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Influence of Bradykinin on Catecholamine Release from the Rat Adrenal Medulla

Dong-Yoon Lim, Il-Hwan Kim, Gwang-Moon Na, Moo-Jin Kang, Ok-Min Kim, Deok-Ho Choi, and Young-Woo Ki

Department of Pharmacology, College of Medicine, Chosun University, Gwangju 501–759, Korea

The present study was undertaken to investigate the effect of bradykinin on secretion of catecholamines (CA) evoked by stimulation of cholinergic receptors and membrane depolarization from the isolated perfused model of the rat adrenal glands, and to elucidate its mechanism of action. Bradykinin (3×10^{-8} M) alone produced a weak secretory response of the CA. however, the perfusion with bradykinin (3×10^{-8} M) into an adrenal vein of the rat adrenal gland for 90 min enhanced markedly the secretory responses of CA evoked by ACh (5.32×10^{-3} M), excess K^+ (5.6×10^{-2} M, a membrane depolarizer), DMPP (10^{-4} M, a selective neuronal nicotinic agonist) and McN-A-343 (10^{-4} M, a selective M_1-muscarinic agonist). Moreover, bradykinin (3×10^{-8} M) in to an adrenal vein for 90 min also augmented the CA release evoked by BAY-K-8644, an activator of the dihydropyridine L-type Ca^{2+} channels. However, in the presence of (N-Methyl-D-Phe^7)-bradykinin trifluoroacetate salt (3×10^{-8} M), an antagonist of BK_2-bradykinin receptor, bradykinin no longer enhanced the CA secretion evoked by Ach and high potassium whereas the pretreatment with Lys-(des-Arg^9, Leu^8)-bradykinin trifluoroacetate salt (3×10^{-8} M), an antagonist of BK_1-bradykinin receptor did fail to affect them. Furthermore, the perfusion with bradykinin (3×10^{-6} M) into an adrenal vein of the rabbit adrenal gland for 90 min enhanced markedly the secretory responses of CA evoked by excess K^+ (5.6×10^{-2} M). Collectively, these experimental results suggest that bradykinin enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization through the activation of B_2-bradykinin receptors, not through B_1-bradykinin receptors. This facilitatory effect of bradykinin seems to be associated to the increased Ca^{2+} influx through the activation of the dihydropyridine L-type Ca^{2+} channels.

Key Words: Bradykinin, Catecholamine release, Adrenal medulla, Bradykinin B_2-recepotors

INTRODUCTION

In neuronal cells and isolated organ preparations, it has been clearly demonstrated that bradykinin (BK), a nonapeptide, stimulates CA release by acting on presynaptic B_2-receptors (Chulak et al, 1995; Dendorfer & Dominiak, 1995; Warashina, 1997). In an attempt to understand the mechanisms underlying neurosecretory processes, several investigators have utilized the rat pheochromocytoma cell line, PC-I2, as a model with which to study the properties of several stimuli in evoking neurotransmitter release (Greene & Tischler, 1976). Much of this work was focused on the ability of such stimuli as elevated extracellular potassium and nicotinic cholinergic agonist to induce neurotransmitter release. These agents were thought to cause an influx of Ca^{2+} through depolarization-induced activation of voltage-sensitive Ca^{2+} channels, thereby triggering exocytosis (Ritchi, 1979; Di Virgilio et al, 1987). In addition to depolarizing stimuli, muscarinic receptor activation was shown to induce neurotransmitter secretion through a Ca^{2+}-dependent process. Increased intracellular free Ca^{2+} levels, $[Ca^{2+}]_i$, were found to occur through agonist-stimulated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield inositol I,4,5-trisphosphate (IP_3) (Vincentini et al, 1985; Rabe et al, 1987). Muscarinic receptor-evoked neurotransmitter release was believed to be dependent on this IP_3-induced Ca^{2+} mobilization. VanCalker et al. (1987) have shown that BK receptors on PC-12 cells can stimulate the rapid accumulation of IP_3, presumably through the hydrolysis of PIP_2. BK-induced release of IP_3 might be expected to mobilize intracellular Ca^{2+}, which then might evoke secretion from these cells. Appell & Barefot (1989) have investigated the effects of BK on $[Ca^{2+}]_i$ and neurotransmitter release in PC-12 cells. In that work, they reported that BK, acting through the BK_2 receptor subtype, rapidly

Corresponding to: Dong-Yoon Lim, Department of Pharmacology, College of Medicine, Chosun University, Gwangju 501-759, Korea. (Tel) 82-62-230-6335, (Fax) 82-62-227-4693, (E-mail) dylim@chosun.ac.kr

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ABBREVIATIONS: PIP_2, phosphatidylinositol 4, 5-bisphosphate; IP_3, inositol I, 4, 5-trisphosphate; BK, bradykinin; CA, catecholamines; PC-12, pheochromocytoma cell; SHR, spontaneously hypertensive rat.

