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Bradykinin-induced secretion of insulin, glucagon and somatostatin

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Bradykinin-induced secretion of insulin, glucagon and somatostatin

by

Chi Yang

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Major Professor

For the Major Department

For the Graduate College

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ABSTRACT

Rat pancreas perfusion was performed to study the effect of bradykinin (BK) on insulin secretion. At the perfusate glucose concentration of 6 mM, BK (0.01 - 1 μM) increased insulin secretion in a concentration-dependent manner. BK (1 μM) induced a biphasic insulin secretion, transiently increased glucagon secretion and decreased somatostatin secretion. In addition, BK (1 μM) enhanced glucose-induced insulin secretion in a glucose-dose-dependent manner. BK-induced insulin secretion was greatly reduced in 1 mM glucose. The level of BK-glucose (10 and 20 mM)-induced sustained insulin secretion was higher than that of transient insulin release peak. Since the sustained phase is due to increased synthesis of insulin, BK may stimulate both the synthesis and release of insulin. HOE140 (0.1 μM), a BK_2-receptor antagonist, abolished BK-induced increase in insulin secretion. HOE140 also decreased basal and glucose (10 mM)-induced increase in insulin secretion. Therefore, BK may play a physiological role in the regulation of insulin secretion via BK_2-receptors. A clonal β-cell line RINm5F was used to study the mechanisms underlying BK-induced rise in $[Ca^{2+}]_i$ and insulin secretion. BK increased insulin secretion and $[Ca^{2+}]_i$ in a concentration-dependently manner. BK (1 μM) induced a biphasic $[Ca^{2+}]_i$ rise which was characterized by a transient Ca^{2+} release and a sustained Ca^{2+} influx phase. The BK-induced Ca^{2+} influx was triggered by Ca^{2+} release. The effects of BK were mediated through BK_2-receptors which were coupled to a pertussis toxin-insensitive G protein, probably G_q. G_q protein activated phospholipase C which promoted the formation of inositol, 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 released $[Ca^{2+}]_i$ from the intracellular Ca^{2+} stores, probably the endoplasmic reticulum, thereby triggering Ca^{2+} influx via voltage-dependent Ca^{2+} channels and increasing insulin secretion. DAG activated protein kinase C which promoted Ca^{2+} efflux and inhibited Ca^{2+} influx, thereby decreasing $[Ca^{2+}]_i$ and thus reducing insulin secretion.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Pancreatic acinar and islet cells secrete different compounds. Acinar cells secrete digestive juice and enzymes into the intestinal tract, while islet cells secrete hormones including insulin, glucagon, and somatostatin, into the blood stream, which are involved in glucose homeostasis. Acinar cells also secrete kallikrein, which releases kinins from the precursor kininogens or converts prohormones to hormone, e.g., proinsulin to insulin. The primary kinin released in the rat is bradykinin (BK). Although the function of BK is well understood in pain, inflammation, hypotension, smooth muscle contraction, the physiological role of BK in the endocrine and exocrine pancreas is still unknown despite the fact that kallikrein is found abundantly in the pancreas and kininogens are rich in the pancreas and blood circulation. In fact, the pancreas is one of the richest sources of kallikrein.

Two kinins, BK and lys-BK (kallidin), increased insulin release in perfused rat and dog pancreata in our preliminary studies. In addition, we found that BK increased glucose-induced insulin release in the perfused rat pancreas (see Chapter 2). The newly synthesized BK_2-receptor blocker, HOE 140, antagonized BK-induced insulin release and diminished glucose-induced insulin release in the perfused rat pancreas. HOE 140 also lowered the basal levels of insulin secretion in the perfused rat pancreas. Our findings support the premise that kinins and kallikrein are important factors involved in the regulation of insulin secretion. Patients with chronic pancreatitis develop diabetes mellitus, but the minor change in β-cells can not account for the impaired insulin secretion. Therefore, we proposed that pancreatic kallikrein promotes the formation of BK which is important for maintaining adequate basal and glucose-induced insulin release.

The islets have α and δ cells in addition to β cells. The effects of BK on these cell types or the paracrine effects between these cells are still not well understood. Therefore, the goal of these studies was to investigate the stimulatory effects and the mechanisms of BK on insulin release, and to investigate the effects of BK on the release of glucagon and somatostatin. The perfused rat pancreas was used as a model to study the following phenomena: 1) BK-induced insulin release in the normal rat pancreas in order to understand the stimulatory effect of BK on insulin release from the β cells of islets, 2) the potentiation of glucose-induced insulin secretion by BK to understand the interaction between BK and glucose in insulin release on β cells, 3) the

inhibition of BK-induced and glucose-induced insulin secretion by the BK_2-receptor blocker, HOE 140, to determine the physiological significance of BK in the regulation of insulin release, 4). the glucose-dependent effect of BK on insulin release in order to investigate the importance of glucose for the action of BK, 5) the effects of BK on α cells and δ cells of islets to explore the responses of these cells to BK in the secretion of glucagon and somatostatin.

In order to investigate the mechanisms underlying BK-induced insulin secretion, a insulin-secreting cell line RINm5F was used to study the mechanisms of action of BK on insulin release. We proposed that BK activates BK_2-receptors which are coupled to a pertussis toxin-insensitive GTP-binding protein (G_q). The G_q is coupled to an intracellular enzyme, phospholipase C (PLC). The activated PLC hydrolyzes phosphatidylinositol biphosphate (PIP_2), resulting in the formation of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). Both IP_3 and DAG may contribute to the intracellular signals and increase the release of insulin.

The information obtained from these studies may enhance our understanding of the role of BK in the regulation of insulin release. In summary, the purpose of these studies was to investigate the stimulatory effects of BK on insulin release from the perfused rat pancreas and the mechanisms of action of BK in rat insulinoma cells, RINm5F cells. In addition, we investigated the effects of BK on the release of glucagon and somatostatin, respectively, from the same samples collected from the pancreatic perfusion to evaluate the interactions between these different cell types in islets with regard to insulin release.

Dissertation Organization

This dissertation is written in an alternative thesis format. It consists of a general introduction including literature review and references cited, four manuscripts, a general conclusion and acknowledgements. Chapter 2, "Stimulatory effect of bradykinin on insulin release from the perfused rat pancreas," has been published in the American Journal of Physiology and the other chapters, Chapter 3, "Glucose dependency of bradykinin-induced insulin secretion in perfused rat pancreas," Chapter 4, "The effect of bradykinin on the secretion of insulin, glucagon and somatostatin from the perfused rat pancreas," and Chapter 5, "Mechanisms of bradykinin-induced insulin secretion and a rise in cytosolic Ca^{2+} in a clonal β-cell line RINm5F," will be submitted to the American Journal of Physiology for publication. The format used for literature cited in the manuscripts is according to the requirement of the submitted journal.

Background and Literature Review

Biosynthesis and Release of Islet Hormones

In rodents, pancreatic tissue is a diffuse, almost lacy network without discrete lobular characteristics (Hazelwood, 1989). It contains two kinds of secretory tissues: the exocrine pancreas and the endocrine pancreas. The endocrine pancreas is composed of the islets of Langerhans which represents ≤ 2% of the pancreas and secretes hormones such as insulin, glucagon, and somatostatin. The exocrine pancreas is formed by acinar cells which represents about 80% of the pancreas and secretes ≥ 20 digestive emzymes.

The islet of Langerhans consists of at least four different hormone-secreting cells. In most species, β cells which secrete insulin are generally located in the central part of the islets that represent 60-80% of the islet cells. They are surrounded by α cells which secrete glucagon and represent 15-20% of the islet cells, and somatostatin-secreting cells (δ cells) that represent 5-10% of the islet cells. Pancreatic polypeptide-secreting cells (κ cells) that represent 15-20% of the islets are located in the periphery of the islets, mainly in the islets of the head of the pancreas (Bendayan, 1990; Miller, 1981). However, in the horse, α cells tend to localize centrally within the islets as small clusters (Helmstaedter et al., 1976), mainly located in the islets of the dorsal or splenic part of the pancreas.

