Endothelial Factors Involved in the Bradykinin-induced Relaxation of the Guinea-pig Aorta

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Abstract

Endothelial factors involved in the bradykinin (BK)-induced relaxation of the guinea-pig aorta were investigated using isolated aortic rings. In intact aortic rings, higher concentrations of BK (≥ 10^{-7} M) produced contraction, possibly as a direct action on smooth muscle. This BK-induced contraction was enhanced either by N^o-nitro-L-arginine (NOLA), an inhibitor of the production of nitric oxide or by indomethacin (IND), an inhibitor of cyclooxygenase, but not by carbenoxolone (CX), a known inhibitor of gap junctions. In aortic rings contracted with noradrenaline, BK elicited a relaxation with two components; an initial fast relaxation followed by a gradually diminishing slow relaxation, both in an endothelium-dependent manner. The BK-induced relaxation was inhibited in a drug specific manner by either NOLA, IND or CX. NOLA either abolished the fast relaxation, or sometimes converted it into a contractile response. IND reduced the amplitude and duration of the relaxation, by inhibiting the fast relaxation and abolishing the following slow relaxation. CX reduced both components of the relaxation. In the presence of both NOLA and CX, the BK-induced relaxation was converted to a contractile response followed by an IND-sensitive slow relaxation. In the presence of NOLA and IND together, BK stimulation caused a contraction with no following relaxation. These results indicate that in aortic rings of the guinea-pig, BK stimulates endothelial cells to release nitric oxide and prostanoids that produce the fast and slow components of the relaxation respectively. The effects of CX suggest that the contribution of EDHF to the BK-induced relaxation is weak.

Key words: Endothelium-dependent relaxation, Bradykinin, Nitric oxide, Prostanoids, EDHF

Introduction

Vascular smooth muscles contracted with agonists or high-potassium solutions are relaxed by acetylcholine or other physiologically active substances, in an endothelium-dependent manner, and the mediator involves endothelium-derived relaxing factor (EDRF), endothelium-derived hyperpolarizing factor (EDHF) or prostanoids (Furchgott, 1983; Vanhoutte et al., 1986; Suzuki and Chen, 1990; Moncada et al., 1991). EDRF may be nitric oxide (NO), as its production is prevented by L-arginine analogues such as N^o-monomethyl-L-arginine (L-

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NMMA), N\textsuperscript{\textomega}-nitro-L-arginine methyl ester (L-NAME) or N\textsuperscript{\textomega}-nitro-L-arginine (NOLA) (Moncada et al., 1991). Prostanoids involved in the endothelium-dependent relaxation are mainly prostacyclin (PG\textsubscript{I\textalpha}), and their production is prevented by chemicals that inhibit the enzyme cyclooxygenase (Moncada et al., 1991). The chemical nature of EDHF remains unclear, and an involvement of the metabolites of arachidonic acid such as epoxyeicosatrienoic acids (Hecker et al., 1994) and cannabinoids (Randall and Kendall, 1997) or potassium ions (Edwards et al., 1998) has been proposed. In some arteries, EDHF may be electrotonic signals conducted from endothelial cells and the effects could be attenuated by inhibition of gap junctions (Chaytor et al., 1998; Yamamoto et al., 1999). Contribution of unidentified humoral factor and gap junctional electrical signals from endothelial cells in the EDHF-mediated relaxation is also noted in the rat small arteries (Edwards et al., 1999).

Bradykinin (BK) induces an endothelium-dependent relaxation in many vascular tissues, through activation of B\textsubscript{2} subtype receptors, and the relaxation is attenuated by the inhibition of NO synthesis (Mombouli and Vanhoutte, 1995). This means that the BK-induced relaxation is produced mainly by EDRF. However, the actions of vasoactive agonists vary between vascular beds and also animal species. For example, acetylcholine (ACh) is a potent agonist for the endothelium-dependent relaxation in the aorta of the rabbit (Furchgott, 1983) or rat (Chen et al., 1988), but not in the guinea-pig (Hozumi et al., 1997). The endothelium-dependent relaxation produced by substance P is associated with a hyperpolarization in the carotid artery (Zhang et al., 1994), but not in the mesenteric artery of the guinea-pig (Bolton and Clapp, 1986).

