Possible role of bradykinin on stimulus-secretion coupling in adrenal chromaffin cells

Hitoshi Houchi*, Mami Azuma*, Masanori Yoshizumi†, Toshiaki Tamaki†, and Kazuo Minakuchi*

*Division of Pharmacy, University Hospital, and †Department of Pharmacology, The University of Tokushima School of Medicine, Tokushima, Japan

Abstract : Nonapeptide bradykinin is known to be a central nervous system neurotransmitter and to play a role in regulation of neuronal function. However, few details are known of the function of its peptide on stimulus-secretion coupling in neuronal cells. In this article, the role of bradykinin on catecholamine biosynthesis, secretion and Ca^{2+} movement in adrenal chromaffin cells as a model for catecholamine-containing neurons are examined. Bradykinin receptors are classified as B_1 and B_2 receptor subtypes. These receptors are present on the adrenal chromaffin cell membrane. Bradykinin increases the influx of Ca^{2+} and the turnover of phosphoinositide through the stimulation of bradykinin B_2 receptor. The secretion of catecholamine from the cells is initiated by the raise of $[Ca^{2+}]i$. An increase in $[Ca^{2+}]i$ and production of diacylglycerol stimulate the activation of calcium-dependent protein kinases. These kinases stimulate the activation of tyrosine hydroxylase, a rate-limiting enzyme in the biosynthesis of catecholamine. Otherwise, bradykinin increases Ca^{2+} efflux from the cells through the stimulation of the bradykinin-B_2 receptor. This action may be explained by an extracellular Na^+-dependent mechanism, probably through acceleration of Na^+/Ca^{2+} exchange. It is interesting that bradykinin, which stimulates the biosynthesis and secretion of catecholamine in adrenal chromaffin cells, plays a role in the termination of calcium-signal transduction through the stimulation of Ca^{2+} efflux from the cells. J. Med. Invest. 46 : 1-9, 1999

Key words **:** *Bradykinin, Catecholamine, Biosynthesis, Secretion, Calcium*

INTRODUCTION

The nonapeptide bradykinin has been found to influence several physiological processes including pain generation (1), blood pressure (2) and cardiovascular regulation (3). It has also been suggested to be a central nervous system neurotransmitter and to play a role in the regulation of neuronal function (4, 5).

Adrenal chromaffin cells and their cancer cell line, pheochromocytoma PC-12 cells, are useful for studying the mechanism of the stimulus-secretion coupling, and are regarded as a model for catecholamine-containing neurons. Physiological stimulations of the cells cause an increase in the levels of intracellular free Ca^{2+} ($[Ca^{2+}]i$) which comes from both the intracellular and extracellular pools. The increase in $[Ca^{2+}]i$ causes exocytosis including the stimulation of catecholamine secretion and activation of its biosynthesis (6, 7).

In this article, the role of bradykinin on catecholamine biosynthesis, secretion and Ca^{2+} movement in adrenal chromaffin cells and pheochromocytoma PC-12 cells are examined.

General stimulus-secretion coupling in adrenal chromaffin cells

Chromaffin cells synthesize, store and secrete catecholamines (dopamine, noradrenaline and adrenaline) in nerve stimulations. The cells have an excitable action in response to acetylcholine or to electrical

Received for publication November 30, 1998 ; accepted December 15, 1998.

Address correspondence and reprint requests to Hitoshi Houchi, Ph. D., Division of Pharmacy, University Hospital, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-633-7472.

stimulation of the splanchnic nerve. Following activation of acetylcholine nicotinic receptors, acetylcholine causes the opening of the receptor-mediated ion channel, allowing the influx of Na^+, and to a lesser extent Ca^{2+} (8, 9). This influx into the chromaffin cells of Na^+ results in a mild depolarization of the cell membrane sufficient to activate voltage-dependent Na^+ channels (10). The opening of Na^+ channels induces the activation of voltage-dependent Ca^{2+} channels (11). The opening of Na^+ and Ca^{2+} channels causes the firing of action potentials and the entry of Ca^{2+} from extracellular spaces (12). An increase in $[Ca^{2+}]i$ is the trigger for exocytosis of chromaffin granules (secretion of catecholamine) and stimulation of biosynthesis of catecholamine. Acetylcholine nicotinic and muscarinic receptors are present in chromaffin cells, and these receptors in most species stimulate secretion of catecholamine. In bovine adrenal chromaffin cells, however, only nicotinic receptor stimulation evoke secretion of catecholamine. Catecholamine secretion can also be evoked by high K^+ which directly activate the voltage-dependent channels. The depolarization induced by high K^+ directly opens the Ca^{2+} channels without the contribution of the Na^+ channel. The role of bradykinin, a putative neuropeptide, on stimulus-secretion coupling including biosynthesis and secretion of catecholamine in adrenal chromaffin cells and pheochromocytoma PC-12 are discussed below.

Catecholamine biosynthesis regulated by bradykinin

Tyrosine hydroxylase, which is a rate-limiting enzyme in the biosynthesis of catecholamine, is phosphorylated and activated by cAMP-dependent protein kinase and calcium-dependent protein kinases (calcium/calmodulin-dependent protein kinase and protein kinase C) (13-16) : it is activated on incubation of cells with cAMP analogs or forskolin (an activator of adenylate cyclase), phorbol esters (activators of protein kinase C) or compounds that elevate the $[Ca^{2+}]i$ concentration (compounds causing nicotinic receptor stimulation or K^+-induced depolarization or calcium ionophores). Increased activation and phosphorylation of tyrosine hydroxylase on depolarization with high K^+ or treatments with cholinergic agonists, dibutyryl cAMP, phorbol esters and bioactive neuropeptides (vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide) in adrenal chromaffin cells were reported (17-32).