The islets of Langerhans are richly vascularized, both the islet cells and blood vessels are closely associated with a variety of autonomic nerves. The pancreas receives its blood supply from the superior and inferior pancreatic-duodenal arteries and from the pancreatic branches of the splenic artery. As the arteriole enters the islets, it forms a dense capillary network and the major afferent arteriole to the islet branches to supply the core of β cells first, then outward to the mantle in which the α cells are perfused before the δ cells (Samols and Stagner, 1990). Thus, the cellular order of perfusion, β cells to α cells to δ cells is responsible for the regulation of release of islet hormones. The islet capillaries are fenestrated which allow for the rapid translocation of islet products intothe blood stream and for maintaining glucose homeostasis.

Insulin

In β cells, insulin is initially synthesized as a much larger peptide, called pre-proinsulin. The pre-proinsulin molecule consists of an insulin molecule which has the A (21 amino acids) and B (30 amino acids) chains, a connecting peptide (31-35 amino acid residues, depending on species) and a leading sequence of 23 amino acid residues (24 in rats) in the N-terminal of B chain. As pre-proinsulin enters the rough endoplasmic reticulum, the leading sequence is cleaved by microsomal enzymes, and forms proinsulin, an intermediate in the formation of active insulin (Steiner et al., 1967). During the translocation process within the microvesicles, proinsulin is cleaved by proteolytic enzymes including kallikrein to release the connecting peptide, insulin, and the four basic residues. Some of the cleavage occurs during Golgi apparatus processing of the granules-to-be, and a small fraction occurs after the formation of secretory granules. Proinsulin has only about 10% of the activity of insulin.

Most of the insulin, including the newly synthesized proinsulin, is stored in secretory granules and is released only after stimulation by secretagogues. This is called the classic or regulated pathway. However, some of the insulin is directly secreted from the Golgi apparatus to the plasma membrane after being synthesized, which allows the hormones to leak out. This is called the constitutive pathway (Rhodes and Halban, 1987). Insulin secretagogues promote the unidirectional movement of insulin granules away from the Golgi apparatus toward the cell membrane. The intracellular microtubular-microfilamentous system is involved in the transport of secretory granules toward the plasma membrane. In addition, subcellular components including tubulin, actin, and cytosolic Ca^{2+} are involved in the exocytosis of insulin granules. When β cells are stimulated with glucose, it produces oscillations of $[Ca^{2+}]_i$ and is followed by the synchronization of these signals which evoke a pulsatile release of insulin (Hellman et al., 1994).

The rat insulin molecular structure is similar to that of human insulin which is shown in Fig. 1 as the shaded polypeptide A and B chains. The A and B chains are connected by two interchain disulfide bonds which occur at positions A_7-B_7 and A_{20}-B_{19}. In addition, an intrachain disulfide bond occurs at positions A_6-A_{11} (Hazelwood, 1989). In rats, there are two insulin-producing genes, thus they produce two variants of insulin, rat insulin I and II, which are different at B_{29} (Gross et al., 1988). Rat insulin I and II are synthesized in a ratio of as approxmately 60 : 40 (Gishizky and Grodsky, 1987)

C-PEPTIDE
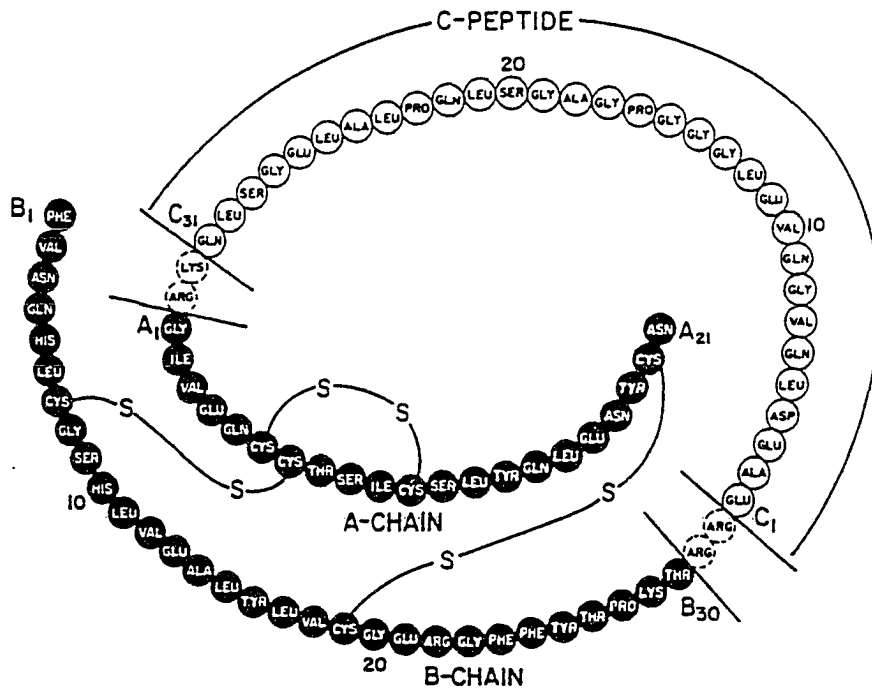
A-CHAIN

B-CHAIN

Fig. 1. Human proinsulin structure. Insulin is shown as the shaded polypeptide A and B chains. The rat insulin differs from the human insulin by having a lysine and serine instead of a arginine and threonine at positions B_3 and B_{30}, respectively (Bunzli et al., 1972).

Glucagon

Glucagon is a straight-chain polypeptide with 29 amino acid residues without any disulfide bonds. It is synthesized in α cells as a larger protein called pre-proglucagon with 179 amino acid residues. The biosynthesis of glucagon in α cells is similar to that of insulin in β cells. It involves translocation of new synthesized pre-proglucagon from polysomes into endoplasmic reticulum, cleavage of leader peptide and formation of proglucagon before the encapsulation within microvesicles, transportation of microvesicles to Golgi apparatus, formation of secretory glucagon granules, and exocytosis by stimulation (Noe et al., 1981). The amino acid sequence of rat and human glucagon are identical.

Somatostatin

Somatostatin secreted by pancreatic δ cells is identical in structure to that found in other parts of the body. The pancreas contains the highest concentration of tissue somatostatin. The biosynthesis of somatostatin is similar to that of insulin in β cells and glucagon in α cells. The rat prosomatostatin has 121-125 amino acid residues. When the prosomatostatin is packaged in the Golgi apparatus, most of the prosomatostatin is cleaved into somatostatin with 28 amino acid and then it is cleaved again into somatostatin with 14 amino acid residues (Hazelwood, 1989).

Interactions Between Islet Hormones

The intra-islet blood flow is from β cells to α cells and from α cells to δ cells. δ Cells are located in the islet mantle interspaced among α cells, and the endocrine pancreatic hormones are released into the circulation. Thus insulin regulates the release of glucagon, and then both insulin and glucagon regulate the release of somatostatin (Samols and Stagner, 1990). However, the islet hormones are actually released into the extracellular space which may produce a paracrine effect on the surrounding cells through local diffusion.

Insulin inhibits the release of glucagon, but stimulates the release of somatostatin (Greenbaum et al., 1991). In addition, insulin promotes glucose transport which inhibits the release of glucagon (Greenbaum et al., 1991). Insulin secretion is inhibited by the secreted insulin through a local feedback mechanism (Ammon et al., 1991). Glucagon increases the release of insulin and somatostatin, but both insulin and somatostatin inhibit the release of glucagon (Unger and Orci, 1977).