stimulates the mobilization of calcium which then leads to neurotransmitter release. Furthermore, BK-induced responses involve the influx of extracellular Ca^{2+}, but not through the activation of voltage-dependent Ca^{2+} channels. Moreover, it has been reported that BK modulates the sympathetic system in various ways. It can stimulate sympathetic neurotransmission directly through presynaptic receptors (Llona et al, 1991) and indirectly via its hypotensive or nociceptive effects, which activate central and ganglionic mechanisms (Dray et al, 1988; Kuo & Keeton, 1991). However, it has been found that BK can also liberate prostaglandins or nitric oxide in peripheral tissues, thereby attenuating the release of CA (Starke et al, 1977; Schwieler et al, 1993). Therefore, the aim of the present study was to determine the effect of BK on CA secretion evoked by cholinergic stimulation and membrane depolarization in the isolate perfused model of the rat adrenal gland and to establish its mechanism of action.

METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180~300 g, were anesthetized intraperitoneally with thiopental sodium (50 mg/kg). The adrenal gland was isolated by modification of the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by the placement of three-hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads, and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations. A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Before ligating vessels and cannulations, heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. The adrenal gland, along with ligated blood vessels and the cannula, was then carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at $37 \pm 1^{\circ}C$.

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic pump (WIZ Co.) at a rate of 0.3 ml/min in rats. The perfusion was carried out with Krebs-bicarbonate solution of following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95% O_2 + 5% CO_2, and the pH of the solution was maintained at 7.4~7.5. The solution contained disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of CAs.

Drug administration

The perfusions of DMPP (100 μM) and McN-A-343 (100 μM) for 2 minutes, and Bay-K-8644 (10 μM) for 4 minutes were made into perfusion stream, respectively. A single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml was injected into perfusion stream via a three-way stopcock, respectively.

In the preliminary experiments, it was found that, upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343, and Bay-K-8644 returned to pre-injection level in about 4 min, but the responses to DMPP in 8 min.

Collection of perfusate

Prior to stimulation with various secretagogues, perfusate was routinely collected for 4 min to determine spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, the perfusates were continuously collected in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated samples were collected for 4 to 8 min. The amounts secreted in the background sample have been subtracted from that secreted from the stimulated sample to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effects of BK on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing BK for 90 min immediately after the perfusate was collected for a certain minute (background sample). And the medium was then changed to the one containing the stimulating agent, and the perfusates were collected for the same period as that for the background sample. Generally, the adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

CA content of perfusate was fluorospectrophotometrically (Kontron Co. Italy) measured directly by the fluorometric method of Anton & Sayre (1962) without intermediate purification on alumina for the reasons described earlier (Wakade, 1981).

A volume of 0.2 ml perfusate was used for the reaction. The CA content in the glands perfusate stimulated by secretogagues in the present work was high enough to obtain several folds greater readings than that of control samples (unstimulated). The sample blanks were also the lowest for perfusates of stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical significance between groups was determined by utilizing the Student's t-test. A P-value of less than 0.05 was considered to represent statistically significant changes, unless specifically noted in the text. Values given in the text refer to means and standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida & Murray (1987).

Drugs and their sources

Bradykinin, Lys-(Des-Arg^9-Leu^8)-bradykinin trifluoroacetate salt, (N-methyl-D-Phe^7)-bradykinin trifluoroacetate

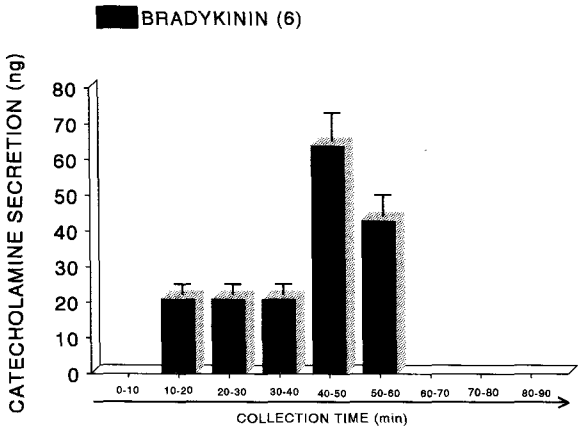

Fig. 1. Time-course effects of continuous infusion of bradykinin on the secretion of catecholamines (CA) from perfused rat adrenal glands. Bradykinin (3×10^{-8} M) was infused continuously for 90 min into an adrenal vein. Bradykinin infusion was carried out after perfusion for one hour with normal Krebs-bicarbonate solution prior to the initiation of the experimental protocols. The data are expressed as the mean±S.E. from 6 rat adrenal galands. Abscissa: collection time (min). Ordinate: secretion of CA in ng for 10 min. The vertical columns and bars denote the means and the standard errors of the corresponding means, respectively. Number in the upper bracket indicates the number of animals used in the experiments.

