ORIGINAL

Vascular action of bradykinin is mediated by B₂- subtype receptors in the canine lingual artery

Katsufumi Nanba, Kazuo Todoki and Eiichiro Okabe*

*Department of Pharmacology, Kanagawa Dental College
82 Inaoka-Cho, Yokosuka, Kanagawa 238, Japan*

[Received on September 18, 1995 ; Accepted on March 4, 1996]

Key words : lingual artery/bradykinin/B₂-receptors for kinins/NO/signal transduction

Abstract : The mechanisms underlying the responses of the canine lingual artery to bradykinin (BK) have been examined. In endothelium-intact lingual artery ring preparations contracted to a stable plateau tension by the addition of norepinephrine (NE), BK produced B₂-receptor antagonist [D-Phe⁷]-BK-sensitive and endothelium-dependent relaxations. The observed effect was significantly inhibited by nitric oxide (NO) inactivator hemoglobin, methylene blue, a good inhibitor of soluble guanylate cyclase, and by N^G-monomethyl-L-arginine, a NO synthase inhibitor, but not by B₁-receptor antagonist des-Arg⁹-[Leu⁸]-BK, suggesting endothelial production of the vasodilator agent NO by B₂-receptors activation. In endothelium-denuded rings in the absence of active tension caused by NE, BK increased muscle tension and cytosolic Ca²⁺ level ; these were inhibited by [D-Phe⁷]-BK. The concentration-dependent contraction produced by BK of endothelium-denuded rings was also attenuated by [D-Phe⁷]-BK ; protein kinase C inhibitor H-7 and phospholipase C inhibitor neomycin B reduced the BK-induced contraction. Indomethacin was without effect. Hence, it can be concluded that BK can cause relaxation and contraction in the canine lingual artery via B₂-receptors activation. The relaxation response to BK might be mediated by endothelial production of NO. BK has the potential to be a vasoconstrictor by exerting an effect on vascular smooth muscle cells, but not on endothelial cells, via B₂-receptors activation. It is also postulated that the contractile response to BK may be due to the production of second messengers derived from the action of phospholipase C, but not due to contractile prostanoids, when B₂-receptors are activated.

抄録 : Bradykinin (BK) に対するイヌ舌動脈の反応メカニズムを検討した。BK は norepinephrine (NE) によって前収縮した舌動脈輪状標本を内皮依存性に弛緩させ、この反応は B₂-受容体アンタゴニスト [D-Phe⁷]-BK で抑制された。また、BK に反応して引き起こされる内皮依存性弛緩は、一酸化窒素 (NO) を不活化する hemoglobin、可溶性 guanylate cyclase 阻害薬 methylene blue、そして NO 合成酵素阻害薬 N^G-monomethyl-L-arginine によっても有意に抑制された。NE 非存在下で、BK によって生ずる内皮除去標本平滑筋張力と細胞内 Ca²⁺ 濃度の増加は、[D-Phe⁷]-BK 抑制性であった。BK は内皮除去標本を濃度依存性に収縮させるが、これもまた [D-Phe⁷]-BK によって抑制され、同様に、protein kinase C 阻害薬 H-7、phospholipase C 阻害薬 neomycin B によっても抑制された。しかし、indomethacin は効果的ではなかった。

以上の結果から、BK は内皮細胞 B₂-受容体を刺激することによる NO 産生を介して舌動脈を弛緩させ、一方、平滑筋の B₂-受容体にリンクした phospholipase C 活性化に連動して舌動脈を収縮させるが、この収縮反応には prostanoids は関連しないことが示唆された。

Introduction

Normal orofacial blood flow is important for the maintenance of healthy teeth and gums. The blood vessels and tissues of the oral cavity are continuously exposed to foods, spices, bacteria, and endogenous substances (e. g., kallikreins and kinins), which stimulate and inhibit the production of endogenous prostanoids^{1,2)}. Locally produced kinins and prostanoids may have pronounced effects on orofacial blood flow^{3,4)}; however, a paucity of information exists on the sensitivity of the orofacial blood vessels to kinins.