We observed the mechanical responses produced by BK in the guinea-pig aorta. In this artery, the relaxation produced by ACh is mediated mainly by EDRF while that produced by substance P is mediated by EDRF and EDHF (Hozumi et al., 1997). It has also been shown that the EDHF-mediated relaxation is attenuated by 18\beta-glycyrrhetinic acid, a known inhibitor of gap junctions (Fukuta et al., 1999). Attempts were made to identify the endothelial factors involved in the BK-induced mechanical responses in the guinea-pig aorta. The involvement of EDRF, EDHF or prostanoids in the BK-induced response was eliminated by using appropriate inhibitors. Synthesis of EDRF (or NO) was inhibited by NOLA (Moncada et al., 1991). Production of prostanoids was inhibited by indomethacin (IND), an inhibitor of cyclooxygenase, (Moncada et al., 1991). The effects of carbeneoxolone (CX), an inhibitor of gap junctions (Edwards et al., 1999), were tested to assess possible involvement of gap junctional signals in the EDHF-induced responses. The results indicated that BK relaxes aortic rings mainly by production of endothelial NO and prostanoids, and that the EDHF-induced component is small.

Materials and Methods

Male albino guinea-pigs (body weight, 250-300 g) were anesthetized with ethyl ether, and decapitated. The protocols used conformed with guidelines on the conduct of animal experiments issued by The Physiological Society of Japan, and were approved by The Committee on the Ethics of Animal Experiments in Nagoya City University Medical School. The thoracic aorta was excised, cleaned by removing the surrounding connective tissues, and cut into 1-2 mm
wide rings. In some preparations, the endothelial cells were removed mechanically by rubbing the surface with moistened filter paper, after the aortic rings were turned inside out. The ring preparations were mounted in a recording chamber, and two stainless steel wires (0.3 mm thick) inserted in parallel into the lumen of the rings, each from opposite directions. One wire was connected to a force-transducer (TB-612T, Nihon Kohden, Tokyo, Japan) and the other was anchored to the bottom. The preparation was perfused with oxygenated, warmed (35.5 °C) Krebs solution (composition in mM: Na⁺ 137.4, K⁺ 5.9, Mg²⁺ 1.2, Ca²⁺ 2.5, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134, glucose 15.5), at a constant flow rate of about 3 ml/min. The pH of the solution was maintained at 7.2–7.3 by aeration with O₂ containing 5% CO₂. Isometric mechanical responses of aortic rings were measured in the direction of the circular muscle.

Aortic rings were stimulated repetitively (each for 5 min, every 20 min) with a solution containing 38.8 mM [K⁺]o (high-K solution, prepared by replacing NaCl with KCl), until the amplitude of contraction reached a stable value (usually the stabilization required 2–3 h). In the stabilized aortic ring preparations, responses elicited by bradykinin (BK) were measured in the absence or presence of noradrenaline (10⁻⁷–5 × 10⁻⁶ M). Amplitude of contractions produced by BK was measured as relative to the contraction produced by high-K solution. The amplitude of the BK-induced contraction was expressed as the percentage of noradrenaline-induced contractions produced before application of bradykinin. Measured values were expressed as the mean±standard deviation (S.D.). Statistical significance of the values was assessed using the Student’s t-test, with probabilities of less than 5% (P < 0.05) considered to be significant.

Chemicals used were bradykinin (Peptide Institute, Osaka, Japan), carbenoxolone, indomethacin, noradrenaline hydrochloride and Nω-nitro-L-arginine (Sigma Chemicals, MO, USA). These chemicals, except indomethacin, were dissolved in distilled water at concentrations of 10⁻²–10⁻⁴ M. Indomethacin was dissolved in ethanol at a concentration of 10⁻² M. The dissolved chemicals were diluted further with Krebs solution. These procedures did not alter the pH of the Krebs solution.

Results

Effects of bradykinin on aortic rings at rest

In intact aortic rings, bradykinin (BK, < 10⁻⁷ M) produced no detectable mechanical response. Increasing concentration of BK to 10⁻⁷ M resulted in a contraction with an amplitude of 5.2±2.6% (n=20) of the contractions produced by high-K solution. Nω-nitro-L-arginine (NOLA, 10⁻⁵ M) itself did not produce any mechanical response (n=12), but doubled the BK-induced contraction (10.5±5.8%, n=12). The BK-induced contraction was not significantly increased by 5 × 10⁻⁶ M indomethacin (IND) (5.7±1.1%, n=5, P > 0.05) or by 3 × 10⁻⁴ M carbenoxolone (CX) (7.4±4.2%, n=2, P > 0.05).

Bradykinin-induced relaxing responses

The effects of BK were observed in aortic rings contracted with noradrenaline (NA) to about half the amplitude of contraction produced by high-K solution (mean amplitude, 55.2±
16.9%), $n=52$). The concentrations of NA required to achieve such contraction were between $1 \times 10^{-8} - 5 \times 10^{-8}$ M. BK (10^{-7} M) applied during the sustained contraction with NA produced a relaxation with two components; an initial fast relaxation followed by a slow relaxation (Fig. 1, A). The peak amplitude of the initial relaxation reached $38.1 \pm 13.9\%$ ($n=51$). The amplitude of relaxation decreased gradually and sometimes the initial part of the following slow component was unclear. The maximum amplitude of the measurable slow relaxation was $17.3 \pm 11.7\%$ ($n=36$) of the NA-induced contraction.