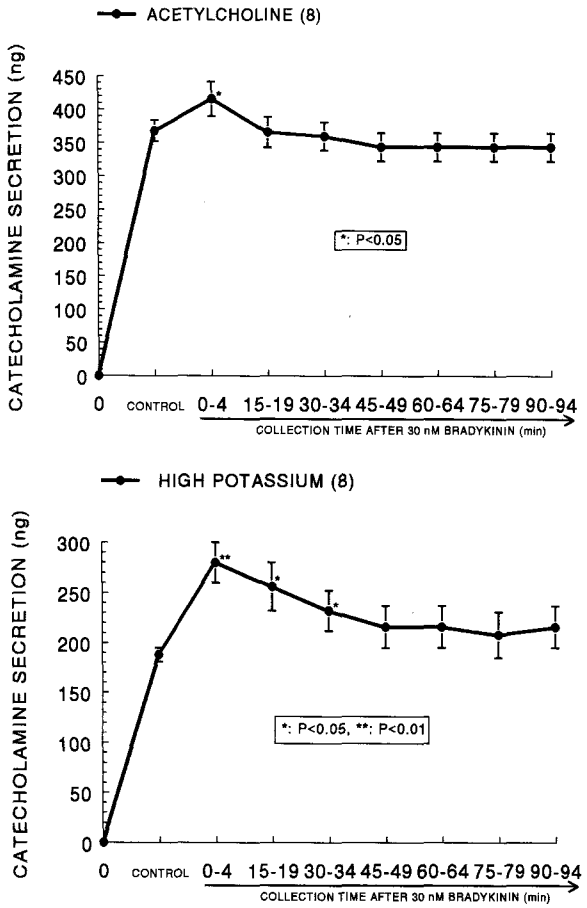

Fig. 2. Effects of bradykinin on the secretory responses of catecholamines evoked by acetylcholine (upper) and high potassium (lower) from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32×10^{-3} M) or high K^+ (5.6×10^{-2} M) was induced "BEFORE (CONTROL)" and "AFTER" preloading with bradykinin (3×10^{-8} M) for 90 min. Statistical difference was obtained by comparing the corresponding "CONTROL" with each period "AFTER" the intiation of bradykinin perfusion. Perfusates were collected for 4 minutes at 15 min intervals. Other legends are the same as in Fig. 1.

salt, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, and ethyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (BAY-K-8644) were purchased from Sigma Chemical Co., U.S.A. (4-(N-[3-Chlorophenyl] carbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) was purchased from RBI Co., U.S.A. Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644, which was dissolved in 99.5% ethanol and diluted appropriately (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

The secretory effect of CA evoked by bradykinin

When the adrenal gland was perfused with oxygenated Krebs-bicarbonate solution for 60 min before experimental protocol is initiated, the spontaneous CA secretion reached steady state. The basal CA release from the perfused rat adrenal medulla amounted to 21±2 ng for 2 min from 6 experiments. The releasing effects to the initial perfusion of BK (3×10^{-8} M) for 90 min are shown in Fig. 1. Time-course effect of BK (3×10^{-8} M) infusion into the perfusion stream for 90 min produced the significant responses of CA secretion over the background release, leading to the peak secretion at 40~50 min period. This result seems to be similar to the findings that BK increases $[Ca^{2+}]_i$ and neurotransmitter release in PC-12 cells (Appell and Barefot, 1989). In 6 rat adrenal glands, this BK-evoked CA secretory responses were 21~64 ng (0~90 min), which seemed to be a very weak secretagogue. The tachyphylaxis to releasing effects of CA evoked by BK was not observed (Data not shown). However, in all subsequent experiments, BK was not administered more than twice only at 120 min interval.

Effect of bradykinin on CA secretion evoked by ACh, high K^+, DMPP, and McN-A-343 from the perfused rat adrenal glands

In order to examine the effects of BK on CA release, the dose-dependent effect of BK on CA seretory responses evoked by ACh and high potassium was examined. 30 nM of BK concentrations produced the most effective enhancement of CA secretory responses evoked by ACh and high K^+ (data not shown). Therefore, in all subsequent experiments, 30 nM BK was used. In the present experiment, ACh (5.32×10^{-3} M)-evoked CA release prior to the perfusion with BK (3×10^{-8} M) was 368±16 ng (0~4 min) from 8 rat adrenal glands. However, in the presence of BK (3×10^{-8} M) for 90 min, it was significantly increased to 416±26 ng ($P<0.05$) only for first 0~4 min, but following the first period, it was not affected (366~344 ng) compared