It is now widely recognized that bradykinin (BK) and related peptides participate in important physiological and pathological states. The development of a series of peptide BK analogs which are selective agonists or antagonists at BK receptors has revealed that BK actions can be mediated by two subtypes of membrane receptors, named B_1 and B_2 ^{5,6)}. Current experimental evidence suggests that the vasodilator action of BK in some vessels is mediated primarily by B_2 -receptor activation, although there is also evidence for B_1 -receptor mediation⁷⁻⁹⁾. In addition, there are receptors by which BK increases endothelial production of vasodilator agents, such as nitric oxide (NO)⁹⁾.

Despite (a) the potential importance of BK to orofacial blood flow and its relationship to the maintenance of normal gingival, periodontal, and oral biology, and (b) the susceptibility of the lingual-gingival area to bacterial and other inflammatory states that can modulate BK, little is known about the effects of BK on the function of the blood vessels serving orofacial blood flow. The purpose of the present study was to determine the vasoactive effect of BK on canine lingual artery and to investigate the role of BK B_1 - and B_2 -subtype receptors, and NO in mediating this effect. Our findings indicate that BK can produce concentration-dependent relaxation and contraction; the effect is mediated by B_2 -receptors activation and NO production.

Materials and Methods

Animals

Mongrel dogs of either sex (10-15 kg) were used for all studies.

Vessel preparation and isometric tension recording

In accordance with our institutional Animal Care Committee guidelines, the animals were anesthetized with sodium pentobarbital (30 mg/kg, i. v.) and then sacrificed by rapid exsanguination. The tongue was cut out at the level of the papillae vallate, and the lingual artery, which is the largest collateral branch of the external carotid artery, was carefully isolated from sub-deep longitudinal fiber, and then dissected into several ring segments without disturbing the intimal layer after immersion in ice-cold modified Krebs-Ringer solution (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 25.0 mM NaHCO_3 , 0.026 mM calcium disodium ethylenediaminetetraacetic acid and 11.1 mM glucose, aerated with 95% O_2 -5% CO_2 ; pH 7.2-7.3). The endothelium was removed from some rings by gently rubbing the luminal surface with a wooden implement.

The rings were suspended in a 20 ml water-jacketed tissue bath (37°C) with one end tied to a fixed point and the other, to a force transducer (TB-612T; Nihon Kohden, Tokyo); changes in isometric force were recorded with an amplifier (AP-601T, Nihon Kohden) attached to a recorder (RM-6000, Nihon Kohden). Before the start of the experiment, the rings were allowed to equilibrate for 90 min in a modified Krebs-Ringer solution. During this time, the rings were stretched to a passive tension of 15.3 millinewton (mN) (1.5 g). Endothelial integrity was checked in all experiments by the presence of the characteristic relaxation response to 10^{-6}M acetylcholine.

Cytosolic Ca^{2+} level

Cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) was measured simultaneously with muscle contraction as reported by Ozaki *et al.*¹⁰⁾ and Sato *et al.*¹¹⁾ The acetoxymethyl ester of fura-2 (fura-2/AM) was added to a modified Krebs-

Ringer solution to achieve a final concentration of 5 mM. A small amount (0.5%) of cremophor EL was also added to increase the solubility of fura-2/AM. The prepared spiral strips (2~3 mm width and 5~6 mm length) were loaded with fura-2/AM for 4 to 5 hr at room temperature (23~25°C). After fura-2/AM loading, the preparations were washed with modified Krebs-Ringer solution in a tissue bath (7 ml) at 37°C for 20 min to remove unhydrolyzed fura-2/AM. The preparations were illuminated alternately (48 Hz) at excitation wave lengths of 340 and 380 nm, and fluorescence at 500 nm was measured with a fluorimeter (CAF-100, Jasco, Tokyo, Japan). The ratio of fluorescence induced by excitation at 340 nm and that induced by excitation at 380 nm ($R_{340/380}$) was used as an indicator of $[Ca^{2+}]_i$.