Fig. 1 compares the activation and phosphorylation of tyrosine hydroxylase produced by bradykinin

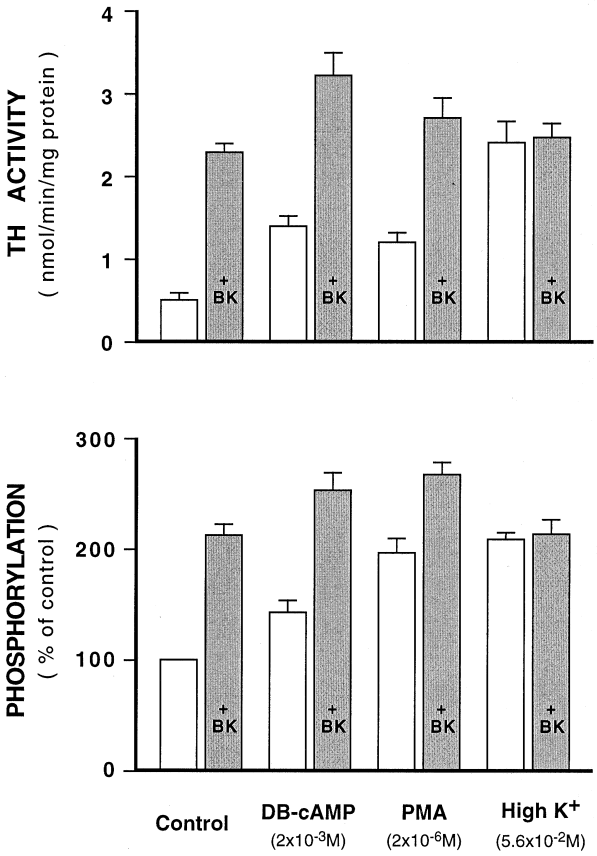

Fig.1. Effects of bradykinin on dibutyryl cAMP-, PMA- and high K^+-stimulated activation and phosphorylation of tyrosine hydroxylase in PC-12 cells. The PC-12 cells were incubated for 15 min with concentrations of dibutyryl cAMP ($2x10^{-3}M$), PMA ($2x10^{-6}M$) or high K^+ ($5.6x10^{-2}M$). These concentrations had previously been shown to produce maximal activation and phosphorylation of tyrosine hydroxylase. The PC-12 cells were incubated with bradykinin ($10^{-5}M$) for 5 min. (28)

with the activation and phosphorylation of tyrosine hydroxylase produced by treatment with dibutyryl cAMP, phorbol ester phorbol myristate acetate (PMA) and high K^+ in pheochromocytoma PC-12. Concentrations of these compounds were chosen to produce maximal activation and phosphorylation of tyrosine hydroxylase and the additivity of this activation and phosphorylation with that produced by bradykinin was evaluated. The increase in tyrosine hydroxylase activity produced by bradykinin (356%) and dibutyryl cAMP (181%) were additive (544%). Likewise, the enhanced phosphorylation of tyrosine hydroxylase produced by bradykinin (114%) and dibutyryl cAMP (44%) were additive (153%). PMA increased by 19% the activation of tyrosine hydroxylase produced by bradykinin. Likewise, the phosphorylation of tyrosine hydroxylase was increased to a similar degree. High K^+ had no effect on the increase in tyrosine hydroxylase activity or

phosphorylation produced by bradykinin. These data suggest that the mechanism of activation and phosphorylation of tyrosine hydroxylase produced by bradykinin is similar to that produced by high K^+ and to a minor extent by PMA.

In order to evaluate this further, the sites on tyrosine hydroxylase that were phosphorylated by bradykinin were determined. PC-12 cells were incubated with ^{32}P for 1 hr to label the cellular ATP stores. After exposure of the cells to bradykinin, tyrosine hydroxylase was isolated, subjected to SDS-polyacrylamide gel electrophoresis, eluted from the gels, and digested with trypsin for 12 to 18 hr at 37°C. The ^{32}P-labeled phosphopeptides derived from tyrosine hydroxylase were separated by HPLC. Fig. 2 illustrates the HPLC elution pattern of free ^{32}P-labeled peptide peaks that were produced by bradykinin, peptide peak A (retention time, 16min), peak B (19min) and peak C (27min). The phosphorylation of peak A and B was increased markedly when PC-12 cells were treated with bradykinin, whereas peak C was unaffected. It is known that peak B is phosphorylated by activators of protein kinase C (PMA or 1-oleyl-2-acetylglycerol), peak C is phosphorylated by activators of cAMP-dependent protein kinase (dibutyryl cAMP or forskolin), and peak A is phosphorylated by compounds that increase $[Ca^{2+}]i$ concentration and possibly activate calcium/calmodulin-dependent protein kinase (high K^+ or ionomycin). As bradykinin selectively stimulated the phosphorylation of peaks A and B, the possibility exists that bradykinin may activate tyrosine hydroxylase by an action on both calcium/calmodulin-dependent protein kinase and protein kinase C.

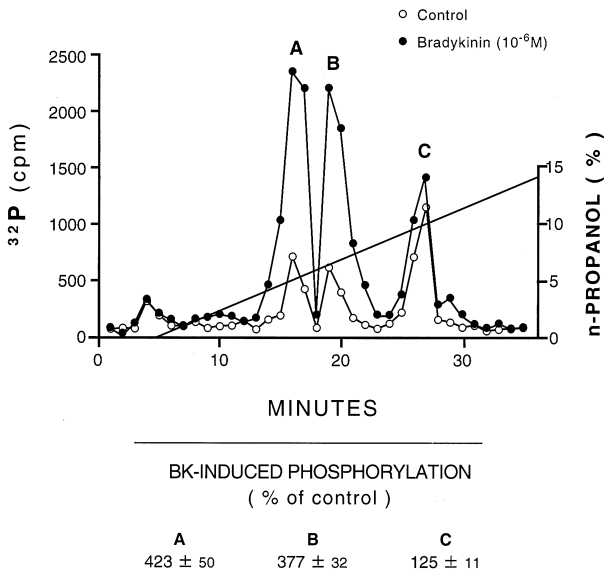

Fig. 2. HPLC analysis of tryptic phosphopeptide isolated from phosphorylated tyrosine hydroxylase obtained from PC-12 cells after treatment with bradykinin. ^{32}P-labeled tryptic peptides from tyrosine hydroxylase were separated by HPLC. (28)

Catecholamine secretion regulated by bradykinin

Activation of acetylcholine nicotinic receptors with cholinergic agents or membrane depolarization with high K^+ is known to initiate transient rapid catecholamine secretion accompanied by an increase in Ca^{2+} influx into the cells (6, 33, 34).