The effects of NOLA, IND or CX on the BK-induced relaxation were observed in NA-contrasted aortic rings. In the presence of NOLA (10^{-5} M), but not IND (5 \times 10^{-6} M) or CX (3 \times 10^{-8} M), the amplitude of NA-induced contraction was increased by 2-4 fold, as also occurred in the guinea-pig carotid artery (Suzuki et al., 1992). Therefore, the aortic rings were contracted to comparable amplitudes (nearly 40-60% of high-K induced contraction) by reducing the concentration of NA, since the amplitude of the endothelium-dependent relaxation is a function of tension levels (Ibgwe & Suzuki, 1986; Suzuki et al., 1992). In the presence of NOLA, the concentration of NA required to contract aortic rings to levels similar to those in the absence of NOLA (mean value, 55.9 \pm 2.8\% of high-K induced contraction, $n=11$) was between $1 \times 10^{-7} - 5 \times 10^{-7}$ M. NOLA caused marked inhibition of the fast component of the BK-induced relaxation (Fig. 1, A), and in 5 out of 11 preparations it was converted to a contractile response with an amplitude of 2-10% of the NA-induced contraction (data not shown). The slow component of the BK-induced relaxation was not markedly altered by NOLA (control, 14.7 \pm 10.7\%; in NOLA, 17.3 \pm 11.7\%, $n=36$ for each; $P > 0.05$). IND reduced
the fast component by about 35% and abolished the following slow component, and as a consequence altered the relaxation from a gradually decreasing form to a brief transient form (Fig. 1, B). CX reduced the amplitude of both components of the BK-induced relaxation, with no significant alteration of the form of relaxation (Fig. 1, C).

The effects of NOLA, IND or CX on the amplitude of BK-induced fast relaxation are summarized in Figure 2. The effects of NOLA on the relaxation were summarized only for those aortic rings that demonstrated relaxation alone. All of these drugs reduced the amplitude of the fast relaxation significantly ($P < 0.05$). NOLA most potently inhibited the BK-induced relaxation. IND and CX reduced the amplitude of the fast component of the BK-induced relaxation to a similar extent.

The effects of combined application of NOLA, IND or CX on the BK-induced relaxation were also observed in the NA-contracted aortic rings. In the presence of NOLA and IND, BK produced a contractile response without a following slow relaxation (Fig. 3, Ab). The BK-induced response was also converted from a relaxation to a contractile response in the presence of NOLA and CX, with a following small but sustained relaxation (Fig. 3, Ba and Bb). This relaxation was abolished by the additional application of IND (Fig. 3, Bc), suggesting it was produced by prostanoids. In the combined presence of CX and IND, BK produced a relaxation similar to that produced in the presence of IND alone (data not shown). The summarized data for the effects of the combined application of NOLA, IND or CX on the BK-induced fast relaxation (Fig. 2) indicated that the amplitude of the BK-induced contraction reached nearly 30% of the NA-induced contraction in the presence of NOLA and CX. In the presence of IND and CX, the amplitude of the BK-induced relaxation did not differ significantly from that

![Fig. 2. Effects of NOLA, IND or CX on the BK-induced relaxation](image)

Aortic rings contracted with NA were relaxed by BK, in the absence (Control) and presence of NOLA ($10^{-5}$ M), IND ($5 \times 10^{-8}$ M) or CX ($3 \times 10^{-8}$ M) alone, or in the combined presence of NOLA plus IND, NOLA plus CX or IND plus CX. E (−), endothelium-denuded preparation ($n = 20$). The peak amplitude of the relaxation or contraction was measured as relative to the NA-induced contraction. Mean ± S.D. ($n = 6$–51). Significant inhibition ($P < 0.05$): Control vs. NOLA, IND, CX or IND+CX. No significant difference ($P > 0.05$): IND vs. CX, IND vs. IND+CX, or CX vs. IND+CX.
produced in the presence of IND alone \( (P > 0.05) \).

In aortic rings in which the endothelial cells had been removed mechanically, BK produced contractions with an amplitude similar to those produced in intact tissues in the presence of NOLA and IND (Fig. 2).