Recently, somatostatin receptors were found in β cells (Thermos et al., 1990). Somatostatin inhibits the release of insulin and glucagon from β and α cells, respectively (Hoyer et al., 1994), enhances insulin sensitivity, and increases utilization of glucose (Moller et al., 1995). These actions may be mediated by a paracrine effect (Unger and Orci, 1977).

Effect of Islet Hormones on Exocrine Pancreas

Due to the islet-acinar portal system and the dispersion of islets in the exocrine pancreas there may be interaction between these two tissues. In addition, islet hormone (insulin and somatostatin) receptors exist on the plasma membrane of pancreatic acinar cells (Bendayan, 1990) and the periacinal islet hormone concentrations are at least 20-fold higher in concentration than those in the systemic circulation (Bendayan, 1993). Thus, pancreatic islet hormones may regulate

the secretion of the exocrine pancreas (Nakagawa et al., 1995; Hasegawa et al., 1993).

Insulin directly regulates the function of exocrine pancreas including the release of zymogen, glucose transport, and protein synthesis (Williams and Goldfine, 1985). In addition, insulin increases secretin-induced pancreatic juice release, but does not affect the release of protein. However, both glucagon and somatostatin inhibit secretin-induced pancreatic juice and protein secretion (Hasegawa et al., 1993).

RINm5F Insulinoma Cells

Establishment of RIN-m Cell Line

Because the pancreatic islet contain other types of cells in addition to insulin-secreting β cells, it is not suitable for studying the mechanisms of action of agents affecting insulin release. In addition, it is difficult to study the subcellular and molecular components from the small amounts of islet β cells. Thus, the RIN-m cell line was established for these studies.

The RIN-m cell line was developed from a transplantable insulinoma in inbred albino rats of the New England Deaconess Hospital (NEDH) strain by exposing the pancreas to x-ray irradiation. The original tumor, about 1 cm in diameter, came from a male parabiont. Subsequently, the small fragments were transplanted and continuously grew in NEDH rats for 9 transplantations. After 7 transplantations, the period for the tumor growth to 0.5-1.5 cm was decreased from 5-8 months in the first transplantation to 2-5 months. The tumor in male rats is larger than in female rats (Chick et al., 1977).

The cells kept in inbred rats are called RIN-r, whereas, the cells maintained in athymic nude mice are called RIN-m. After RIN-m was transplanted in athymic nude mice for four series, five cell clones designated 1 to 5 were selected. Thus, the cell line, RINm5F which secretes high concentrations of insulin, is one of the clones of RIN-m (Gazdar et al., 1980).
The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. Usually, the cell population doubling time is 60-80 hours. The RINm5F cells are epithelioid and tumorigenic and have a hypodiploid chromosome number. The cells grow faster at later passages than at earlier passages. At earlier passages, RIN cells have neuron-like processes, but they change to round-shaped cells after long-term culture (Willingham, 1976).

Functional Characterizations

RIN cells secrete both insulin and somatostatin, but they have a relatively high capacity to secrete insulin when they are maintained in the RPMI-1640 medium (Gazdar et al., 1980; Bhathena et al., 1984). The amount of insulin secretion varies depending on the duration and culture medium. Furthermore, the variation in insulin production is related to the stability of mRNA which subsequently affects the synthesis of insulin (Muschel et al., 1986).

RINm5F cells contain 0.19 pg insulin/cell which represents approxmately 1% of the insulin content of normal β cells. When RINm5F cells are cultured with 2.8 mM glucose for 60 minutes, they releases 7-12% of their cellular content of insulin. By increasing glucose concentrations from 2.8 to 16.7 mM, RINm5F cells increase the utilization of glucose, but do not increase insulin release even when glucose concentration is increased to 33.4 mM (Praz et al., 1983). Thus, they have a relatively high basal insulin secretion but a modest, monophasic increase in insulin secretion in response to glucose (Leclercq-Meyer et al., 1988).

Although RIN cells secrete somatostatin, the rate of somatostatin secretion is stable and maintained at the range from 3.15 to 8.70 ng/million cells/24 hrs. The simultaneous secretion of somatostatin may influence insulin secretion during culture (Basabe et al., 1977).

Biochemical Characterizations

Insulin secretion from RINm5F cells is increased by Ca^{2+}, K^+, tolbutamide, theophylline, glucagon (Bhathena et al., 1984), leucine, and dibutyryl cAMP, but not by glucose (Sopwith et al., 1981). Insulin secretion is inhibited by oxidative phosphorylation inhibitors (cyanide and 2,4-dinitrophenol), Ca^{2+} flux blockers (EGTA, verapamil, and Mg^{2+}), calmodulin antagonist (trifluoperazine), microtubule inhibitors (vinblastine and colchicine) as well as epinephrine and somatostatin. Thus, the exocytotic mechanisms of insulin secretion in these insulinoma cells are similar to those of normal islet cells (Sopwith et al., 1981). The difference between RINm5F cells and normal islet β cells is that RINm5F cells do not response to glucose or arginine (Bhathena et al., 1984).

In normal pancreatic β cells, glucokinase which contributes about 75% of the total glucose phosphorylation capacity regulates the metabolic flux rate through the glycolytic chain and generates the signal for glucose-induced insulin release (Lenzen et al., 1987a). The activity of glucokinase is decreased in RINm5F cells; it appears to result from a loss of the ability of insulin to stimulate this enzyme. Thus, RINm5F cells show a single low-Km (hexokinase-like activity)

component of glucose utilization and lack the ability to stimulate insulin release in response to glucose (Lenzen et al., 1987b). However, in normal islet β cells, glucose stimulates insulin release with the high-Km (glucokinase-like activity) component of glucose utilization (Halban et al., 1983).

RINm5F cells also express a high level of amine precursor uptake and decarboxylation, (APUD) cell enzyme, L-dopa-decarboxylase and L-amino acid decarboxylase (Oie et al., 1983). Thus, they resemble the APUD cells series in biochemical, functional and morphological features (Pearse, 1980),

Kallikrein-Kinin System

Kallikrein

Kallikrein was first found in the extracts of porcine, bovine, and human pancreata. Thus, it was named kallikrein after an old greek synonym for that organ, kallikréas (Frey et al., 1950). Kallikreins are serine proteases which enzymatically release smooth muscle-contracting substances, kinins, from endogenous substrates, kininogens (Müller-Ester, 1989). Kallikreins are involved in the processing of prohormones into biological active hormones (Seidah et al., 1988; Bothwell et al., 1979), such as the formation of insulin from proinsulin (Ole-Moiyoi et al., 1979) and renin from prorenin (Sealey et al., 1978).

The kallikrein-kinin system can be divided into the plasma kallikrein-kinin system and the tissue kallikrein-kinin system (Fiedler, 1979). At the molecular level, tissue and plasma kallikreins are two completely different proteins. They differ in their molecular weights, substrate kininogens, types of kinin released, and physiological functions (Bhoola, 1992). Recently, another kininogenase, T-kininogenase (Bedi et al., 1983; Damas and Adam, 1985) which releases T-kinin from T-kininogen, was found to occur only in the rat (Okamato and Greenbaum, 1983).

Plasma Kallikrein

Chemistry and Synthesis

Plasma kallikrein molecule has 619 amino acids. After cleaving the single peptide bond between Arg^{371} and Ile^{372}, it forms a two-chain (heavy and light chain) protease which is held together by a disulfide bond with an enzyme catalytic site in the light chain (35 or 38 KDa)

(Bhoola et al, 1992) and a Hageman factor binding region in the heavy chain (Veloso et al., 1987).

