evidence suggest that activation of ATP-sensitive K^+ channels and Ca^{2+}-activated K^+ channels exert an important role in vasodilation (Nelson *et al.*, 1990a; Nichols and Lederer, 1991). Nevertheless, there is some controversy over whether the K^+ channel openers exert relaxation in the rat cerebral vasculature. McCarron *et al.* (1991) and McPherson and Stork (1992) reported that K^+ channel openers did not relax the small resistance arteries from the rat cerebral vessels, whereas Nagao *et al.* (1991) demonstrated cromakalim-induced relaxation of the rat posterior cerebellar artery. In our results, CGRP exerted a strong vasorelaxant effect which was markedly inhibited by charybdotoxin or

iberiotoxin but not by glibenclamide or apamin, suggesting that the vasorelaxation to CGRP appears to result from the presence and activation of the large conductance Ca^{2+}-activated K^+ channels in the rat basilar artery. However, several different K^+ channels was demonstrated to be responsible for the actions of K^+ channel openers in various vascular beds (Gelband *et al.*, 1989; Kajioka *et al.*, 1991; Standen *et al.*, 1989).

Recently, we have observed in the in vivo experiment that CGRP as well as levcromakalim, a benzopyran K^+ channel opener, exert dilating effects in the rat pial arterioles which are inhibited by not only glibenclamide but also charybdotoxin, and concluded the vasodilation in response to either CGRP or levcromakalim results from the activation of the ATP-sensitive K^+ channels and Ca^{2+}-activated K^+ channels in these arterioles (Hong *et al.*, 1996)

A question arises as to why glibenclamide could not exert the inhibitory effect on the CGRP-induced vasorelaxation and decrease in $[Ca^{2+}]_i$ in the rat basilar artery in vitro as contrasrt to the finding in the rat pial arterioles as reported by Hong *et al.* (1996). Edwards *et al.* (1988) reported the heterogeneity in the electrophysiological properties in rat middle cerebral arteries, which is dependent upon its origin along the vascular tree. They described that the proximal segments displayed a stable resting membrane potential of approximately −69 mV whereas segments cut more distally showed an unstable membrane potential around −40 mV. Otherwise, McPherson and Keily (1995) have reported that the electrophysiological and mechanical properties of the isolated rat middle cerebral artery depend on the passive resting conditions under which the vassel is studied.

Although it is not defined as to why these different responses are resulted, the discrepancy appears to be attributed to the several factors such as regional difference, the situation of the tissues (in vivo or in vitro), different conditions (depolarization or tension states), or the presence of different K^+ channels (Gelband *et al.*, 1989; Kajioka *et al.*, 1991).

It is not known, at the present time, how CGRP activates the large conductance Ca^{2+}-activated K^+ channels in the basilar artery. Thus, further study is required to identify electrophysiologically whether the K^+ channels activated by CGRP in the rat basilar arteries share the properties with both ATP-sensitive K^+ channels and calcium-regulated K^+ channels. Recently, Trezise and Weston (1992) have compared the relaxant and hyperpolarizing actions of CGRP with those of BRL 38227 (levcromakalim) in the rabbit basilar arteries. In their results, CGRP consistently evoked a glibenclamide-inhibitable hyperpolarization, but in less degree than BRL 38227. Since the tonic increase in $[Ca^{2+}]_i$ induced by high K^+ (90 mM) was not abolished by CGRP, it does not seem to be due to membrane hyperpolarization. Thus the inhibitory mechanism of CGRP on Ca^{2+} influx induced by U46619 in basilar arterial strips may not be explained by its ability to hyperpolarize the plasma membrane resulting in the reduction of Ca^{2+} channel activation.

Vasorelaxation induced by CGRP has been proposed to be mediated by two interesting pharmacological actions: one is by stimulation of adenyl cyclase activity with a subsequent accumulation of intracellular cAMP (Edvinsson, 1985; Edwards *et al.*, 1991), and the other is by activation of the K^+ channels and subsequent hyperpolarization (Nelson *et al.*, 1990b; Zschauer *et al.*, 1992). Role of the K^+ channels was reported in the vascular response to several endogenous vasodilators (Bevan and Brayden, 1987; Brayden *et al.*, 1991). It is possible that the activation of large conducatance Ca^{2+}-activated K^+ channel may be linked to the activation of adenylate cyclase. This possiblity is supported by the recent report (Schultz *et al.*, 1992) that a hyperpolarization activated K^+ conductance of the cell membrane of Paramecium directly stimulates adenyl cyclase activity as a carrier of the K^+ resting conductance. However, it has not been reported whether the K^+ conductance-coupled cAMP-producing system exists in the smooth muscle cells.