**Discussion**

The present experiments indicate that in the guinea-pig aortic rings, the BK-induced relaxation may be produced by EDRF (or NO), EDHF and prostanoids, since the relaxation is significantly attenuated by NOLA, CX or IND. Absence of a relaxation response to BK in aortic rings with the endothelium-removed indicates that these factors may be released mainly from the endothelial cells. As the relaxation is almost abolished in the presence of NOLA, the main factor involved may be NO. The inhibition of the slow component of the BK-induced relaxation by IND suggests that this component is caused mainly by prostanoids. The significant inhibitory effects of CX on the BK-induced relaxation suggest that signals from endothelial cells through gap junctions are also involved in the endothelial component as an EDHF. These properties of the BK-induced relaxation differ from the endothelium-dependent relaxation produced by ACh or substance P in the guinea-pig aorta, in that the ACh-induced relaxation is produced mainly by NO while the substance P-induced relaxation is produced by NO and EDHF (Hozumi et al., 1997). Thus, the present experiments clearly indicate that the factors involved in the endothelium-dependent relaxation differ among agonists. However,
the transient nature of the BK- induced relaxation is similar to that of substance P but not to that of ACh.

Stimulation of BK B₂ subtype receptors distributing in vascular tissues activates phospholipase C to increase production of inositol 1, 4, 5-trisphosphate (InsP₃) and diacylglycerol (DG) in endothelial cells (Monbouli and Vanhoutte, 1995). InsP₃ stimulates release of Ca²⁺ from internal stores and elevates intracellular Ca²⁺ concentrations, while DG increases production of prostanoids through activation of phospholipase A₂ (Berridge, 1993). As a consequence, BK may induce production of NO and prostanoids. This may be the case in the guinea-pig aorta, since the BK-induced relaxation is attenuated by IND, in addition to the strong inhibition by NOLA. The present experiments show that endothelial NO and prostanoids are indeed involved in the BK-induced relaxation.

IND not only reduced the amplitude of the fast component but also abolished the slow component of the BK-induced relaxation, suggesting an involvement of endothelial prostanoids for both components of the relaxation. The IND-sensitive component appeared a long period of time after removal of the stimulant. Similar relaxing actions of endothelial prostanoids are noted in the human pulmonary artery, in which the relaxation continues for up to 20 min after removal of the stimulant (Zhang et al., 1996). In the coronary artery of guinea-pigs, the hyperpolarizations produced by endogenous and exogenous prostanoids are both slow in onset and are maintained for a long time, even after removal of stimulants (Yajima et al., 1999). Thus, long-lasting relaxation may be one of the characteristics of the actions of prostanoids.

In some arteries, BK hyperpolarizes vascular smooth muscle membranes in an endothelium-dependent manner, and the mediator is possibly EDHF (Beny, 1990). In small arterioles, the EDHF-induced hyperpolarization may be produced by an electrotonic spread of potential from endothelial cells, since the inhibition by 18β-glycyrrhetinic acid of gap junctional connections between endothelial and smooth muscle cells attenuates the endothelium-dependent hyperpolarization (Yamamoto et al., 1999). The EDHF-mediated component of the relaxation is also attenuated by CX, a soluble form of 18β-glycyrrhetinic acid (Edwards et al., 1999). BK hyperpolarizes the membrane in arterial endothelial cells (Beny, 1990; Yamamoto et al., 1991). Thus, it is reasonable to speculate that EDHF is involved in the BK-induced relaxation. In fact, EDHF is one of the factors to induce the BK-induced relaxation in the canine coronary artery (Mombouli et al., 1992). In the guinea-pig aorta, substance P elicits relaxation mainly by EDHF, and the relaxation is attenuated by 18β-glycyrrhetinic acid (Fukuta et al., 1999). Thus, the CX-sensitive component of the relaxation may be produced mainly by EDHF.

It would be reasonable to speculate that single endothelial factors may be involved in the BK-induced relaxation produced in the combined presence of NOLA, IND or CX. For example, the relaxation produced in the presence of IND and CX may be elicited mainly by NO. EDHF may be the main factor to produce the BK-induced relaxation evoked in the presence of NOLA and IND. However, the combination of NOLA with either IND or CX caused a contraction by BK of aortic rings to the level similar to that produced in endothelium-removed aortic rings (Fig. 2). Thus, these data indicate that in the guinea-pig aorta the major endothelial relaxing factors are NO and prostanoids, and an involvement of EDHF in the BK-induced relaxation may be negligibly small. This contradicts the observation that CX significantly
inhibits the BK-induced relaxation (Fig.1C). Inhibition by 18β-glycyrrhetinic acid of gap junctions is accompanied by unidentified effects on smooth muscle, such as the inhibition of relaxation elicited by a K⁺-channel opener or modulation of contractions produced by NA or high-K solutions (Fukuta et al., 1999). In submucosal arterioles of the guinea-pig, 18β-glycyrrhetinic acid shows inhibitory actions not only on gap junctions but also on the relaxation produced by NO donor (Imaeda et al., 2000). Therefore, an alternative hypothesis is that CX inhibits the actions of NO in the guinea-pig aorta.

In conclusion, BK stimulates aortic endothelial cells to release NO and prostanoids and produces the fast and slow components of the relaxation respectively. Any contribution by EDHF to the BK-induced relaxation appears to be negligible.

References


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