Drugs

Fura-2/AM (Dojindo Laboratories, Kumamoto, Japan) was dissolved in dimethyl sulfoxide. The following drugs were dissolved in water: bradykinin acetate salt (Sigma Chemical, St. Louis, MO, U.S.A.), des-Arg⁹-[Leu⁸]-bradykinin acetate salt (Sigma), [D-Phe⁷]-bradykinin acetate salt (Sigma), ($\pm$) norepinephrine hydrochloride (Sigma), acetylcholine chloride (Sigma), methylene blue (Sigma), 1-(5-soquinoliny-sulfonyl)-2-methyl-piperazine (H-7; Sigma), neomycin B sulfate (Sigma), *N*^G-monomethyl-L-arginine acetate salt (L-NMMA; Sigma), hemoglobin (Sigma), and cremophor EL (Nakarai Chemicals, Kyoto, Japan). Indomethacin (Sigma) stock solution was prepared by dissolving three parts indomethacin and one part sodium bicarbonate in distilled water. In preliminary experiments, we confirmed that test drugs used in the fura-2 experiments did not interfere with the fura-2- Ca^{2+} fluorescence measurement.

Statistical analyses

Means $\pm$ SEM are given throughout. Student's *t*-test was used for statistical analysis of the results. Differences were considered to be statistically significant when *P* was <0.05.

Results

Concentration-response relationship

Endothelium-intact lingual artery rings exhibited biphasic responses to the cumulative application of bradykinin (BK) consisting of relaxations followed by contractions in the absence of active tone induced by norepinephrine (NE) (Fig. 1A, left). At stable plateau tension by the addition of NE, the cumulative application of BK produced relaxation up to 10^{-7} M in endothelium-intact rings; at 10^{-6} M, BK exerted only contraction but not relaxation (Fig. 1A, right). When the endothelium was rubbed off, the relaxant response to BK was almost completely abolished in the presence or absence of active tone induced by NE (Fig. 1B). Contractile response of endothelium-denuded rings to BK in the absence of NE-induced active tone was concentration-dependent (Fig. 2A), and the response of endothelium-intact rings to BK at 10^{-10} to 10^{-7} M resulted in a concentration-dependent relaxation (Fig. 2B). On the basis of this, preparations used to assess the response to BK were chosen. We used endothelium-intact rings for assessing the relaxant response at stable plateau tension by the addition of NE. The contractile response was assessed in endothelium-denuded ring preparations in the absence of NE-induced active tension.

We next determined whether or not the responses to BK were altered by BK antagonists. BK B_2 -receptor antagonist [D-Phe⁷]-BK¹² significantly attenuated the observed responses of endothelium-intact and denuded ring preparations with rightward shifts of the concentration-relaxation and contraction curves (Fig. 3), respectively. Under similar experimental conditions, the inhibitory effect of BK B_1 -receptor antagonist des-Arg⁹-[Leu⁸]-BK¹³ was negligible (Fig. 4) in comparison with the observed effect of [D-Phe⁷]-BK.

$[Ca^{2+}]_i$ level

Having established that BK causes B_2 -receptor-mediated responses, and that the relaxant response is endothelium-dependent, we next determined the $[Ca^{2+}]_i$ levels in endothelium-denuded muscle strips of the lingual artery. As shown in Figure 5, BK in-

Fig. 1 Responses to bradykinin of endothelium-intact (A) and endothelium-denuded (B) canine lingual artery rings. Concentration-response curves were determined by the method of stepwise cumulative addition of bradykinin in the presence or absence of norepinephrine (NE; 10^{-5} M)-induced active tone; the functional integrity of the endothelium was checked by the addition of acetylcholine (ACh; 10^{-6} M) before the assessment of bradykinin effect. Two ring preparations (for endothelium-intact and endothelium-denuded rings) obtained from the same vessel were studied in parallel. Numbers indicate minus logarithm of molar dose.

duced an increase in muscle tension and $[Ca^{2+}]_i$. Both these changes were inhibited by [D-Phe⁷]-BK (percentage of inhibition: tension, 16.7%; $[Ca^{2+}]_i$, 44.4%).