All of the kallikreins are synthesized in a precursor form called prekallikrein. Plasma prekallikrein is synthesized in the liver from a single gene expression and circulates in the plasma (Seidah et al., 1989). It is a single-chain glycoprotein and exists in two forms with molecular weights of 85 and 88 Kda, respectively (Mandle and Kaplan, 1977). Plasma prekallikrein can be activated by the Hageman-factor or by negative charged surfaces like collagen and endotoxin (Bönner et al., 1986) and is involved in the blood clotting cascade (Bhoola et al., 1992).

Functions

Kallikreins liberate kinins from kininogens. In mammals, there are three types of kininogens: 1) a high molecular weight kininogen (HMK) found in the blood, 2) low molecular weight kininogen (LMK) found in the blood and in various tissues (Jacobsen, 1966), and 3) T-kininogen which was discovered recently and found only in rats (Okamoto and Greenbaum, 1983). T-kininogen is markedly increased as an acute phase protein in cases of trauma, surgery, and toxin exposure (Mindroiu et al., 1987). It inactivates cysteine proteases involved in inflammation (Potempa et al., 1988).

The plasma kallikrein releases BK from HMK by hydrolysis of the Lys-Arg and Arg-Ser bond to form a nonapeptide with arginine residues on both N- and C- terminals. LMK is not a good substrate for plasma kallikrein, but in the presence of neutrophil elastase which acts on LMK and forms a kinin-containing fragment (26 amino acid residues), plasma kallikrein can release BK from the fragment (Sato and Nagasawa, 1988).

Tissue Kallikrein

Chemistry and Synthesis

Tissue kallikreins have been purified and characterized from the pancreas of pigs (Kutzbach and Schmidt-Kastner, 1972), rodents, and dogs (Hojima et al., 1975). They also exist in the rat aorta (Oza et al., 1990), rat mesenteric and tail arteries (Nolly and Lama, 1982), and other tissues. They are acidic glycoproteins with pI values ranging from 3.5-4.4 and have a molecular weights ranging from 24 to 45 KDa (Fiedler, 1979). The catalytic reaction is similar to that of trypsin and the other serine proteases (Fiedler, 1979; Pisano, 1975). The half-life of tissue kallikrein is 16 minutes in normal animals (Ward and Mills, 1974).

Tissue (glandular) prekallikreins are synthesized by different genes in different tissues (Mason et al., 1983; Qin et al., 1991). They are similar but not identical enzymes in different organs of one species, such as in the rat. The tissue kallikrein multigene family contains 20 closely related genes and they are synthesized in different expression patterns (Shai et al., 1989). Recently, the mRNA of tissue kallikrein has been isolated in the pancreas (Swift et al., 1982), kidney (Inoue et al., 1989), and other tissues of the rat. Thus, tissue kallikrein is synthesized in the rat pancreas including acinar cells (Ørstavik, 1981; Ørstavik et al., 1976) and islet β cells (Pinkus et al., 1983),. The concentration of tissue kallikrein is influenced by pathological inflammation. For example, such as the concentration of tissue kallikrein in the blood was increased in acute pancreatitis with shock induced by taurocholic acid in piglets (Kortmann, 1984).

Functions

In most species, tissue kallikrein (except the rat and mouse enzyme) forms a decapeptide kallidin (Lys-BK) from LMK by cleavage of the Met-Lys bond at the N-terminus and Arg-Ser bond at the C-terminus (Iwanaga et al., 1977). However, tissue kallikrein is considered to release kinins from both HMK and LMK (Iwanaga et al., 1977; Girolami et al., 1986).

In rats, the formation of BK is catalyzed by tissue kallikrein, because in the rat HMK, at the N-terminal sequence of the BK, an Arg-Arg sequence replaces the Met-Lys-Arg sequence in other species (Kato et al., 1985). Therefore, both kallidin and BK are found in human and other species, whereas BK, but not kallidin is found in rats. However, the bioactivities of kallidin and BK are similar (Bhoola et al., 1992). The lys-BK is rapidly converted into BK through the removal of the N-terminal lysine by an unspecific aminopeptidase (Bhoola et al., 1992).

Kininogens

Chemistry

The molecular structures and functional domains of HMK and LMK have been determined by Müller-Esterl and coworkers in 1986 (Fig. 2). The kininogens are single-chain glycoproteins. They consist of a heavy chain including domains 1, 2, and 3 in the N-terminal, a light chain which contains domains 5 and 6 in the C-terminal, and the kinin moiety (domain 4) that is located between the two chains (Kellermann et al., 1987). HMK has 626 amino acids which forms 6 domains with molecular weights ranging from 88 to 120 KDa (Kato et al., 1976; Yamamoto,

1987), and LMK has 409 amino acids which only consists of 5 domains with the molecular weight ranging from 50 to 68 KDa (Kato et al, 1976). The heavy chains of all three kininogens have a similar basic structure (Bhoola et al., 1992; Higashiyama et al., 1986). In contrast, light chains distal to the kinin moiety have different lengths and different molecular weights, 45 to 58 KDa in HMK and 4 to 5 KDa in LMK, respectively (Kitamura et al., 1983; Takagaki et al., 1985).

(a) H-Kininogen   (b) L-Kininogen

Heavy Chains : D1,D2 & D3 = inhibitor domains for cysteine proteinases

                D4 = Kinin domain

H-kininogen   D5 = histidine rich domain
Light chain * D6 = binding site for plasma pre-kallikrein and clotting factor XI

L-kininogen
Light chain : D5 = unknown function
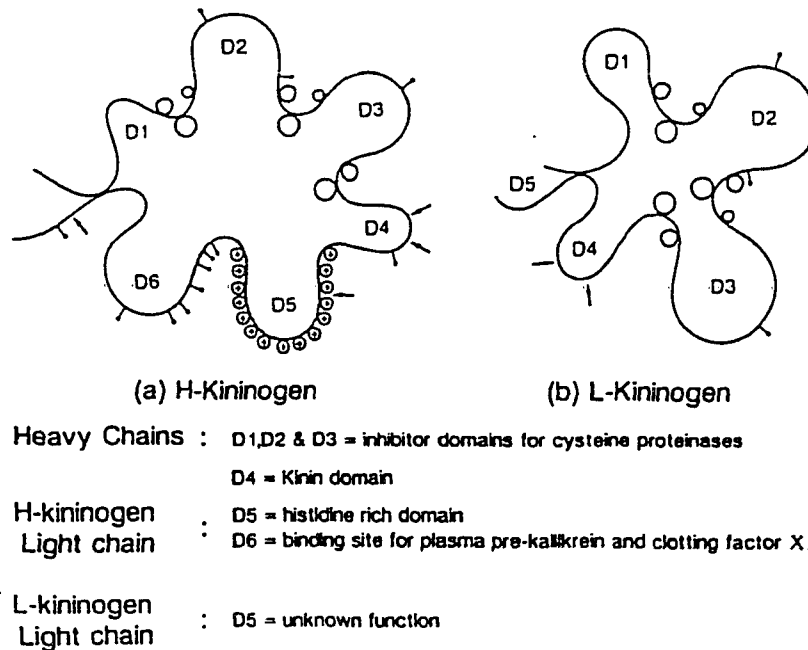
Fig. 2. Structure of human kininogens, indicating the domains of high molecular weight kininogen (a) and low molecular weight kininogen (b). o, potential disulphide loop; ↑, carbohydrate attachment site; ⊕, histidine-rich region; ↓, cleavage site for kallkrein. From Müller-Esterl et al. (1986)

Synthesis

Kininogens are primarily synthesized as prekininogens by hepatocytes, and subsequently modified through posttranslational glycosylation and stored prior to secretion into the circulation (Kitamura et al., 1983; Takagaki et al, 1985). However, T-kininogen is released immediately after

it is synthesized (Baussant et al., 1988; Bhoola et al., 1992). The synthesis of kininogens also occurs in the cortex and medulla of the kidney (Iwai et al., 1988) and endothelial cells (Schmaier et al.,1988), because the present and expression of kininogen mRNA have been demonstrated in these tissues. After synthesis, HMK is transported to the plasma membrane (Schmaier et al, 1983) and attaches there by the domains 3 and 5 of the heavy chain and light chains, respectively (Schmaier et al., 1987). The plasma concentration of total HMK is 55 µg/ml (Bhoola et al., 1992).