to the corresponding control (Fig. 2-upper). Also, KCl, a direct membrane-depolarizing agent, sharply stimulates CA secretion. In the present work, high K^+ (5.6×10^{-2} M)-evoked CA release in the presence of BK (3×10^{-8} M) for 90 min was significantly enhanced to 280~232 ng (0~34 min, $P<0.01$), whereas the third period later it was not affected (208~216 ng) compared to the corresponding control secretion (188±76 ng, 0~4 min) from 8 glands (Fig. 2-lower). In 8 rat adrenal glands, DMPP (10^{-4} M), a selective nicotinic receptor agonist in autonomic sympathetic ganglia, when perfused through the rat adrenal gland, evoked a sharp and rapid increase in CA secretion. As shown in Fig. 3 (upper), DMPP-stimulated CA secretion following the loading with BK (3×10^{-8} M) was potentiated to 384~392 ng (0~28 min), but the second period later it was rather reduced to 320~360 ng (40~88 min) compared to the corresponding control secretion (364±12 ng, 0~8 min). As illustrated in Fig. 3 (lower), McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-receptor agonist (Hammer & Giachetti, 1982), perfused into an adrenal vein

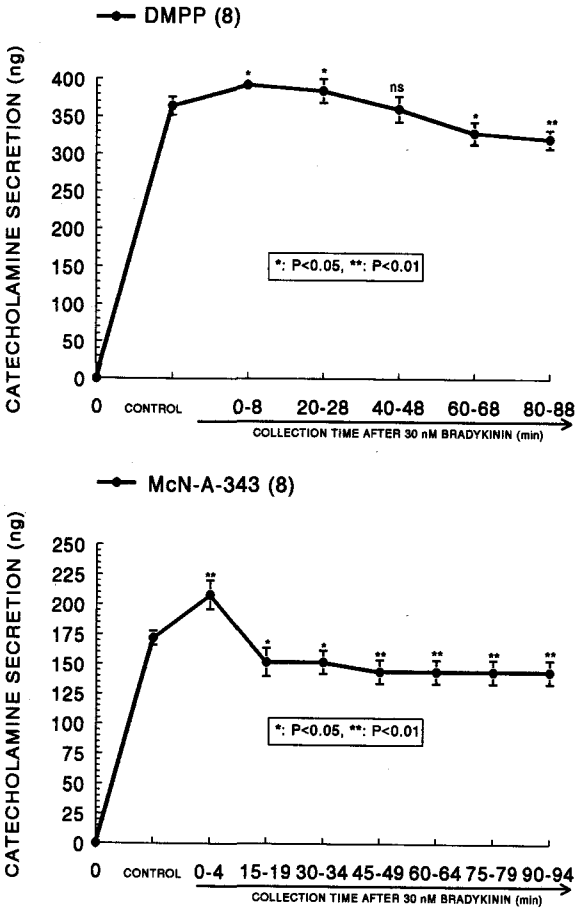

Fig. 3. Effects of bradykinin on the secretory responses of catecholamines evoked by DMPP (upper) and McN-A-343 (lower) from the isolated perfused rat adrenal glands. CA secretion by perfusion of DMPP (10^{-4} M) or McN-A-343 (10^{-4} M) was induced before (CONTROL) and after perfusion with bradykinin (3×10^{-8} M) for 90 min. DMPP- and McN-A-343-induced perfusates were collected for 8 and 4 minutes at 20 and 15 min interval, respectively. Other legends are the same as in Fig. 1.

for 4 min caused an increased CA secretion to 172±6 ng (0~4 min) from 8 experiments. However, in the presence of BK (3×10^{-8} M), McN-A-343-evoked CA secretion was significantly increased to 208±10 ng ($P<0.01$) only for first 0~4 min, but the first period later it was rather gradually inhibited to 144~152 ng (15~94 min) compared to the corresponding control.

Effect of bradykinin on CA secretion evoked by Bay-K-8644 from the perfused rat adrenal glands

It has been found that Bay-K-8644 is a selective L-type calcium channel activator, which causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm et al, 1982; Wada et al, 1985) and enhances basal Ca^{2+} uptake (Garcia et al, 1984). Therefore, it was of interest to determine the effects of BK on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. In the absence of BK, Bay-K-8644 (10^{-5} M) given into the perfusion stream produced CA secretion of 192±17 ng (0~4 min) from 8 rat adrenal glands. However, in the presence of BK (3×10^{-8} M), Bay-K-8644-stimulated CA secretion was significantly increased to 213~267 ng (0~34 min), but the third period later it was not affected (197±17 ng) compared to the corresponding control, as shown in Fig. 4.