Effect of protein kinase inhibitors on contractile response

The contractile effect of kinins on the rabbit isolated aorta is partially inhibited by treatment with protein kinase C inhibitors¹⁴⁾. Therefore, we tested the effects of protein kinase inhibitors, in particular, to evaluate the possible role of protein kinase C following the stimulation of BK-receptors in BK-induced contraction of the endothelium-denuded lingual artery rings. The effect of protein kinase C inhibitor H-7¹⁵⁾ was analyzed by constructing full cumulative concentration-effect curve for BK in its presence (Fig. 6A). The contractile effect of BK (10^{-8} to 10^{-5} M) was significantly depressed by H-7. Furthermore, phos-

pholipase C inhibitor neomycin B¹⁶⁾ inhibited the contraction induced by BK (10^{-6} M) in a concentration-dependent fashion (Fig. 6B).

Effect of hemoglobin, methylene blue and L-NMMA on relaxant response

BK B₂-receptors are found on the endothelium of a number of vessels⁶⁾. Activation of these receptors leads to the release of endothelium-derived NO and prostacyclin, the balance between these two relaxing factors varying according to the vessel type⁶⁾. Therefore, we proposed that endothelium-dependent relaxation produced by BK reported here could be due to NO released in response to BK in our system. A test of this hypothesis is presented in Figure 7. The relaxation of endothelium-intact rings produced by BK at a stable plateau tension induced by NE was significantly attenuated when NO (released from endothelium in response to BK) had been inactivated by

Fig. 2 Dose-response contractions of endothelium-denuded rings (A) and relaxations of endothelium-intact rings (B) induced by bradykinin. The experimental conditions were similar to those described in Figure 1. Contractile and relaxant responses were assessed in the absence and presence of active tone induced by NE (10^{-5} M), respectively. Contractile responses to 10^{-5} M bradykinin are taken as 100%, and other data are plotted in relation to it. Relaxant responses were expressed as percentage of the stable plateau contraction evoked by NE. Each point represents the mean, and vertical lines show SEM ($n=4\sim 8$); n refers to the number of dogs from which the lingual artery was taken.

hemoglobin¹⁷⁾ (Fig. 7A), when a component of soluble guanylate cyclase had been oxidized by methylene blue¹⁸⁾ (Fig. 7B), and when NO synthase had been inhibited by L-NMMA (Fig. 7C). Indomethacin (10^{-6} M) had no effect on this system (data not shown).

Discussion

BK is a potent vasodilator *in vivo*⁵⁾. This vasodilation has been reported to be both dependent^{19,20)} and independent^{21,22)} of the endothelium. These observations are of potential interest since the initial post-receptor signaling events in vascular smooth muscle cells are the same for BK and several vasoconstrictor agents, including NE²³⁾, angiotensin II²⁴⁾, and endothelin²⁵⁾. Specifically, all of these agents, including BK, are known to increase $[\text{Ca}^{2+}]_i$ in vascular smooth muscle cells within seconds of their application^{26,27)}. In the present study, in endothelium-intact canine lingual artery rings *in vitro*, BK produced BK B_2 -receptor antagonist [D-Phe⁷]-BK-