Functions

There are specific receptors for HMK on the plasma membrane of platelets (Gustafson et al., 1986), human endothelial cells (Schmaier et al., 1988), and neutrophils (Gustafson et al., 1989). HMK can bind to the surface receptors of human umbilical endothelial cells by its heavy and light chains, which provides the source of BK , inhibits cysteine proteinases (Higashiyama et al., 1986) and has procoagulant properties (Nishikawa et al., 1992; Reddigari et al., 1993).

The binding of HMK to the platelet membrane may provide a surface to regulate the contact phase activation of platelets (Gustafson et al., 1986), since HMK is the major inhibitor for the thrombin-induced activation of platelets (Puri et al., 1987) by interfering with the binding of fibrinogen to specific membrane receptors (C3R) of activated platelets and neutrophils (Gustafson et al., 1989).

The heavy chains of all three kininogens have a similar ability to inhibit SH-proteinases such as calpains and papain (Higashiyama et al., 1986). The domain 5 of the light chain in HMK which is rich in histidine, proline, and lysine can bind to the negative charge on the damaged surfaces of endothelial cells and enhance the blood clotting cascade (Kaplan, 1978). The binding of domain 5 to specific receptors through a Zn^{2+} bridge promotes the internalization of circulating kininogen into endothelial cells (Bhoola et al., 1992). Domain 6 possesses the binding capacity to plasma prekallikrein or clotting factor XI (Tait and Fijikawa,1987). Domain 6 also enhances the binding of plasma prekallikrein to the plasma membrane of neutrophils. The light chains of LMK and T-kininogen are formed by only one domain and their function is still unknown.

Kinins

Chemistry

The autacoid bradykinin (BK) is a potent bioactive peptide which is released from kininogens by the enzymatic action of kallikreins. The amino acid sequence of BK is H_2N-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH with arginine on both N- and C- terminals.

There are many endogenous BK analogues, such as Lys-BK, Met-Lys-BK, Gly-Arg-Met-Lys-BK. Met-Lys-BK is released by another two enzymes: pepsin and uropepsin (Hial et al., 1976). The relative potency of these BK analogues on smooth muscle (guinea pig ileum) is in the following ranking order: BK > Lys-BK > Met-Lys-BK > Gly-Arg-Met-Lys-BK, but the activity on vascular permeability and blood pressure is in inverse order (Reiss et al., 1971).

There are a large amount of kinins in tissues and up to 13 μg/ml of kinins can be produced with trypsin in human plasma (Rocha e Silva, 1960). The range of plasma BK concentrations is from undetectable to 3 ng/ml in normal animals (Talamo et al., 1969). The formation of BK is increased in endotoxic shock (Kimball et al., 1972), but decreased in alcoholic cirrhosis (Wong et al., 1972).

Another two mammalian kinins, Ile-Ser-BK (T-kinin) and Met-Ile-Ser-BK, are found only in the plasma and pouch fluid of rats (Okamoto and Greenbaum, 1983). T-kinin is released from T-kininogen by T-kininogenase (Bedi et al., 1983; Damas and Adam, 1985). T-kinin can be converted into BK by aminopeptidase M in the rat kidney (Vieira et al., 1994). The plasma concentration of T-kinin and T-kininogen are too low to be detected (Campell et al., 1993).

Breakdown

After kinins are produced, they are rapidly degraded within a few seconds by kininases which are abundant in body fluids. The half-lives of kinins are estimated to be less than 30 seconds in the blood, they thus act as local hormones or autacoids (McCarthy et al., 1965).

There are at least three peptidases called kininases: kininase I (KI), kininase II (KII), and endopeptidase. The kininase I family, including plasma kininase I-carboxypeptidase N (KI-CPN) (Erdös, 1979) and the cell membrane enzyme kininase I-carboxypeptidase M (KI-CPM) (Skidgel et al., 1984), acts on the C-terminus of kinin molecules by removing the terminal Arg^9 and produces a BK_1-receptor agonist, des[Arg^9]-BK or des[Arg^9]lys-BK (Bhoola et al., 1992). The released arginine may provide the substrate for the formation of nitric oxide by nitric oxide synthase within

the endothelial cells (Erdös, 1990; Moncada et al., 1988).

The kininase II family, including kininase II-angiotensin I-converting enzyme (KII-ACE) (Erdös, 1979) and kininase II-neutral endopeptidase (KII-NEP) (Almenoff and Orlowski, 1983), cleaves the Pro^7-Phe^8 bond and releases a dipeptide, Phe^8-Arg^9. Then KII-ACE liberates Ser^6-Pro^7 by cleaving the Phe^5-Ser^6 bond from the kinin molecules (Erdös, 1979). The other kininases include prolidase (aminopeptidase P) which cleaves the Arg^1-pro^2 bond of BK, and another two endopeptidases (kininases A and B).

Circulating BK is primarily degraded in the lung (Vane, 1969; Ferreira and Vane, 1967). The plasma concentrations of kinin are primarily controlled by KI-CPN, but in the pulmonary vascular bed the kinin concentrations are controlled by KII-ACE in rat plasma (Ishida et al., 1989).

Functions

Kinins are potent vasoactive peptides that influence a number of biological processes including the regulation of blood pressure (Nwator and Whalley; 1989; Pan et al., 1993), inflammation and pain (Carter et al., 1974; Whalley et al., 1987), contraction of smooth muscle (Collier et al., 1962; Bhoola et al., 1992), neurotransmission (Higashida, 1988; Liona et al., 1991), hypoglycemia (Wicklmayr and Dietze, 1977), cell proliferation (Goldstein et al., 1984; Marceau and Trembley, 1986), transportation of electrolytes (Brock et al., 1988; Sharp and Debnam, 1992) and other cellular functions. All of these studies suggest that kinins are involved in numerous normal and patho-physiological functions (Pisano and Austin, 1974).

Regulation of Blood Pressure

The vasodilatory effects of kinins have been increasingly studied. Kinins may be useful for treating clinic cardiac, cerebral, or renal ischemia by reducing or limiting the size of the infarct, preventing cardiovascular contraction, improving blood perfusion and the function of organs (Linz et al., 1986, 1990).

The coronary infusion of BK reduces reperfusion injury and prevents arrhythmias in isolated ischemic hearts (Linz et al., 1986;1990). This protective effect may be related to the reduction in the release of cardiac norepinephrine (Ribuot et al., 1994). In addition, intracoronary infusion of BK transiently inhibits myocardial contractions, and the effect in contractile performance can be reduced by an ATP-sensitive K^+ channel blocker, glibenclamide, suggesting

that it acts through the opening of K^+ channels (Fort and Lewis, 1993). In the kidney of diabetic patients and streptozotocin-induced diabetic rats, kinins mediate the renal vasodilation, increased glomerular filtration rate and renal blood flow (Jaffa et al., 1995).

The hypotensive response and increased local blood flow are mediated through the BK_1-receptors in the aorta of rabbits (Nwator and Whalley, 1989). However, the exercise pressor reflex is mediated through the BK_2-receptors located on the nerve endings of the muscle afferent nerves in cats (Pan et al., 1993). The effects of BK on vascular endothelial cells depend on the presence of endothelium-relaxing factors, nitric oxide (NO), and prostaglandin E_2 (Furchgott, 1981).