Effect of BK_2 antagonist on bradykinin-induced potentiation of CA release evoked by ACh and high K^+ from the perfused rat adrenal glands

As illustrated in Fig. 2, 3, it was found that BK enhanced the CA secretory responses evoked by cholinergic stimulation and membrane-depolarization. Therefore, it was of much interest to examine the effects of BK, in the presence of (N-methyl-Phe^7)-BK, a highly selective B_2 receptor antagonist, on CA secretion evoked by the cholinergic receptor stimulation and membrane depolarization in the isolated rat adrenal glands. It has also been found that (N-methyl-Phe^7)-BK, representing the basic structure of a new type of BK B_2 receptor antagonists, inhibits both *in vitro*

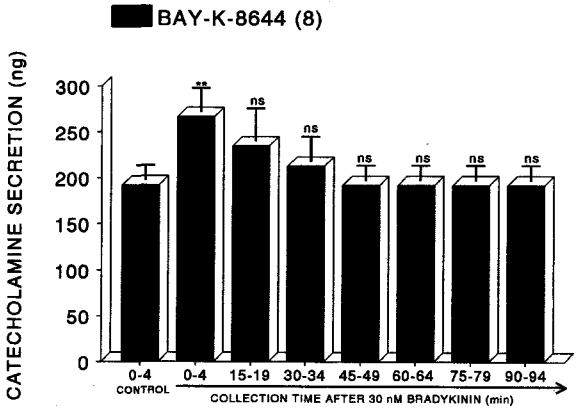

Fig. 4. Effects of bradykinin on the secretory responses of catecholamines evoked by Bay-K-8644 from the isolated perfused rat adrenal glands. CA secretion by perfusion of Bay-K-8644 (10^{-5} M) was induced before (CONTROL) and after perfusion with bradykinin (3×10^{-8} M) for 90 min. Bay-K-8644 -induced perfusates were collected for 4 minutes at 15 min interval. Other legends are the same as in Fig. 1. ns: Statistically not significant.

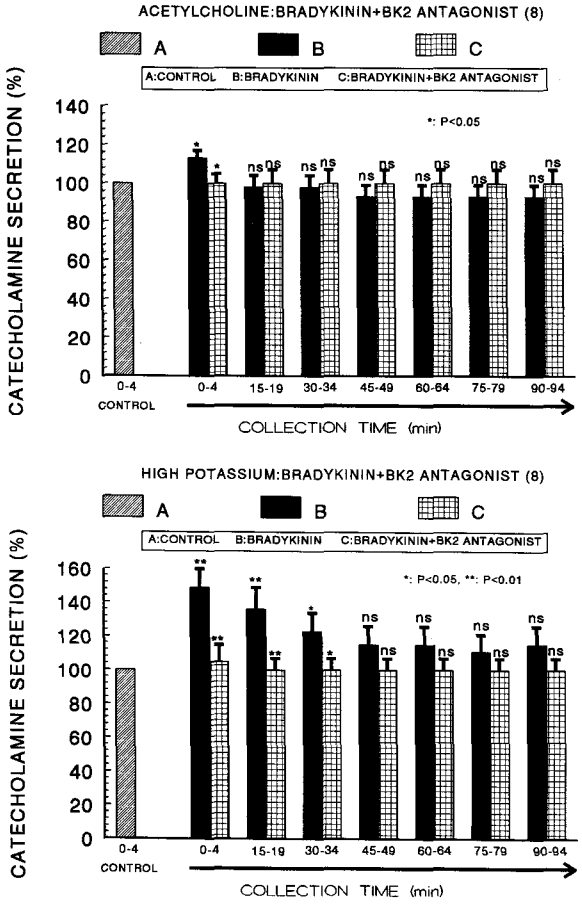

Fig. 5. Effects of bradykinin plus BK_2-antagonist on catecholamine release evoked by acetylcholine (upper) and high potassium (lower) from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32×10^{-3} M) or high potassium (5.6×10^{-2} M) was induced before (CONTROL, A) and after preloading with 3×10^{-8} M bradykinin only (B) or 3×10^{-8} M bradykinin + 3×10^{-8} M BK_2-antagonist (C) for 90 min. Ordinate: the amounts of CA secreted from the adrenal gland (%). Abscissa: Collection time of perfusate (min). Statistical BK_2-antagonist: (N-Methyl-D-Phe^7)-bradykinin trifluoroacetate salt. ns: Statistically not significant.