Fig. 3 Effect of [D-Phe⁷]-bradykinin on bradykinin-induced contractions of endothelium-denuded rings (A) and relaxations of endothelium-intact rings (B). The experimental conditions were similar to those described in Figure 2 except that 10^{-6} M [D-Phe⁷]-bradykinin (●) was added 5 min before stepwise cumulative addition of bradykinin (○). In this series of studies, four ring preparations (for endothelium-intact rings in the presence and absence of the antagonist, and endothelium-denuded rings in the presence and absence of the antagonist) obtained from the same vessel were studied in parallel. Contractile responses to 10^{-5} M bradykinin are taken as 100%, and other data are plotted in relation to it. Relaxant responses were expressed as percentage of the stable plateau contraction evoked by NE. Each point represents the mean, and vertical lines show SEM ($n=4$); n refers to the number of dogs from which the lingual artery was taken. *Significantly ($P<0.05$) different from corresponding values without antagonist used.

sensitive (Fig. 3B) and endothelium-dependent relaxations (Fig. 1). The observed effect of BK was significantly inhibited by hemoglobin, methylene blue, or by L-NMMA (Fig. 7). These findings are compatible with endothelial production of vasodilator agent NO by B_2 -receptors activation. The importance of a rise in $[\text{Ca}^{2+}]_i$ for contraction has been previously investigated.²⁸⁾ In the endothelium-denuded lingual artery strips, BK increased $[\text{Ca}^{2+}]_i$ and muscle tension (Fig. 5). These were inhibited by B_2 -receptor antagonist [D-Phe⁷]-BK (Figs. 3A and 5), but not by B_1 -receptor antagonist des-Arg⁹-[Leu⁸]-BK (Fig. 4A). These observations raised the possibility that, although the events in vascular smooth muscle con-

Fig. 4 Effect of des-Arg⁹-[Leu⁸]-bradykinin on bradykinin-induced contractions of endothelium-denuded rings (A) and relaxations of endothelium-intact rings (B). The experimental conditions were similar to those described in Figure 2 except that 10⁻⁶ M des-Arg⁹-[Leu⁸]-bradykinin (●) was added 5 min before stepwise cumulative addition of bradykinin (○). In this series of studies, four ring preparations (for endothelium-intact rings in the presence and absence of the antagonist, and endothelium-denuded rings in the presence and absence of the antagonist) obtained from the same vessel were studied in parallel. Contractile responses to 10⁻⁵ M bradykinin are taken as 100%, and other data are plotted in relation to it. Relaxant responses were expressed as percentage of the stable plateau contraction evoked by NE. Each point represents the mean, and vertical lines show SEM (n=6); n refers to the number of dogs from which the lingual artery was taken.

traction and relaxation are shared, both relaxation and contraction responses can occur through BK B₂-receptors activation, and that Ca²⁺ signaling events in vascular smooth muscle cells may dictate B₂-receptors-mediated contraction. This was a possibility worthy of testing, since several of the known signaling events involved in BK-induced vascular smooth muscle contraction are not clear in lingual artery.

We have previously reported that the indomethacin-insensitive BK-induced contraction of canine lingual artery rings, also mediated by B₂-receptors, is highly dependent on extracellular Ca²⁺. This conclusion is based on experiments conducted in a Ca²⁺-free physiological medium and on the modest inhibitory effects of nifedipine and verapamil²⁹⁾, suggesting the activation of receptor-controlled Ca²⁺ channels following B₂-

Fig. 5 Effect of [D-Phe⁷]-bradykinin (10⁻⁶ M) on muscle tension (upper trace) and [Ca²⁺]_i (lower trace, indicated by R_{340/380}) in canine lingual artery strip stimulated by 10⁻⁶ M bradykinin.