The time courses of catecholamine secretion induced by 10^{-6}M bradykinin and 10^{-5}M ouabain, an Na^+/K^+ ATPase inhibitor, separately or in combination were carried out in adrenal chromaffin cells. Bradykinin alone induced slight, transient secretion of catecholamine within 2 min, followed by slower, sustained secretion for at least 30 min. Ouabain alone also induced slight secretion for at least 30 min. The secretion was markedly higher with bradykinin plus ouabain, about 15% of the total intracellular catecholamine being secreted in 30 min. Stimulation of catecholamine secretion in either the presence or absence of 10^{-5}M of ouabain was observed with more than 10^{-8}M bradykinin and was maximal with 10^{-5}M bradykinin. The concentration of ouabain necessary to potentiate the bradykinin-induced catecholamine secretion was 10^{-6}M to 10^{-4}M. Catecholamine secretion stimulated by bradykinin in the presence of ouabain was inhibited by 10^{-6}M D-Arg-[Hyp^3, Thi^{5,8}, D-Phe^7]-bradykinin, a bradykinin-B_2 receptor antagonist (35), but not by 10^{-6}M Des-Arg^9-[Leu^8]-bradykinin, a bradykinin-B_1 receptor antagonist (3), and was reduced in medium without Ca^{2+}. These results indicate that the stimulatory effect of bradykinin plus ouabain on catecholamine secretion was mediated via the bradykinin-B_2 receptor and dependent on the presence of Ca^{2+} in the medium. It is further indicated that stimulation of the bradykinin-B_2 receptor may stimulate the influx of Ca^{2+} into the cells.

We examined the $^{45}Ca^{2+}$ influx into the cells stimulated by bradykinin and ouabain separately or together. Bradykinin or ouabain alone increased $^{45}Ca^{2+}$ influx into the cells slightly, whereas bradykinin plus ouabain increased $^{45}Ca^{2+}$ influx markedly. The time courses of $^{45}Ca^{2+}$ influx were similar to those of catecholamine secretion, indicating that continuous $^{45}Ca^{2+}$ influx is associated with catecholamine secretion.

To determine whether Na^+, as well as Ca^{2+}, is involved in the secretion of catecholamine induced by bradykinin and bradykinin plus ouabain, we examined their effects in Na^+-depleted sucrose medium. We found that Na^+-depleted sucrose medium containing Ca^{2+} itself induced secretion about 5-6% of

the total catecholamine by increasing Ca^{2+} influx into the cells. Therefore, we incubated the cells with bradykinin for 30 min in Ca^{2+}-free medium in the presence or absence of ouabain and then stimulated them for 15 min with Ca^{2+}-medium (containing 0.55-2.2mM Ca^{2+}) without bradykinin and ouabain. As shown in Fig. 3, prior stimulation of the cells with bradykinin increased the catecholamine secretion induced by each concentration of Ca^{2+} tested, and this stimulatory effect of bradykinin was greatly potentiated by the presence of ouabain. The stimulatory effect of bradykinin plus ouabain on catecholamine secretion in Na^+-free sucrose medium was much lower. During the first stimulation of the cells with bradykinin plus ouabain in Ca^{2+}-free medium, $^{22}Na+$ accumulation in the cells was significantly higher than that with bradykinin or ouabain alone, the levels induced by 10^{-6}M bradykinin, 10^{-5}M ouabain and 10^{-6}M bradykinin plus 10^{-5}M ouabain being 25±4,210±24 and 285±31 (nmol/dish in 30 min), respectively. These results indicated that activation of the bradykinin-B_2 receptor and inhibition of the Na^+ pump by ouabain both increase the accumulation of Na^+ in the cells, resulting in increases in Ca^{2+} influx, the $[Ca^{2+}]i$ level and catecholamine secretion (36).

Calcium efflux from chromaffin cells regulated by bradykinin

After stimulation of catecholamine secretion from adrenal chromaffin cells induced by various secretagogues, the increase in $[Ca^{2+}]i$ should rapidly return to the resting level to enable response to a subsequent stimulation (37-43). In this part, the mechanism of decrease in elevated $[Ca^{2+}]i$ and the effect of bradykinin on Ca^{2+} efflux from adrenal chromaffin cells are discussed.

Fig. 4 shows the effluxes of $^{45}Ca^{2+}$ from adrenal chromaffin cells in culture induced by various concentrations of bradykinin. The stimulatory effect of bradykinin on $^{45}Ca^{2+}$ efflux was dose-dependent at concentrations of 10^{-9}-10^{-6}M bradykinin. The efflux of $^{45}Ca^{2+}$ increased to a peak value within about 1min after bradykinin addition. The peak value with 10^{-6}M bradykinin was 8.2±0.7% of the total $^{45}Ca^{2+}$ in the cells. After the peak, the efflux decreased rapidly for the next 5 min. The effects of the bradykinin-receptor antagonists Des-Arg^9-[Leu^8]-bradykinin (B_1-receptor antagonist (3)) and D-Arg-[Hyp^3, Thi^{5,8}, D-Phe^7]-bradykinin (B_2-receptor antagonist (35)) on the submaximal $^{45}Ca^{2+}$ efflux from the cells induced by 10^{-7}M bradykinin were evaluated. This efflux was inhibited 81% by 10^{-6}M D-Arg-[Hyp^3, Thi^{5,8}, D-Phe^7]-bradykinin, but was not inhibited by Des-Arg^9-[Leu^8]-bradykinin. This result suggests that the $^{45}Ca^{2+}$ efflux