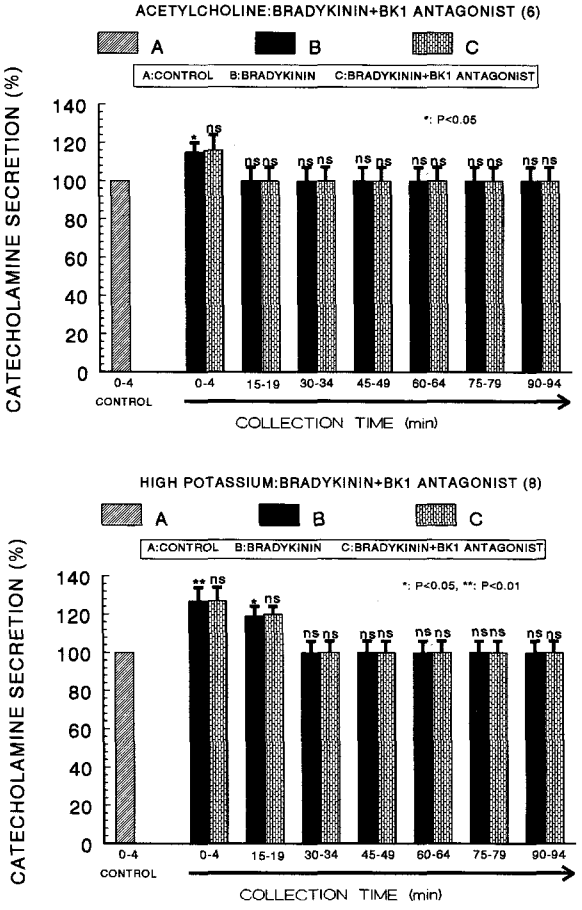

Fig. 6. Effects of bradykinin plus BK_1-antagonist on catecholamine release evoked by acetylcholine (upper) and high potassium (lower) from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32×10^{-3} M) or high potassium (5.6×10^{-2} M) was induced before (CONTROL, A) and after preloading with 3×10^{-8} M bradykinin only (B) or 3×10^{-8} M bradykinin + 3×10^{-8} M BK_1-antagonist (C) for 90 min. Ordinate: the amounts of CA secreted from the adrenal gland (%). Abscissa: Collection time of perfusate (min). Statistical difference was obtained by comparing "A" with "B", and by comparing "B" with "C". BK_1-antagonist: Lys-(Des-Arg^9-Leu^8)-bradykinin trifluoroacetate salt. ns: Statistically not significant.

BK-induced contraction of the guinea pig lung strip and *in vivo* BK-induced bronchoconstriction. Furthermore, it shows analgesic activity, blocks in a dose-dependent manner the BK-induced Ca^{2+} release from macrophages and inhibits at a concentration of 10^{-13} M the BK-induced cytokine release from mononuclear cells (Reissmann et al, 1996).

When introduced into the adrenal vein in 6 rat adrenal glands, the ACh (5.32×10^{-3} M)-evoked CA releasing responses, in the presence of BK (3×10^{-8} M) along with (N-methyl-Phe^7)-BK (3×10^{-8} M) for 90 min, were not enhanced ($100\pm5\%$ for 0~4 min of their control secretion, $P<0.05$) compared to their secretory responses in the presence of BK (3×10^{-8} M) only ($113\pm4\%$ of their control secretion) as shown in Fig. 5 (upper). However, treatment with (N-methyl-Phe^7)-BK (3×10^{-8} M) only failed to alter the basal CA secretory response (data not shown).

As depicted in Fig. 5 (lower), high K^+ (5.6×10^{-2} M)-evoked CA secretory enhanced by BK to 115~149% of the control response (100%) were significantly inhibited to 100~105% of the control release by the concurrent treatment with (N-methyl-Phe^7)-BK (3×10^{-8} M) for 90 min.

Effect of BK_1 antagonist on bradykinin-induced potentiation of CA release evoked by ACh and high K^+ from the perfused rat adrenal glands

In order to examine the effect of BK B_1 receptor antagonist on BK-induced potentiation of CA release evoked by ACh and high K^+, Lys-(Des-Arg^9-Leu^8)-BK, a BK B_1 receptor antagonist (MacNeil et al, 1997), was perfused simultaneously along with BK intsroduced into the adrenal vein for 90 min. As shown in Fig. 6 (upper), in 6 rat adrenal glands, the ACh (5.32×10^{-3} M)-evoked CA releasing responses, in the presence of BK (3×10^{-8} M) along with Lys-(Des-Arg^9-Leu^8)-BK (3×10^{-8} M) for 90 min, were not enhanced, as compared to the secretory response in the

presence of BK (3×10^{-8} M) only (115±5% of their control secretion). However, the treatment only with Lys-(Des-Arg^9-Leu^8)-BK (3×10^{-8} M) did not also alter the basal CA secretory response (data not shown). Moreover, high K^+ (5.6×10^{-2} M)-evoked CA secretory responses enhanced by BK to 119~127% (0~19 min) of the control response (100%) were not affected by the concurrent treatment with (N-methyl-Phe^7)-BK (3×10^{-8} M) and BK (3×10^{-8} M) for 90 min, as compared to the control release (Fig. 6-lower).

DISCUSSION

These experimental results demonstrate that BK enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization through the activation of B_2-BK receptors. This facilitatory effect of bradykinin seems to be associated to the increased Ca^{2+} influx through the activation of the dihydropyridine L-type Ca^{2+} channels.