receptor stimulation. Contractility data (Fig. 6) in the present study also suggest the involvement of protein kinase(s) in BK-induced contraction. Protein kinase C is susceptible to inhibition by H-7¹⁵⁾; accordingly, this compound effectively suppresses the contractile effect due to the protein kinase C activator, phorbol myristate acetate. In our system, H-7 significantly reduced the contractile effect of BK in endothelium-denuded rings (Fig. 6A); furthermore, the phospholipase C inhibitor neomycin B was also effective (Fig. 6B), suggesting that the signal transduction of B₂-receptors may be physiologically regulated by diacylglycerol derived from the phospholipase C pathway. The activation of phospholipase C following the activation of B₂-receptors is consistent for the participation of diacylglycerol-regulated protein kinase C in the contractile effect of BK that was proposed above, in reference to the effects of protein kinase C and phospholipase C inhibitors (Fig. 6). The inositol-1, 4, 5-trisphosphate (IP₃)-mediated release of intracellular Ca²⁺ stores in the cells could also be a distinctive feature of B₂-receptor pharmacology in blood vessels. However, the contraction of the canine lingual artery failed to be evoked by BK in Ca²⁺-free experimental conditions²⁹⁾. Thus, second messengers derived from IP₃ may not be involved in the B₂-receptor signal transduction process. Obviously, the definition of the roles of IP₃-mediated signal transduction in contraction induced by BK of canine lingual artery awaits further study.

The use of protein kinase C inhibitors has some limitations, which include lack of selectivity and they

Fig. 6 Effects of H-7 (A) and neomycin B (B) on bradykinin-induced contractions of endothelium-denuded rings. The experimental conditions were similar to those described in Figure 2 (for contractile responses) except that 10^{-6} M H-7 (A, ●) was added 5 min before stepwise cumulative addition of bradykinin (A, ○), and that inhibitory effect of neomycin B (cumulative concentrations, 1 to 5 mM) was assessed in the rings contracted to a stable plateau tension by the addition of 10^{-6} M bradykinin. In this series of studies, three endothelium-denuded ring preparations (for bradykinin control, bradykinin plus H-7, and bradykinin plus neomycin B) obtained from the same vessel were studied in parallel. Contractile responses to 10^{-5} M bradykinin are taken as 100%, and other data are plotted in relation to it (A). Effect of neomycin B was expressed as percentage of inhibition of contraction elicited by 10^{-6} M bradykinin (B). Each point represents the mean, and vertical lines show SEM ($n=6$); n refers to the number of dogs from which the lingual artery was taken. ***Significantly ($*P<0.02$); $**P<0.01$) different from corresponding values without inhibitor used.

may also affect several distinct protein kinases. For example, H-7 inhibits cyclic nucleotide-dependent protein kinases¹⁵. Indomethacin, at low concentrations, reportedly inhibits a cyclic AMP-dependent protein kinase, in addition to suppressing the synthesis of prostanoids³⁰. The lack of effect of this drug, as well as the known relaxant effect of both cyclic AMP and cyclic GMP in vascular smooth muscle, suggest that the B_2 -receptors are not linked directly to cyclic nucleotide metabolism in the canine lingual artery. Therefore, the results tend to eliminate eicosanoids, notably the contractile compounds (thromboxane A_2

Fig. 7 Effects of hemoglobin (A), methylene blue (B) and L-NMMA (C) on the bradykinin-induced relaxations of endothelium-intact rings. The experimental conditions were similar to those described in Figure 2 (for relaxant responses) except that 10^{-5} M hemoglobin (A, ●), 10^{-5} M methylene blue (B, ●) or 10^{-4} M L-NMMA (C, ●) was added 5 min, 5 min or 50 min, respectively, before stepwise cumulative addition of bradykinin (○). In this series of studies, two endothelium-intact rings preparations (for bradykinin control and each test drug used) obtained from same vessel were studied in parallel in each experimental protocol (A, B, and C). Relaxant responses were expressed as percentage of the stable plateau contraction evoked by NE. Each point represents the mean, and vertical lines show SEM ($n=4$); n refers to the number of dogs from which the lingual artery was taken. ***Significantly ($*P<0.02$; $**P<0.01$) different from corresponding values without inhibitors used.

and PGH_2) from consideration as secondary mediators of BK action.