Inflammation and Pain

Kinins are potent pain-producing substances (Whalley et al., 1987) and there are many studies about the role of kinins in inflammation, tissue damage, antigen-antibody reactions, and anaphylactic and septic shock. Recently, tissue kallikrein was found in neutrophils (Figueroa and Bhoola, 1989) and the attachment of LMK, HMK, and plasma prekallikrein to the surface of the neutrophil plasma membrane provides evidence that kinins are produced and are involved in the inflammatory processes (Figueroa,1990). Kinins are the primary components involved in inflammation. Kinins produce pain by stimulating nociceptive sensory C-fiber terminal (Steranka et al., 1988). Kinins promote the release of substance P, followed by an increase of vascular permeability, leakage of serum proteins and fluid from the capillaries, and local vasodilation. Tissue edema and flare are then observed (Reis et al., 1971; Carter et al., 1974). Subsequently, monocytes release cytokines which attract leukocytes to the injection site (Bhoola et al., 1992) and inflammatory processes are initiated.

Kinins are produced in airway secretions in some pulmonary diseases and inflammation, such as allergic or viral rhinitis, and asthma. The release of kinins activates the plasma and tissue kallikrein systems, increases vascular permeability, and stimulates sensory nerves to produce symptoms of nasal obstruction, rhinorrhea, and airway irritation (Proud, 1994).

Contraction of Smooth Muscle

In most species, kinins initiate a biphasic response on isolated airway smooth muscle. Kinins relax the airway smooth muscle at low concentrations (Bhoola et al., 1989), but produce contractions at higher concentrations (Collier et al., 1962). The mechanisms of BK-induced bronchocontraction may involve prostanoids, thromboxanes, substance P, and neuropeptides, the

stimulation of bronchial C-fiber, and direct stimulation (Bhoola et al., 1992).

Kinins also initiate the contraction of intestine and uterus via BK_2-receptors (Figueiredo et al., 1990; Bhoola et al., 1992). The contraction of the small intestine is partly mediated through the direct action of BK and partly mediated by the stimulatory effect of acetylcholine released from the efferent cholinergic nerves (Palov and Ovsiannikov, 1988). However, BK relaxes rat duodenum which is mediated by BK_2-receptors (Bhoola, 1961).

Neurotransmission

The cellular actions of kinins are modified by their ability to stimulate the release of many second signal mediators, eg., platelet activating factors and prostaglandins (Cahill et al., 1988), endothelium derived relaxing factor (EDRF) (Liao and Homcy, 1992), leukotrienes, substance P (White and Zimmermann, 1988), acetylcholine (Higashida, 1988), catecholamines (Liona et al., 1991), and cytokines (interleukin-I,IL-I; Tumor necrosis factor,TNF) (Tiffany and Burch, 1989).

Kinins also stimulate the secretion of other hormones, eg., renin from the kidney (Beierwaltes et al., 1985), and vasopressin from the neurohypophysis (Baertschi et al., 1981). BK stimulates the release of interleukin-1 which enhances the receptor-mediated release of prostaglandin E_2 by more than 10 times through the activation of phospholipase A_2 and cyclooxygenase (Burch et al., 1988).

Modulation of Glucose Metabolism

Kinins decrease the blood glucose concentration in type II diabetic humans and dogs (Bhoola et al., 1992; Wicklmayr and Dietze, 1977). The infusion of BK increases the blood glucose uptake of the skeletal muscle by 2-fold without increasing the formation of lactate in patients with postoperative stress syndrome (Jauch et al., 1986, 1988).

BK also stimulates the absorption and metabolism of glucose in rat jejunum (Kellett and Barker, 1989). The BK-induced increase in absorption of glucose is mediated by Na^+-dependent glucose uptake at the brush-border and carrier-mediated transport across the basolateral membrane (Sharp and Debnam, 1992).

Other Functions

BK increases the transport of electrolytes by stimulating the Na^+-K^+-Cl^- cotransporter in cultured endothelial cells (Brock et al., 1988). BK promotes protein synthesis and cell division

(Goldstein and Wall, 1984; Marceau and Tremblay, 1986). In addition, kinins stimulate the proliferation and enhance the metastases of tumor cells by increasing vascular permeability (Roberts, 1989; Bhoola et al., 1992).

Receptor Classification

BK receptors are classified according to the relative potency of kinin agonists on isolated smooth muscle preparations (Bhoola et al., 1992) (Table 1). So far, at least two types of kinin receptors, BK_1- and BK_2-receptors, have been characterized (Regoli et al., 1990). These receptors are encoded by distinct mRNAs (Webb et al., 1994).

BK_1-Receptors

Among receptor agonists, desArg^9-BK has a great affinity for BK_1-receptors (Regoli et al., 1977), while BK has low affinity for BK_1-receptors (Bhoola et al., 1992).

BK_1-receptor protein contains 353 amino acid residues and couples to a G-protein for its function. There is 36% homology in amino acid sequence between BK_1- and BK_2-receptors. The cloned BK_1-receptors have high affinity for desArg^{10}-kallidin, but not for BK. The binding of desArg^{10}-kallidin is antagonized by desArg^{10},Leu^9-kallidin, a BK_1-receptor antagonist. Therefore, the expressed receptors have the pharmacological characterics of BK_1-receptors (Menke et al., 1994).

Under normal condition, BK_1-receptors seem to be absent, but are expressed in smooth muscle cells, fibroblasts, and pain fibers in some pathological states, e.g., inflammation and tissue damage (Regoli et al., 1981; Perkins and Kelly, 1993), and are expressed in isolated rabbit vascular smooth muscle in a time-dependent manner (Barabe et al., 1982; deBlois et al., 1991). DesArg^9-BK, a BK_1-receptor agonist, relaxes the aorta smooth muscle of rabbits, an action that is mediated by prostaglandins, but it also contracts the rabbit aorta by direct effect on BK_1-receptors (Churchill and Ward, 1986; 1987). The functions of BK_1-receptors are modulated by interleukins (Regoli et al., 1993). The activation of BK_1-receptors increase the release of IL-1 and TNF (tumor necrosis factor) from macrophage cells (Tiffany and Burch, 1989). IL-I is the endogenous trigger for the induction of BK_1-receptors and the mediator for increasing the effect of desArg^9 BK in inflammation (deBlois et al., 1991).

BK$_2$-Receptors

BK$_2$-receptors have high affinity for BK and kallidin, but do not respond to desArg^9-BK or other related peptides (Bhoola et al., 1992). Most of the actions of kinins are mediated through the BK$_2$-receptors which are responsible for arterial vasodilation, plasma extravasation, pain, and the release of prostaglandins, EDRF, and catecholamines (Regoli et al., 1993). The muscle relaxation in the dog carotid artery (Regoli et al., 1986), rabbit mesentery artery and human basilar artery (Nwator and Whalley, 1989; Whalley et al., 1987) and the venoconstriction in rabbit jugular vein are all mediated by BK$_2$-receptors.

BK$_2$-receptor has 366 amino acid residues with a molecular weight of 41 KDa, three N-linked glycosylation sites, and a Ser318 as a protein kinase A phosphorylation site. The receptor protein has high homology with seven-transmembrane domain G protein-coupled family (McEachern et al., 1991). Human BK$_2$-receptor cDNA has 84% homology with mouse BK$_2$-receptor genomic DNA. Both genes have been expressed in a mammalian cell line, COS-7 cells, which lack endogenous BK receptors. The expressed receptor protein has the same properties of BK$_2$-receptor, including high binding affinity with BK (K_D = 0.13 nM), seven-transmembrane domains and coupled to G-protein (Hess et al., 1994). But the gene cloned from human genomic library encodes a receptor protein with 364 amino acid residues forming seven-transmembrane domains, shares high identity with rat and mouse BK$_2$-receptors, and is found in most human tissues (Ma et al., 1994).