The effectiveness of BK in stimulating the release of endogenous CA, although was very weak in the rat adrenal gland, may be due to the simultaneous actions of several intracellular mechanisms provoking transmembraneous Ca^{2+} influx, which acts as the most important mediator for exocytosis (Kim & Westhead, 1989). On the one hand, it has been found that the adrenal medulla possesses characteristics of postganglionic sympathetic neurons, and both L- and N-type voltage-dependent Ca^{2+} channels (VDCCs) have been identified in medullary chromaffin cells (Gandia et al, 1995). Adrenal CA secretion has been found to be mediated by muscarinic receptors as well as nicotinic receptors in various species (Nakazato et al, 1988), including the dog (Kimura et al, 1992). However, little is known about the involvement of VDCCs in contributing to the muscarinic receptor-mediated CA secretion (Uceda et al, 1994). The receptor-coupled Ca^{2+} channels also exist in PC12 cells, which are directly opened by BK (Weiss & Atlas, 1990). On the other hand, intracellular events such as generation of the second messenger IP_3 and depletion of intracellular Ca^{2+} stores, which are both provoked by BK in PC12 cells (Fasolato et al, 1988; Clementi et al, 1992), can activate Ca^{2+} influx (Berridge & Irvine, 1989; Clementi et al, 1992). Besides the predominant role of Ca^{2+} influx for stimulating secretion processes, a norepinephrine (NE) release independent of extracellular Ca^{2+} has been observed in PC12 cells involving activation of protein kinase C (Pozzan et al, 1984). In the present investigation, the results that BK enhanced CA secretion evoked by stimulation of muscarinic receptors with McN-A-343, a selective muscarinic M_1-receptor agonist, suggest that Ca^{2+} mobilization from intracellular store by the activation of muscarinic M_1-receptors might be involved in the BK-induced enhancement of the CA secretory response in the rat adrenal medulla. In support of this hypothesis, the muscarinic receptor-mediated secretion of adrenal CA seems to be caused by Ca^{2+} mobilized from intracellular storage sites (Nakazato et al, 1988; Misbahuddin & Oka, 1988; Yamada et al, 1988). Furthermore, it has been shown that muscarinic stimulation generates a depolarizing signal, which triggers the firing of action potentials, resulting in the increased CA release in the rat chromaffin cells (Akaike et al, 1990), guinea pigs (Inoue & Kuriyama, 1991) and the perfused rat adrenal gland (Lim & Hwang, 1991). These observations are in line with a previous report showing that Bay-K-8644 almost trippled the peak secretory response to muscarine in perfused cat adrenal glands (Uceda et al, 1994). In the present experiment, BK also potentiated the CA secretion induced by Bay-K-8644, which is found to evoke the release of CA by increasing Ca^{2+} influx through L-type Ca^{2+} channels in chromaffin cells (Garcia et al, 1984). Also, BK greatly enhanced CA secretions evoked by high K^+, a direct membrane-depolarizing agent. These findings that BK potentiated CA secretion evoked by Bay-K-8644 as well as by high K^+ suggest that BK activates directly the voltage-dependent Ca^{2+} channels. In the bovine chromaffin cells, stimulation of nicotinic, but not muscarinic ACh receptors is known to cause CA secretion by increasing Ca^{2+} influx largely through voltage- dependent Ca^{2+} channels (Burgoyne, 1984). In the light of this notion, the present finding that BK enhances DMPP-evoked CA secretion is thought to be due to the increased Ca^{2+} influx through voltage-dependent Ca^{2+} channels activated by nicotinic ACh receptors.

It is felt that the facilitatory effect of BK on CA secretory responses evoked by cholinergic stimulation as well as by membrane-depolarization may be associated with the mobilization of intracellular Ca^{2+} from the cytoplasmic calcium store. This indicates that BK increases the release of Ca^{2+} from the intracellular pools induced by stimulation of muscarinic ACh receptors as well as BK receptors, which is weakly responsible for the secretion of CA. It has been shown that Ca^{2+}-uptake into intracellular storage sites susceptible to caffeine (Ilno, 1989) is almost completely abolished by treatment with cyclopiazonic acid during the proceeding Ca^{2+} load (Suzuki et al, 1992). This is consistent with the findings obtained in skinned smooth muscle fibers of the longitudinal layer of the guinea-pig ileum, where Ca^{2+}-uptake was also inhibited by cylopiazonic acid (Uyama et al, 1992). Suzuki and his coworkers (1992) have shown that cyclopiazonic acid easily penetrates into the cytoplasm through the plasma membrane and reduces Ca^{2+}-ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in increase in the subsequent Ca^{2+} release from those storage sites. Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors is also proposed to cause activation of phosphoinositide metabolism, resulting in the formation of inositol 1,4,5-trisphosphate, which induces the mobilization of Ca^{2+} from the intracellular pools (Challis et al, 1991). Furthermore, intracellular events such as generation of the second messenger IP_3 and depletion of intracellular Ca^{2+} stores, which are both provoked by BK in PC12 cells (Fasolato et al, 1988; Clementi et al, 1992) have been already found. In the light of this notion, it is plausible that BK enhances the CA secretory responses evoked by cholinergic stimulation and membrane depolarization through the mobilization of the intracellular Ca^{2+}. However, in the present study, it is uncertain whether the stimulatory effect of BK on Ca^{2+} movement from intracellular pools is due to its direct effect on the PI response or the indirect effects.