In summary, BK can cause relaxation via endothelial production of NO by B_2 -receptors activation. In addition to this, BK has the potential to be a vasoconstrictor by exerting the effect on vascular smooth muscle cells, also via B_2 -receptors activation. The contractile response to BK may be due to the production of second messengers derived from the action of phospholipase C when B_2 -receptors are activated. In this regard, receptor-controlled Ca^{2+} influx is primarily increased by BK, and may initiate the contractile responses. The maintenance of the contractile state involves protein kinase C activity and is consistent with a current model of smooth muscle cell function. Speculatively, an exposure to BK, in the absence of

endothelium or potentially with endothelial damage as may occur with diabetes, trauma and other insults, may result in sustained lingual artery contraction, which could compromise blood flow to the tongue and the gingival subserved by tributaries of the lingual artery.

Acknowledgments

This study was supported in part by a Grants-in-Aid for Science Research from Ministry of Education, Science and Culture of Japan.

References

- 1) Greenberg, S. and Palmer, C. : Biochemical basis of analgesia : metabolism, storage, regulation and action. *Dent. Clin. North. Am.* **22** : 31~46, 1978.
- 2) Samuelsson, B. : Prostaglandins, thromboxanes, and leukotrienes : formation and biological roles. *Harvey Lect.* **75** : 1~40, 1979.
- 3) Ito, H., Matsukawa, H., Takahashi, K. and Cho, Y. W. : The effects of catecholamines, acetylcholine and bradykinin on gingival circulation in dogs. *Arch. Oral Biol.* **18** : 321~328, 1973.
- 4) Matsukawa, H., Takahashi, K., Maehara, K. and Ito, H. : Effect of catecholamines, acetylcholine and bradykinin on buccal capillary flow in dogs. *J. Int. Res. Commun.* **2** : 1016, 1974.
- 5) Regoli, D. C. and Barabe, J. : Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.* **32** : 1~46, 1980.
- 6) Farmer, S. G. and Burch, R. M. : Biochemical and molecular pharmacology of kinin receptors. *Ann. Rev. Pharmacol. Toxicol.* **32** : 511~536, 1992.
- 7) Churchill, L. and Ward, P. E. : Relaxation of isolated mesenteric arteries by des-Arg⁹-bradykinin stimulation of B₁-receptors. *Eur. J. Pharmacol.* **130** : 651-657, 1986.
- 8) D'Orleans-Juste, P., de Nucci, G. and Vane, J. R. : Kinins act on B₁ or B₂ receptors to release conjointly endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells. *Br. J. Pharmacol.* **96** : 920~926, 1989.
- 9) Farmer, S. G. and Burch, R. M. : The pharmacology of bradykinin receptors. In : *Bradykinin Antagonists : Basic and Clinical Research*. (Edited by Burch, R. M.), pp. 1~31, Marcel Dekker Inc, New York, 1991.
- 10) Ozaki, H., Sato, K. and Karaki, H. : Simultaneous recordings of calcium signals and mechanical activity using fluorescent dye fura 2 in isolated strips of vascular smooth muscle. *Jpn. J. Pharmacol.* **45** : 429~433, 1987.
- 11) Sato, K., Ozaki, H. and Karaki, H. : Changes in cytosolic calcium level in vascular smooth muscle strip measured simultaneously with contraction using fluorescent Ca⁺⁺ indicator fura 2. *J. Pharmacol. Exp. Ther.* **246** : 294~300, 1988.
- 12) Steranka, L. R., Farmer, S. G. and Burch, R. M. : Antagonists of B₂ bradykinin receptors. *FASEB. J.* **3** : 2019~2025, 1989.
- 13) Gaudreau, J., Barabe, J., St-Pierre, S. and Regoli, D. C. : Pharmacological studies of kinins in venous smooth muscle. *Can. J. Physiol. Pharmacol.* **59** : 371~379, 1981.
- 14) Calixto, J. B. and Medeiros, Y. S. : Effect of protein kinase C and calcium on bradykinin-mediated contractions of rabbit vessels. *Hypertension* **19** : II87~II93, 1992.
- 15) Hidaka H., Inagaki, M., Kawamoto, S. and Sasaki, Y. : Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinases and protein kinase C. *Biochemistry* **23** : 5036~5041, 1984.
- 16) Slivka, S. R. and Insel, P. A. : Phorbol ester and neomycin dissociate bradykinin receptor-mediated arachidonic acid release and polyphosphoinositide hydrolysis in Madin-Darby canine kidney cells. *J. Biol. Chem.* **263** : 14640~14647, 1988.
- 17) Martin, W., Villani, G. M., Jothianandan, D. and Furchgott, R. F. : Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. Exp. Ther.* **232** : 708~716, 1985.
- 18) Kukovetz, W. R., Holzmann, S., Straka, M. and Pösch, G. : On the molecular mode of action of nitroglycerin. In : *Vascular Neuroeffector Mechanisms*. (Edited by Bevan, J. A., Fujiwara, M., Maxwell, R. A., Mohri, K., Shibata, S. and Toda, N.), pp. 145~150, Raven Press, New York, 1983.
- 19) Cherry, P. D., Furchgott, R. F., Zawadzki, J. V. and Jothianandan, D. : Role of endothelial cells in relaxation of isolated arteries by bradykinin.