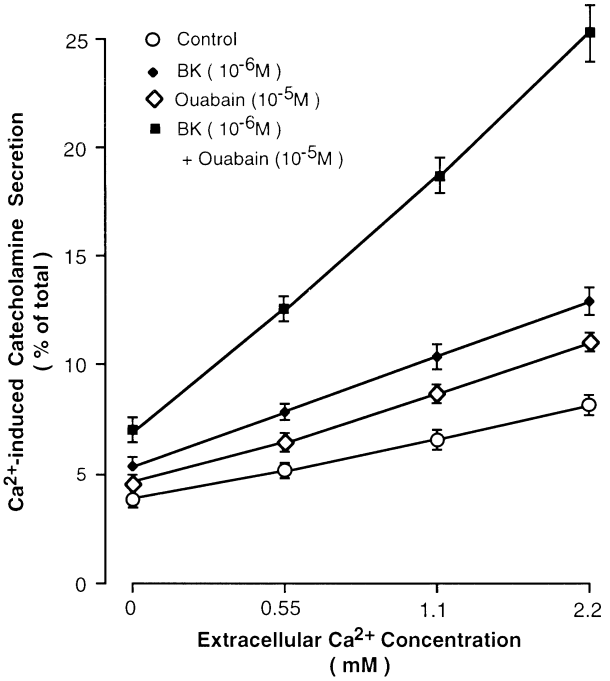

Fig. 3. Effects of extracellular Ca^{2+} on catecholamine secretion from bradykinin-and ouabain-pretreated adrenal chromaffin cells. Cells were incubated with 10^{-6}M bradykinin for 30 min in Ca^{2+}-free medium in the presence or absence of 10^{-5}M ouabain and then stimulated for 15 min with Ca^{2+}-medium (Ca^{2+}concentration: 0.55 to 2.2mM) without bradykinin and ouabain. (36)

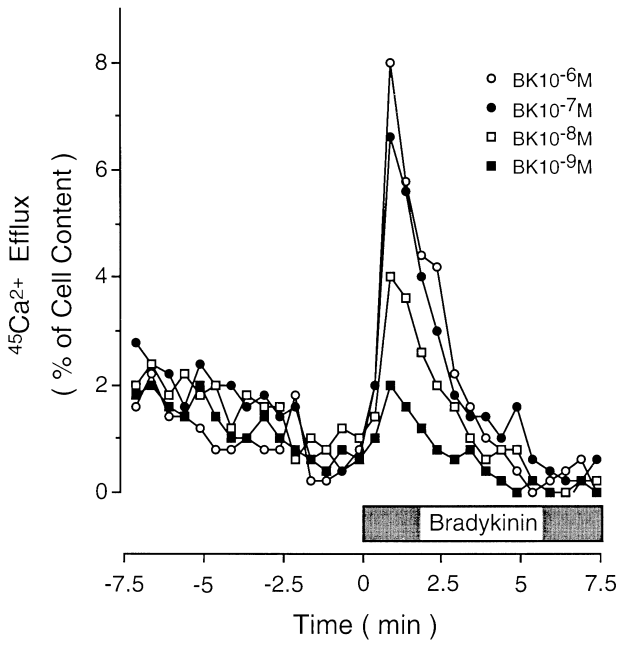

Fig. 4. Effects of different concentrations of bradykinin on $^{45}Ca^{2+}$ efflux from cultured bovine adrenal chromaffin cells. Cells were preloaded with $^{45}Ca^{2+}$ for 1 hr, and were washed 30 times at intervals of 30 sec. They were then incubated 15 times with 1 ml volumes of reaction mixture. (53)

induced by bradykinin was mediated through the bradykinin B_2-receptor.

To determine whether bradykinin-stimulated $^{45}Ca^{2+}$ efflux is mediated by activation of Ca^{2+} channels, we examined whether it was inhibited by Ca^{2+} channel blockers. Nifedipine, an organic voltage-dependent Ca^{2+} channel blocker, had no effect on $^{45}Ca^{2+}$ efflux from the cells induced by bradykinin (Fig. 5). Nor did the inorganic Ca^{2+} channel blockers Co^{2+} and Cd^{2+}, which inhibit voltage-dependent and receptor-operated Ca^{2+} channels, inhibit bradykinin-stimulated $^{45}Ca^{2+}$ efflux (Fig. 5). Thus stimulation of $^{45}Ca^{2+}$ efflux by bradykinin is probably not due to increased Ca^{2+} flux through Ca^{2+}channels.

The stimulations of bradykinin, histamine and muscarinic acetylcholine receptors are reported to induce breakdown of phosphatidyl inositol 4,5-bisphosphate (PIP_2) in bovine adrenal chromaffin cells (44, 45). The breakdown products of PIP_2 (inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol) increase the [Ca_{2+}]i level and stimulate protein kinase C (24, 46, 47). The effects of bradykinin, histamine, acetylcholine and PMA (an activator of protein kinase C) on $^{45}Ca^{2+}$ efflux from the cells were examined. Bradykinin (10^{-6}M) and histamine (10^{-5}M) increased the $^{45}Ca^{2+}$ efflux to about 460% and 350% of the control level, respectively. Acetylcholine (10^{-4}M) increased $^{45}Ca^{2+}$ efflux from the cells slightly, but significantly, whereas PMA (10^{-6}M) had no effect on $^{45}Ca^{2+}$ efflux. These results suggest that bradykinin-stimulated $^{45}Ca^{2+}$ efflux from the cells is related to the formation of IP_3, but not to activation of protein kinase C.