Despite the responsiveness of the rat adrenal medulla to BK, the B-receptors involved in mediating CA secretion have not yet been characterized. Studies using fluorimetric measurements of intracellular Ca^{2+} (Fasolato et al, 1988; Grohovaz et al, 1991; Ransom et al, 1991) have shown the B_2-ligand Arg^0-[Hyp^3, Thi^{5,8}, D-Phe^7]-BK to antagonize BK effectively. In agreement with this, the activation of CA secretion by nanomolar concentrations of BK found in PC12

cells also indicated an involvement of the B_2 receptor subtype. Apparently, receptors of the B_1 subtype do not contribute to the secretory response to BK since dA-BK, an agonist with high affinity for this receptor subtype (e.g. EC5o=50 nM) in rabbit aorta (Regoli et al, 1993), did not increase NE secretion at concentrations up to 10 μM (Dendorfer & Dominiak, 1995). Therefore, either B_1 receptors are not present under physiological conditions, or they are not coupled to CA secretion in the PC12 cell model. The B_2 receptors of PC12 cells have been characterized using the B_2-ligand [Thi^{5,8}, D-Phe^7]-BK which was shown to exert agonistic activity both in neuroblastoma cells (Braas et al, 1988) and in adrenergic neurons of the rat vas deferens, but acts as an antagonist at postsynaptic receptor sites in the latter preparation (Llona et al, 1987). Based on these results, in the present study, BK-induced enhancement of CA secretion evoked by cholinergic stimulation and membrane depolarization was blocked in the presence of (N-methyl-Phe^7)-BK, a highly selective B_2 receptor antagonist (Reissmann et al, 1996). This result indicates that BK enhances CA release through the activation of B_2 receptors located on the rat adrenomedullary chromaffin cells. However, the fact that Lys-(Des-Arg^9-Leu^8)-BK, a BK B_1 receptor antagonist (MacNeil et al, 1997), did not affect the CA release evoked by them, demonstrates that BK B_1 receptors are not associated with BK-induced potentiation of CA secretion evoked by cholinergic stimulation and membrane depolarization.

In support of this notion, under the conditions of effective kininase inhibition, exogenous bradykinin potentiates the release of CAs during electrical preganglionic stimulation in the pithed SHR (Dendorfer et al, 1999). Doses $\geq$ 10 μg/kg of the B_2-receptor antagonist HOE 140 are able to completely abolish the BK-induced increase in plasma NE, and to inhibit, although incompletely, the increase in plasma epinephrine. Therefore, exogenous BK induces sympathetic activation in the pithed SHR essentially via B_2-receptors, which are most likely located on peripheral neurons (Dendorfer et al, 1999) and chromaffin cells. In view of the effective inhibitory actions of HOE 140, a significant involvement of the B_1-receptor subtype can be excluded.

Moreover, it has been found that analogous to BK-evoked release of [^{3}H]NE from neuroblastoma cells (McDonald et al, 1994; Purkiss et al, 1995), the BK-induced potentiation of NE exocytosis in myocardial ischemia/reperfusion also may involve an increase in intraneuronal Ca^{2+}. This could result from Ca^{2+} entry from extracellular sources, as well as protein kinase C activation and Ca^{2+} release from intracellular stores (McDonald et al, 1994; Purkiss et al, 1995). Hatta and his co-workers (1999) have found that BK administration (100 nM) markedly enhanced exocytotic and carrier-mediated NE overflow from guinea pig hearts subjected to 10- and 20-min ischemia/reperfusion, respectively. The BK B_2 receptor antagonist HOE140 blocked the effects of BK, whereas the B_1 receptor antagonist des-Arg^9-Leu^8-BK did not. The BK-induced chronotropic effect is mediated by BK B_2 receptors, whereas B_1 receptors do not play a role in mediating this effect (Li et al, 1998).

In conclusion, taken together, these experimental results suggest that bradykinin enhances the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization through the activation of B_2-bradykinin receptors, not through B_1-bradykinin receptors. This facilitatory effect of bradykinin seems to be associated to the increased Ca^{2+} influx through the activation of the dihydropyridine L-type Ca^{2+} channels.

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