- Proc. Natl. Acad. Sci. USA **79** : 2106~2110, 1982.
- 20) Furchgott, R. F. : Role of endothelium in response of vascular smooth muscle. *Circ. Res.* **53** : 557~573, 1983.
- 21) Blumberg, A. L., Denny, S. E., Marshall, G. R. and Deedleman, P. A. : Blood vessel-hormone interactions : angiotensin, bradykinin, and prostaglandin. *Am. J. Physiol.* **232** : H305~H310, 1977.
- 22) Vio, C. P., Bednar, M. M. and McGiff, J. C. : Prostaglandins as mediators and modulators of the kallikrein-kinin system. *Adv. Exp. Med. Biol.* **156** : 501~514, 1983
- 23) Berk, B. C., Canessa, M., Vallega, G. and Alexander, R. W. : Agonist-mediated changes in intracellular pH ; role in vascular smooth muscle cell function. *J. Cardiovasc. Pharmacol.* **12** (Suppl 5) : S104~S114, 1988.
- 24) Caramelo, C. P., Tsai, P. and Schrier, R. W. : Mechanism of cellular effect of phorbol esters on the action of arginine vasopressin and angiotensin II on vascular smooth muscle cells. *Biochem. J.* **254** : 625~629, 1988.
- 25) Resink, T., Scott-Burden, T. and Buhler, F. R. : Endothelin stimulates phospholipase C in cultured vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* **157** : 1360~1368, 1988.
- 26) Dixon, B. S., Breckon, R., Fortune, J., Vavrek, R. J., Stewart, J. M., Marzec-Calvert, R. and Linas, S. L. : Effects of kinins on cultured arterial smooth muscle. *Am. J. Physiol.* **258** : C299~C308, 1990.
- 27) Kremer, S., Harper, P., Hegele, R. and Skorecki, K. : Bradykinin stimulates a rise in cytosolic calcium in renal glomerular mesangial cells via a pertussis toxin insensitive pathway. *Can. J. Physiol. Pharmacol.* **66** : 43~48, 1988.
- 28) Sommerville, L. E. and Hartshorne, D. I. : Intracellular calcium and smooth muscle contraction. *Cell Calcium* **7** : 353~364, 1986.
- 29) Koike, A., Todoki, K., Okabe, E. and Ito, H. : Possible mechanism of the contracting effect of bradykinin on isolated canine lingual artery. In : *Microcirculation*. (Edited by Tsuchiya, M., Asano, M., Mishima, Y. and Oda, M., Vol 1), pp. 273~274, Excerpta Medica, Amsterdam, 1987.
- 30) Kantor, H. S. and Hampton, M. : Indomethacin in submicromolar concentration inhibits cyclic AMP-dependent protein kinase. *Nature* **276** : 841~842, 1978.