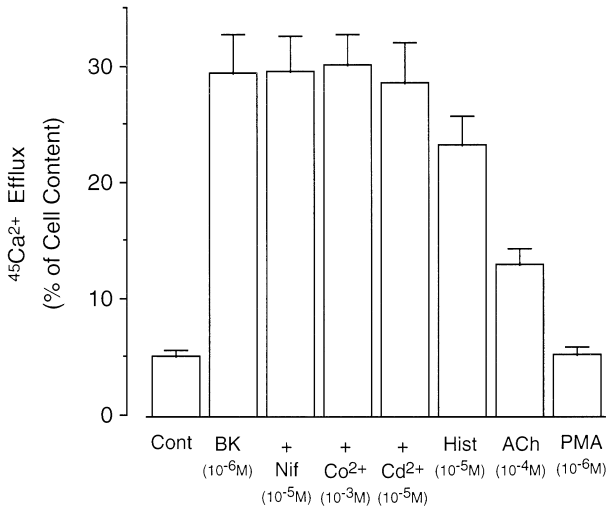

Fig. 5. Effects of various agents on $^{45}Ca^{2+}$ efflux from cultured bovine adrenal chromaffin cells. Cells were incubated in the presence or absence of bradykinin (BK ; 10^{-6}M), histamine (Hist ; 10^{-5}M), acetylcholine (ACh ; 10^{-4}M) or PMA (10^{-6}M). Nifedipine (Nif ; 10^{-5}M), Co^{2+} (10^{-3}M) or Cd^{2+} (10^{-5}M) was added 150 sec before bradykinin (10^{-6}M). $^{45}Ca^{2+}$ efflux 5 min after addition of agents was calculated. (53)

To determine whether the increased $^{45}Ca^{2+}$ efflux induced by bradykinin is dependent on the elevation of [Ca^{2+}]i level, the effects of bradykinin, histamine and acetylcholine on [Ca^{2+}]i concentration were examined. Bradykinin (10^{-6}M) and histamine (10^{-5}M) increased the [Ca^{2+}]i to approximately 430nM and 360nM from 100nM. Acetylcholine (10^{-4}M) increased it to about 890nM. However, acetylcholine-stimulated $^{45}Ca^{2+}$ efflux from the cells was less than bradykinin- or histamine-stimulated $^{45}Ca^{2+}$ efflux (Fig. 5). Therefore, bradykinin-stimulated $^{45}Ca^{2+}$ efflux from the cells may not be dependent on the elevation of the intracellular [Ca^{2+}]i level in the cells.

It has been established that bradykinin increases cyclic GMP (cGMP) in adrenal chromaffin cells through the activation of guanylate cyclase (48). Therefore, the role of cAMP and cGMP on Ca^{2+} efflux from cultured bovine adrenal chromaffin cells was evaluated to determine whether these intracellular messengers are involved in regulation of the Ca^{2+} efflux mechanism. The effects of dibutyryl cAMP (10^{-3}M), forskolin (10^{-6}M : an activator of adenylate cyclase (18)), dibutyryl cGMP (10^{-3}M) and nitroprusside (10^{-3}M : an activator of guanylate cyclase (49, 50)) on $^{45}Ca^{2+}$ efflux from cultured bovine adrenal chromaffin cells preloaded with $^{45}Ca^{2+}$ were examined. All these agents stimulated efflux of $^{45}Ca^{2+}$ from the cells. These stimulations of $^{45}Ca^{2+}$ efflux were clearly observed at concentrations of more than $2x10^{-4}$M of dibutyryl cAMP and dibutyryl cGMP, 10^{-7}M of forskolin and 10^{-4}M of nitroprusside. Since these agents did not affect the [Ca^{2+}]i level measured using the Ca^{2+} indicator fura-2 (control 98±8 ; 10^{-3}M dibutyryl cAMP 103±10 ; 10^{-6}M forskolin 101±9 ; 10^{-3}M dibutyryl cGMP 97±9 ; 10^{-3}M nitroprusside 102±11 nM, respectively), this stimulation of Ca^{2+} efflux was not the result of increase in the [Ca^{2+}]i level. These results suggest that both cAMP and cGMP are involved in stimulation of Ca^{2+} efflux from the cells, through these nucleotide-dependent protein kinases (51).

To determine whether the increased $^{45}Ca^{2+}$ efflux induced by bradykinin is Na^+-dependent, we carried out a series of experiments in the absence of extracellular Na^+. As shown in Fig. 6, complete replacement of Na^+ by sucrose significantly blocked the enhanced $^{45}Ca^{2+}$ efflux from the cells induced by bradykinin. Amiloride, an inhibitor of the Na^+/Ca^{2+} exchanger (52), also significantly inhibited bradykinin-stimulated $^{45}Ca^{2+}$efflux from the cells. Therefore, the effect of bradykinin in stimulating Ca^{2+} efflux across the plasma membrane

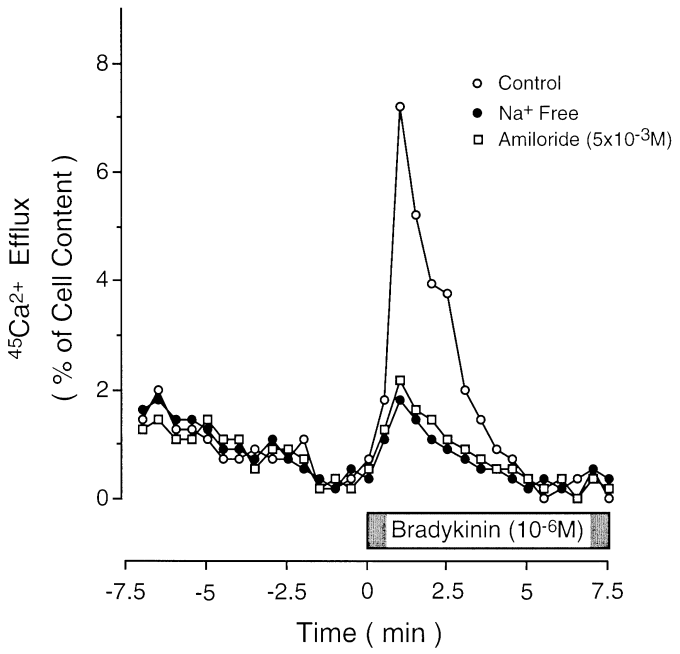

Fig. 6. Effects of Na^+-free medium and amiloride on bradykinin-induced $^{45}Ca^{2+}$ efflux from cultured bovine adrenal chromaffin cells. The medium was changed to Na^+-free medium (with sucrose instead of all Na^+) 150 sec before adding bradykinin (10^{-6}M). In the case of the amiloride experiment, amiloride (5×10^{-3}M) was added 150 sec before bradykinin (10^{-6}M) in normal medium. (53)

may be mediated in part by a Na^+/Ca^{2+} exchange mechanism (53).