Further, the vasodilator response to CGRP has been additionally correlated with increases in cyclic-AMP in the cerebral vessels (Edvinsson *et al.*, 1985; Hong *et al.*, 1996). In their experiment, the CGRP-induced stimulation of cyclic AMP production was inhibited

by charybdotoxin, suggesting a role for CGRP receptor-coupled Ca^{2+}-activated K^+ channel opening. In the present study it is not known on which mechanism the CGRP-evoked attenuation of U46619-induced contraction is more dependent between cyclic AMP and K^+ channel opening. The fact that both vasodilation and decrease in $[Ca^{2+}]_i$ induced by CGRP were markedly inhibited by charybdotoxin and iberiotoxin provide an evidence to support that the large conductance Ca^{2+}-activated K^+ channel are implicated in the CGRP receptor coupled decrease in $[Ca^{2+}]_i$ for vasodilation.

Taken together, it is strongly suggested that the vasodilator effect of CGRP in the rat basilar arteries is mediated by activation of CGRP1 recptor coupled to opening of large conductance Ca^{2+}-activated K^+ channels, thereby leading to inhibit the activation of voltage-dependent Ca^{2+} channels of the plasma membrane and to inhibit the contraction of the smooth muscle.

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=국문초록=

흰쥐의 뇌 기저동맥에서 CGRP에 의한 혈관 이완반응의 기전에 대한 연구

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본 실험에서는 흰쥐의 뇌 기저동맥을 이용하여 K^+과 U46619에 의한 수축과 세포내 Ca^{2+}의 변동을 관찰하고, 이들 반응을 CGRP 전처치시 나타나는 반응과 비교하였다. CGRP (30과 100 nM)는 U46619에 의하여 야기된 수축반응과 세포내 Ca^{2+}의 증가반응을 억제시켰으나, K^+ (90 mM)에 의하여 나타나는 반응에는 영향을 미치지 아니하였다. 게다가, U46619에 의하여 야기되는 장력에 대하여 세포내 Ca^{2+}의 변동 (F_{340}/F_{380})을 도표화하여 세포내 Ca^{2+} 농도와 장력의 발생과의 상관관계를 검토하고, 이들 결과를 CGRP 전처치시 나타나는 결과와 비교하였다. CGRP (30과 100 nM) 전처치군에서 얻어진 직선이 오른쪽으로 치우치지는 않으면서 아래쪽으로 이동하는 점으로 볼 때, CGRP가 Ca^{2+}에 대한 수축기구의 감수성에는 영향을 미치지 않으면서 세포내 Ca^{2+} 농도를 저하시킴에 의하여 U46619에 의한 근수축반응을 억제시키는 것으로 보여진다. 이러한 CGRP의 효과는 CGRP1 수용체 길항제인 CGRP(8～37) 분획(100 nM)의 전처치시 현저히 억제되었다. CGRP에 의한 수축력과 세포내 Ca^{2+}의 저하는 large conductance Ca^{2+}에 의하여 활성화되는 K^+ 통로 봉쇄제인 charybdotoxin (100 nM)과 iberiotoxin (100 nM)의 전처치에 의하여 완전하게 역전되었으나, small conductance Ca^{2+}에 의하여 활성화되는 K^+ 통로 봉쇄제인 apamin (300 nM)과 ATP에 감수성이 높은 K^+ 통로 봉쇄제인 glibenclamide (1 μM)에 의해서는 영향을 받지 아니하였다.

이상의 결과로 볼 때 CGRP1 수용체는 Ca^{2+}에 의하여 활성화되는 K^+ 통로를 개방시킴으로 세포내 Ca^{2+}을 감소시켜 뇌 기저동맥의 이완반응을 매개하는 것으로 사료된다.