Other Receptors

Because the BK-induced contraction of guinea pig tracheal and lung parenchymal strips can not be totally abolished by BK$_1$-receptor blocker, desArg^{9}Leu^8-BK or BK$_2$-receptor blocker, D-ArgHyp^3,D-Phe^7-BK (NPC 567). Thus another BK-receptor (BK$_3$) may exist in pulmonary tissues, particularly in the large airways (Farmer et al., 1989). However, BK$_3$-receptors have not been molecularly characterized.

The subtypes of BK$_2$-receptors may also exist in intestinal epithelium cells, fibroblasts, and primary brain cultures (Lewis et al., 1985; Braas et al., 1988). Two subtypes of BK$_2$-receptors have been characterized by pharmacological studies from smooth muscle and neuron, respectively. The smooth muscle subtype has lower affinity for BK, but has higher affinity for the BK analogue, Thi^{5,8},D-Phe^7-BK which is an antagonist for the smooth muscle subtype and is an agonist for the neuronal subtype (Hess et al., 1992).

Table 1. Kinin receptors in isolated preparations and culture cells (Bhoola et al., 1992)

	Receptor type	Effect
Isolated tissues		
Rat		
Duodenum	BK2	Relaxation
Vas deferens	BK2	Contraction
Uterus	BK2	Contraction
Rabbit		
Aorta	BK1	Contraction
Jugular vein	BK2	Contraction
Guinea pig		
Trachea		
+ epithelium	BK2	Relaxation
− epithelium	BK2	Contraction
Ileum	BK2	Contraction
Dog		
Renal artery	BK2	Relaxation
Carotid artery	BK2	Relaxation
Bladder	BK2	Contraction
Cell type		
Human fibroblasts	BK2	Ca^{2+} increase, cell division
Murine neuroblastoma (N1E-115)	BK2	cGMP increase, phospholipase A_2 activation
Murine neuroblastoma × glioma (NG108-15)	BK2	Phosphotidylinositol turnover, Ca^{2+} and cGMP increase
Bovine aortic endothelial cell	BK1/BK2	cGMP increase
Bovine pulmonary artery endothelial cell	BK2	EDRF release and Ca^{2+} increase
Murine macrophages (P388-D1 RAW 264.7)	BK1	IL-1, TNF release
Murine 3T3 fibroblasts	BK2	PGE_2 synthesis
Madin-Darby canine kidney cells	BK2	Phospholipase A_2 activation, arachidonic acid release

Antagonists

The design and synthesis of new specific antagonists have been obtained from structure-activity analysis by Sterwart and Vavrek. Thus, by replacing the Proline[7] residue of BK with a D-phenylalanine, the agonistic activity of BK is converted into antagonistic activity on the guinea pig ileum (Vavrek and Stewart, 1985). In addition, BK and its antagonists can be converted into BK_1-receptor agonist or antagonists respectively, by carboxypeptidase (Regoli et al., 1986).

The potent action of desArg[9]-BK on the isolated rabbit aorta is antagonized by desArg[9]Leu[8]-BK (Regoli et al., 1986). Thus, desArg[9],Leu[8]-BK is a BK_1-receptor blocker. The antagonist, NPC 567 (D-ArgHyp[3],Thi[5,8],Phe[7]-BK) is a potent BK_2-receptor blocker. The replacement of D-phenylalanine for proline increases resistance to peptidase, and the inclusion of β-2-thienylalanine enhances potency (Bhoola et al., 1992).

In addition, another antagonist, HOE 140 (D-Arg[Hyp^3,Thr^5,D-Tic^7, Oic^8]-BK) is a potent specific BK$_2$-receptor blocker which has high binding affinity for BK$_2$-receptors on guinea pig ileum membranes and has a prolonged half-life (Hock et al., 1991; Wirth et al., 1991; Brenner et al., 1993). The potency of HOE 140 is 40 times greater than that of NPC 567.

HOE 140 inhibits BK-induced hypotension in the rat, bronchoconstriction in the guinea pig, and the carrageenin-induced inflammation in the rat paw (Wirth et al., 1991). HOE 140 also inhibits BK-induced contractions in the guinea pig ileum and pulmonary artery, and in the rat uterus. However, the contraction induced by desArg^9-BK in the rabbit aorta is not inhibited by HOE 140 (Hock et al., 1991; Wirth et al., 1991). Therefore, HOE 140 is considered as a selective BK$_2$-receptor antagonist.

The hypothesis that the C-terminal β turn structure of BK is required for the binding with BK receptors, has led to the design of new agonists and antagonists with more specific and higher binding affinity (Kyle et al., 1994).

Mechanisms of Action

Kinins may promote one or more of the following cellular signal transduction pathways: a) activation of phospholipase C (PLC) which results in the formation of inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG) (van Blitterswijk et al., 1991), and Ca^{2+} mobilization through elevated IP$_3$ concentration (Takeuchi et al., 1988; Simeone et al., 1995); b) an increase in chloride transport in rat colonic epithelia (Cuthbert and Margolius, 1982); c) formation of EDRF, nitric oxide (NO) (Liao and Homcy, 1993); d) activation of phospholipase A$_2$ (PLA$_2$) which forms arachidonic acid, prostaglandins, and leukotrienes (Burch and Axelrod, 1987); e) stimulation of adenylyl cyclase which leads to an increase in intracellular cAMP concentration (Gadd and Bhoola, 1988); and f) other pathways.

The linkage of BK receptors to a second messenger system is through a family of G proteins. Different cellular responses produced by BK may occur because of different binding affinity to the receptors and different signal transduction pathways or species difference (Hess et al., 1994). In comparing human and mouse BK$_2$-receptors, both receptors have similar binding affinity for agonists, but they differ in their binding affinity for antagonists. Thus, species difference can produce variation in pharmacological responses (Hess et al., 1994).

Phospholipase C (PLC) and Ca^{2+} Mobilization

BK increases $[Ca^{2+}]_i$ in several tissues. In vascular smooth muscle, the BK-induced increase of $[Ca^{2+}]_i$ is mediated by the receptor-coupled activation of PLC which hydrolyzes inositol biphosphate, releases IP_3 and DAG. The elevated IP_3 then mobilizes $[Ca^{2+}]_i$ from the intracellular Ca^{2+} stores of endoplasmic reticulum (Takeuchi et al., 1988; Simeone et al., 1995). In bovine pulmonary endothelial cells, both BK_1 agonist (desArg^9-BK) and BK_2 agonist (BK) evoke similar responses (Smith et al., 1995). The effect of PLC can be antagonized by an aminosteroid U-73122 (Simeone et al., 1995) which abolishes BK-induced $[Ca^{2+}]_i$ release, but a structural analogue, U-73343, does not (Jin et al., 1994).

In Ca^{2+} free medium, BK induces only a small transient Ca^{2+} peak which is primarily due to a release from endoplasmic reticulum (Fasolato et al., 1988) and thus is called release-phase. After Ca^{2+} is re-added in the same test sample with the presence of BK, it induces another sustained increase of $[Ca^{2+}]_i$ which results from the influx of extracellular Ca^{2+} (Fasolato et al., 1988) and thus is called influx-phase or second-phase. Furthermore, in Ca^{2+} containing medium, BK induces a biphasic increase of $[Ca^{2+}]_i$, an initial, large transient Ca^{2+} spike followed by a lower sustained Ca^{2+} increase (Buchan and Martin, 1991). The Ca^{2+} influx occurs only after the initial release of Ca^{2+} from internal stores (Yang et al., 1994). Therefore, the Ca^{2+} release from intracellular stores causes the first transient peak, which is followed by Ca^{2+} influx from the extracellular medium (Buchan and Martin, 1991). However, part of the elevated $[Ca^{2+}]_i$ may be due to a release from an IP_3-insensitive Ca^{2+} pool caused by Ca^{2+}-induced Ca^{2+} release (Islam et al., 1992).