Bradykinin-stimulated $^{45}Ca^{2+}$ efflux from the cells may be regulated by nitric oxide (NO)/ cGMP pathway because 1) Bradykinin increases the formation of cGMP (48), 2) an increase in cGMP formation stimulates the calcium efflux from adrenal chromaffin cells (51), and 3) the efflux of calcium is regulated by NO production (54). However, we have no data on whether bradykinin stimulates NO production in adrenal chromaffin cells.

CONCLUSION

The role of bradykinin on stimulus-secretion coupling in adrenal chromaffin cells is illustrated in Fig 7. Bradykinin receptors are classified as B_1 and B_2 receptor subtypes. These receptors are present on the adrenal chromaffin cell membrane. Bradykinin increases the influx of calcium and the activity of phospholipase C through the stimulation of bradykinin B_2 receptor on the cell membrane. Diacylglycerol is produced concurrently with IP_3 on breakdown of PIP_2 by phospholipase C and is thought to activate protein kinase C by increasing the affinity of the enzyme for calcium (46). In the pathway of catecholamine biosynthesis, an increase in $[Ca^{2+}]i$ and production of diacylglycerol stimulate the activation of calcium-dependent protein kinases (calcium/calmodulin-dependent protein kinase and protein kinase C). These kinases phosphorylate and acti-vate tyrosine hydroxylase, a rate-limiting enzyme in the biosynthesis of catecholamine. The catecholamine which is formated by tyrosine hydroxylase is stored in chromaffin granules. In the pathway of catecholamine secretion, the stimulation of the bradykinin-B_2 receptor elevates the accumulation of Na^+ in the cells. An increase in intracellular Na^+ concentration leads to Ca^{2+} influx into the cells through the depolarization of the cell membrane and/or the reverse mode of Na^+/Ca^{2+} exchange mechanism. The secretion of catecholamine from the cells is initiated by the raise of $[Ca^{2+}]i$. However, the detailed mechanism of the calcium-induced catecholamine secretion pathway remains unclear.

Physiological stimulation of adrenal chromaffin cells cause an increase in $[Ca^{2+}]i$, leading to initiation of stimulus-secretion coupling. However, this increased $[Ca^{2+}]i$ should be restored to a physiological level for response to subsequent stimulation. In the pathway of Ca^{2+} efflux, bradykinin increases Ca^{2+} efflux from the cells through the stimulation of the bradykinin-B_2 receptor. Bradykinin-induced Ca^{2+} efflux may be explained by an extracellular Na^+-dependent mechanism, probably through acceleration of Na^+/Ca^{2+} exchange. It is interesting

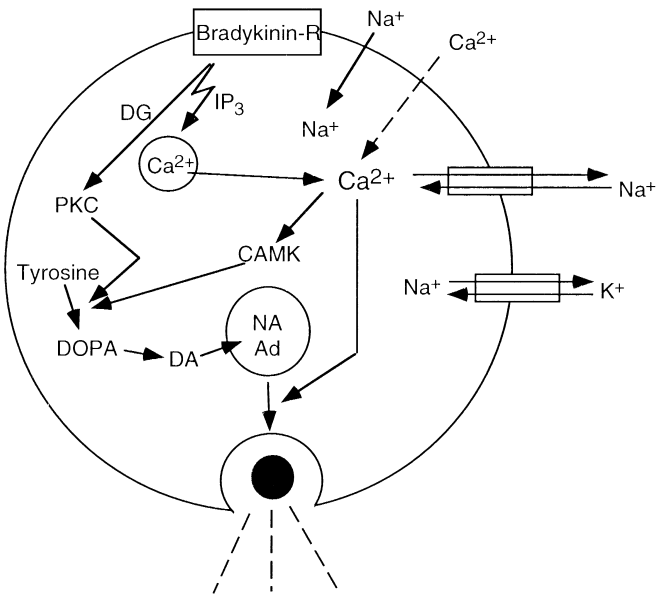

Fig. 7. Schematic stimulus-secretion coupling in adrenal chromaffin cells.

that bradykinin, which stimulates the biosynthesis and secretion of catecholamine in adrenal chromaffin cells, plays a role in the termination of Ca^{2+}-signal transduction through the stimulation of Ca^{2+} efflux from the cells.

REFERENCES

1. Regoli D, Barabe J : Pharmacology of bradykinin and related kinins. Pharmacol Rev 32 : 1-46, 1980
2. Rocha E, Silvia M, Beraldo WT, Rosenfeld G : Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globin by snake venoms and trypsin. Am J Physiol 156 : 261-273, 1949
3. Regoli D, Barabe J, Park WK : Receptors for bradykinin in rabbit aorta. Can J Physiol Pharmacol 55 : 855-867, 1977
4. Snyder SH : Brain peptides as neurotransmitters. Science 209 : 976-982, 1980
5. Perry DC, Snyder SH : Identification of bradykinin in mammalian brain. J Neurochem 43 : 1072-1080, 1984
6. Oka M, Isosaki M, Yanagihara N : Isolated bovine adrenal medullary cells : studies on regulation of catecholamine synthesis and release. In : Usdin E, Kopin IJ, Barchas J, eds. Catecholamines, Basic and Clinical Frontiers, Pergamon Press, Oxford, 1979, pp. 70-72
7. Masserano JM, Vulliet PR, Tank AW, Weiner N : The role of tyrosine hydroxylase in the regulation of catecholamine synthesis. In : Trendelenburg U, Weiner N, eds. Catecholamines, Handbook of Experimental Pharmacology, Springer, Berlin, 1990, pp.427-469
8. Douglas WW, Kanno T, Sampson SR : Effects of acetylcholine and other medullary secretagogues and antagonists on the membrane potential of adrenal chromaffin cells : An analysis employing techniques of tissue culture. J Physiol 188 : 107-120, 1967
9. Douglas WW, Kanno T, Sampson SR : Influence of the ionic environment on the membrane potential of adrenal cells and on the depolarizing effect of acetylcholine. J Physiol 191 : 107-121, 1967
10. Cena V, Nicholas G, Sanchez-Garcia P, Kirpekar SM, Garcia AG : Pharmacological dissection of receptor-associated and voltage-sensitive ionic channels involved in catecholamine release. Neuroscience 10 : 1455-1462, 1983
11. Garcia AG, Sala F, Reig JA, Viniegra S, Frias J, Fonteriz R, Gandia L : Dihydropyridine BAY-K-8644 activates chromaffin cell calcium channels. Nature 309 : 69-71, 1984
12. Artalejo CR, Adams ME, Fox AP : Three types of Ca^{2+} channel trigger secretion with different efficacies in chromaffin cells. Nature 367 : 72-76, 1994
13. Yamauchi T, Fujisawa H : In vitro phosphorylation of bovine adrenal tyrosine hydroxylase by adenosine 3 ': 5 '-monophosphate-dependent protein kinase. J Biol Chem 254 : 503-507, 1979
14. Vulliet PR, Langan TA, Weiner N : Tyrosine hydroxylase : a substrate for cyclic AMP-dependent protein kinase. Proc Natl Acad Sci USA 77 : 92-96, 1980
15. Edelman AM, Raese JD, Lazar MA, Barchas JD : Tyrosine hydroxylase : studies on the phosphorylation of a purified preparation of the brain enzyme by cyclic AMP-dependent protein kinase. J Pharmacol Exp Ther 216 : 647-653, 1981
16. Vulliet PR, Woodgett JR, Ferrari S, Hardie DG : Characterization of the sites phosphorylated on tyrosine hydroxylase by Ca^{2+} and phospholipid-dependent protein kinase, calmodulin-dependent multiprotein kinase and cyclic AMP-dependent protein kinase. FEBS Lett 182 : 335-339, 1985
17. Meligeni JA, Haycock JW, Bennett WF, Waymire JC : Phosphorylation and activation of tyrosine hydroxylase mediate the cAMP-induced increase in catecholamine biosynthesis in adrenal chromaffin cells. J Biol Chem 257 : 12632-12640, 1982
18. Houchi H, Nakagawa H, Oka M, Watanabe J, Isosaki M, Ohuchi T : Increase in cyclic AMP level and stimulation of catecholamine synthesis in isolated bovine adrenal medullary cells caused by forskolin. Jpn J Pharmacol 36 : 97-99, 1984
19. Vulliet PR, Woodgett JR, Cohen P : Phosphorylation of tyrosine hydroxylase by calmodulin-dependent multiprotein kinase. J Biol Chem 259 : 13680-13683, 1984
20. Houchi H, Nakanishi A, Uddin MM, Ohuchi T, Oka M : Phorbol ester stimulates catecholamine synthesis in isolated bovine adrenal medullary cells. FEBS Lett 188 : 205-208, 1985
21. Pocotte SL, Holz RW : Effects of phorbol ester on tyrosine hydroxylase phosphorylation and activation in cultured bovine adrenal chromaffin cells. J Biol Chem 261 : 1873-1877, 1986

22. Houchi H, Morita K, Minakuchi K, Oka M : Inhibition by hydralazine of catecholamine biosynthesis in cultured bovine adrenal medullary cells. Biochem Pharmacol 35 : 2047-2049, 1986
23. Yanagihara N, Tank AW, Langan TA, Weiner N : Enhanced phosphorylation of tyrosine hydroxylase at more than one site is induced by 56mM K^+ in rat pheochromocytoma PC-12 cells in culture. J Neurochem 46 : 562-568, 1986
24. Tachikawa E, Tank AW, Weiner DH, Mosimann WF, Yanagihara N, Weiner N : Tyrosine hydroxylase is activated and phosphorylated on different sites in rat pheochromocytoma PC-12 cells treated with phorbol ester and forskolin. J Neurochem 48 : 1366-1376, 1987
25. Houchi H, Oka M, Misbahuddin M, Morita K, Nakanishi A : Stimulation by vasoactive intestinal polypeptide of catecholamine synthesis in isolated bovine adrenal chromaffin cells. Biochem Pharmacol 36 : 1551-1554, 1987
26. Griffith LC, Schulman H : The multifunctional Ca^{2+}/calmodulin-dependent protein kinase mediates Ca^{2+}-dependent phosphorylation of tyrosine hydroxylase. J Biol Chem 263 : 9542-9549, 1988
27. Houchi H, Masserano JM, Weiner N : Bradykinin activates tyrosine hydroxylase in rat pheochromocytoma PC-12 cells. FEBS Lett 235 : 137-140, 1988
28. Houchi H, Masserano JM, Bowyer JF, Weiner N : Regulation of tyrosine hydroxylase activity in pheochromocytoma PC-12 cells by bradykinin. Mol Pharmacol 37 : 104-110, 1990
29. Houchi H, Yoshizumi M, Minakuchi K, Azuma M, Morita K, Oka M : Idebenone, an agent improving cerebral metabolism, stimulates [^{14}C]tyrosine uptake and [^{14}C]catecholamine formation by cultured bovine adrenal chromaffin cells. Biochem Pharmacol 42 : 951-954, 1991
30. Houchi H, Teraoka K, Oka M, Murakumo Y, Morita K : Substance P inhibits catecholamine biosynthesis stimulated by carbamylcholine in cultured adrenal chromaffin cells. Biochem Pharmacol 45 : 1165-1167, 1993
31. Houchi H, Hamano S, Masuda Y, Ishimura Y, Azuma M, Ohuchi T, Oka M : Stimulatory effect of pituitary adenylate cyclase-activating polypeptide on catecholamine synthesis in cultured bovine adrenal chromaffin cells : involvements of tyrosine hydroxylase phosphorylation caused by Ca^{2+} influx and cAMP. Jpn J Pharmacol 66 : 323-330, 1994
32. Houchi H, Yoshizumi M, Ishimura Y, Oka M : Differential requirements for Ca^{2+} concentrations for catecholamine release and biosynthesis in isolated bovine adrenal chromaffin cells. Naunyn-Schmiedeberg's Arch Pharmacol 353 : 596-598, 1996
33. Houchi H, Azuma M, Oka M, Morita K : Idebenone inhibits catecholamine secretion through its blocking action on Ca^{2+} channels in cultured adrenal chromaffin cells. Jpn J Pharmacol 57 : 553-558, 1991
34. Houchi H, Yoshizumi M, Minakuchi K, Ishimura Y, Morita K, Tamaki T, Oka M : Potentiation of histamine-induced catecholamine secretion by ouabain in cultured bovine adrenal chromaffin cells is dependent on calcium and sodium influx. Life Sci 60 : 2051-2058, 1997
35. Schachter M, Uchida Y, Longridge DJ, Labedz T, Whalley ET, Vavrek RJ and Stewart JM : New synthetic antagonists of bradykinin. Brit J Pharmacol 92 : 851-855, 1987
36. Murakumo Y, Houchi H, Minakuchi K, Yoshizumi M, Oka M : Potentiation by ouabain of bradykinin-induced catecholamine secretion and calcium influx into cultured bovine adrenal chromaffin cells : evidence for involvement of Na^+ influx associated with the bradykinin B_2-receptor. Life Sci 57 : 259-264, 1995
37. Houchi H, Masuda Y, Murakumo Y, Ishimura Y, Ohuchi T, Oka M : Muscarinic receptor-mediated calcium efflux from cultured bovine adrenal chromaffin cells. Biochem Pharmacol 48 : 1982-1985, 1994
38. Houchi H, Kitamura K, Minakuchi K, Ishimura Y, Okuno M, Ohuchi T, Oka M : Mechanism of histamine-induced calcium efflux from cultured bovine adrenal chromaffin cells : possible involvement of an Na^+/Ca^{2+} exchange mechanism. Neurosci Lett 180 : 281-284, 1994
39. Houchi H, Okuno M, Kitamura K, Minakuchi K, Ishimura Y, Ohuchi T, Oka M : Calcium efflux from cultured bovine adrenal chromaffin cells induced by pituitary adenylate cyclase-activating polypeptide (PACAP) : possible involvement of an Na^+/Ca^{2+} exchange mechanism. Life Sci 56 : 1825-1834, 1995
40. Houchi H, Okuno M, Kitamura K, Ishimura Y, Ohuchi T, Tokumura A, Oka M : Stimulatory effect of angiotensin II on calcium efflux from cultured bovine adrenal chromaffin cells. Life Sci 56 : 109-114, 1995
41. Houchi H, Okuno M, Tokumura A, Yoshizumi M, Fukuzawa K, Oka M : Lysophosphatidic acid

as a stimulator of Na^+-dependent Ca^{2+} efflux from adrenal chromaffin cells. Life Sci 57 : 205-210, 1995

42. Houchi H, Okuno M, Yoshizumi M, Oka M : Regulatory mechanism of calcium efflux from cultured bovine adrenal chromaffin cells induced by extracellular ATP. Neurosci Lett 198 : 177-180, 1995
43. Houchi H, Yoshizumi M, Shono M, Ishimura Y, Ohuchi T, Oka M : Adrenomedullin stimulates calcium efflux from adrenal chromaffin cells in culture : possible involvement of an Na^+/Ca^{2+} exchange mechanism. Life Sci 58 : 35-40, 1996
44. Plevin R, Boarder MR : Stimulation of formation of inositol phosphates in primary cultures of bovine adrenal chromaffin cells by angiotensin II, histamine, bradykinin and carbachol. J Neurochem 51 : 634-641, 1988
45. Stauderman KA, Pruss RM : Different patterns of agonist-stimulated increases of ^3H-inositol phosphate isomers and cytosolic Ca^{2+} in bovine chromaffin cells : comparison of the effects of histamine and angiotensin II. J Neurochem 54 : 946-953, 1990
46. Nishizuka Y : The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature (Lond) 312 : 315-321, 1984
47. Misbahuddin M, Isosaki M, Houchi H, Oka M, Muscarinic receptor-mediated increase in cytoplasmic free Ca^{2+} in isolated bovine adrenal medullary cells : effects of TMB-8 and phorbol ester TPA. FEBS Lett 190 : 25-28, 1985
48. Snider RM, Richelson E : Bradykinin receptor-mediated cyclic GMP formation in a nerve cell population (murine neuroblastoma clone N1E-115). J Neurochem 43 : 1749-1754, 1984
49. Bohme E, Graf H, Schultz G : Effects of sodium nitroprusside and other smooth muscle relaxants on cyclic GMP formation in smooth muscle and platelets. Adv Cyclic Nucleotide Res. 9: 131-143, 1978
50. O śullivan AJ, Burgoyne RD : Cyclic GMP regulates nicotine-induced secretion from cultured bovine adrenal chromaffin cells : effects of 8-bromo-cyclic GMP, atrial natriuretic peptide, and nitroprusside (nitric oxide). J Neurochem 54 : 1805-11808, 1990
51. Houchi H, Okuno M, Shono M, Tokumura A, Oka M : Regulations by cyclic nucleotides and phorbol ester of calcium efflux from cultured bovine adrenal chromaffin cells. Life Sci 57: 211-215, 1995
52. Benuck M, Reith M EA, Lajitha A : Evidence for the involvement of Na^+/Ca^{2+} exchange in the stimulation of inositol phospholipid hydrolysis by sodium channel activation and depolarization. Eur J Pharmacol 159 : 187-190, 1989
53. Houchi H, Masuda Y, Ishimura Y, Ohuchi T, Murakumo Y, Oka M : Calcium efflux from cultured bovine adrenal chromaffin cells induced by bradykinin. Biochem Pharmacol 47 : 1309-1313, 1994
54. Houchi H, Yoshizumi M, Tsuchiya K, Hirose M, Kitagawa T, Kujime T, Shimizu E, Niwa Y, Minakuchi K, Tamaki T : Endothelin-1 stimulates sodium-dependent calcium efflux from bovine adrenal chromaffin cells in culture. Bri J Pharmacol 125 : 55-60, 1998