In NG108-15 cells, BK (30 nM) completely depletes the IP_3-sensitive $[Ca^{2+}]_i$ store within 1 min. The refilling of the IP_3-sensitive $[Ca^{2+}]_i$ stores requires the presence of extracellular Ca^{2+} and is blocked by La^{3+} (Lo and Thayer, 1993). Also, the Ca^{2+}-Mg^{2+}-ATPase inhibitor, thapsigargin, irreversibly depletes the $[Ca^{2+}]_i$ stores and prevents their refilling. Thus, the IP_3-sensitive $[Ca^{2+}]_i$ store is refilled by cytoplasmic Ca^{2+} through a thapsigargin-sensitive Ca^{2+}-Mg^{2+}-ATPase and the cytoplasmic Ca^{2+} is refilled by extracellular Ca^{2+} through activated plasmalemmal Ca^{2+} channels (Lo and Thayer, 1993). In eukaryotic cells, the Ca^{2+} influx occurs through at least three pathways: a) voltage-dependent Ca^{2+} channels (VDCCs) controlled by the change of the plasma membrane potential, b) receptor-operated Ca^{2+} channels (ROCs), where the channels are a part of the receptors and are activated by the agonist binding to receptors, and c) second messenger-operated Ca^{2+} channels (SMOCs) activated by the triggered second messengers (Meldolesi and Pozzan,

1987; Berridge and Irvine, 1989). The activation of VDCCs by membrane depolarization initiates a number of cellular responses including muscle contraction, and exocytotic secretion in endocrine and nerves cells.

There are many Ca^{2+} channel blockers. Nimodipine is a L-type VDCC blocker (Baumgarten and Villereal, 1992; ZhuGe et al., 1995) which blocks the BK-induced Ca^{2+} influx in cultured human fibroblasts. SK&F 96365(1-(beta-[3-(4 methoxyphenyl) propoxyl]-4-methoxy-phenethyl)-1H-imidazole) is a ROC blocker (Cabello and Schilling, 1993). In vascular endothelial cells, SK&F 96365 blocks the BK-induced Ca^{2+} influx without affecting the Ca^{2+} release from internal stores (Cabello and Schilling, 1993).

In NG 108-15 cells and N1E-115 neuroblastoma cells, BK induces a transient hyperpolarization which is followed by a prolonged depolarization which may be attributed to the dual effects of IP_3 and DAG formed from the hydrolysis of phosphatidylinositol (Higashida and Brown, 1987). In these cells, the elevated IP_3 releases Ca^{2+} from intracellular stores which activates the Ca^{2+}-dependent K^+ channels and hyperpolarizes the plasma membrane. However, DAG activates protein kinase C (PKC), inhibiting voltage-dependent K^+ channels, and depolarizing the plasma membrane (Higashida and brown, 1986). BK initiates a biphasic increase in intracellular DAG concentration in human fibroblasts (Etscheid et al., 1991). It induces a transient DAG peak by the rapid hydrolysis of phosphoinositdes, which correlates with the accumulation of IP_3 and elevation of $[Ca^{2+}]_i$, followed by a decline to near basal levels, then a second rise to a plateau phase. The second plateau phase of DAG comes from the breakdown of other phospholipids including phosphatidylcholine. The increase of DAG also correlates well with the activation of PKC which phosphorylates the substrate, 80 KDa fibroblast acidic protein (Etscheid et al., 1991). However, the role of PKC is still not well-understood.

Chloride Transport

Kinins increase intestinal smooth muscle contraction and mucosal electrolyte secretion (Gaginella and Kachur, 1989). BK_2-receptors mediate the BK-induced Cl^- secretion in rats and rabbit colonic epithelial cells (Cuthbert and Margolius, 1982), or rabbit and guinea pig ileum (Gaginella and Kachur, 1989). Therefore, the activation of BK_2-receptors may be involved in contraction and secretory processes of the ileum (Gaginella and Kachur, 1989).

Nitric Oxide (NO)

Kinins stimulate the formation of NO in vascular endothelial cells (Liao and Homcy, 1993; Blatter et al., 1995), tracheobronchial epithelial cells, neurons, neutrophils, and macrophages (Salvemini et al., 1989). NO is produced from L-arginine by Ca^{2+}/calmodulin-dependent NO synthase (NOS) (Blatter et al., 1995). The liberated NO plays a role as a signaling molecule which is involved in neuronal cell function, such as the release of neurotransmitter, gene transcription, and neurotoxicity (Clementi et al., 1995).

Both $G\alpha_i$ and $G\alpha_q$ are coupled to BK receptors in bovine aortic endothelial cells but the BK-induced NO release is mediated by $G\alpha_q$ which is insensitive to pertussis toxin (PTX) (Liao and Homcy, 1993). In cultured bovine aortic endothelial cells, BK produces a transient increase in $[Ca^{2+}]_i$ followed by a transient increase in intracellular NO (Blatter et al., 1995). NO may: a) stimulate guanylyl cyclase and transiently increase cGMP level; b) recruit Ca^{2+}, and c) enhance the turnover of phosphatidyl-inositol (Bhoola et al., 1992).

Phospholipase A$_2$ (PLA$_2$)

BK stimulates the synthesis of prostaglandin E_2 (PGE_2) in murine 3T3 fibroblasts. The synthesis of PGE_2 is mediated through the activation of BK-receptors which are coupled to a PTX-insensitive G-protein. Then G-protein activates both PLC and PLA_2, and the activated PLA_2 releases arachidonate for the synthesis of PGE_2 (Burch and Axelrod, 1987).

At 37°C, the formation of lysophosphatidyl-inositol (lyso-PI) is as early as the formation of IP_3. Thus, PLC and PLA_2 are activated at the same time. But at 30°C, lyso-PI is formed before the increase of IP_3, thus the activation of PLA_2 does not require the rise of IP_3. Also the formation of unesterified arachidonic acid occurs earlier than that of DAG. Therefore, the BK-stimulated activation of PLA_2 is independent of the activation of PLC (Kaya et al., 1989). However, DAG increases the activity of PLA_2 and enhances the synthesis of PGE_2 (Burch, 1988) and the BK-induced amplification of PLA_2 is mediated by DAG independently of PKC (Burch et al., 1988). The BK-induced PGE_2 synthesis is potentiated more than 10-fold by interleukin-1 (Burch et al., 1988).

Adenylyl Cyclase

In lung membrane of guinea pigs, BK evokes a dose-dependent increase in cAMP by the stimulation of adenylyl cyclase (Gadd and Bhoola, 1988). BK-induced increase of cAMP requires

the presence of extracellular Ca^{2+} in cultured rat sympathetic neurons. Therefore, BK activates the metabolism of phosphoinositide and elevates cAMP concentration (Suidan et al., 1991). In cultured arterial smooth muscle cells, chronic exposure to cAMP, cholera toxin, forskolin the adenyl cyclase activator or the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) increases the number of cell surface BK receptors and the hydrolysis of phosphatidylinositol. Thus, cAMP enhances the synthesis of new BK receptors (Dixon, 1994). In D384-human astrocytoma cells, BK inhibits the formation of cAMP and the effect is mediated by a Ca^{2+} dependent inhibition of adenylyl cyclase (Altiok and Fredholm, 1993).

Other Mechanisms

In vascular endothelial cells, BK induces the tyrosine phosphorylation of two low molecular weight proteins which are 42- and 44-KDa isoforms of mitogen-activated protein kinase (MAP kinase) (Fleming et al., 1995; Demolle et al., 1988).). In Swiss 3T3 cells, BK induces the tyrosine phosphorylation of focal adhesion-associated protein such as p125FAK and paxillin (Leeb-Lundberg et al., 1994). Also, the phosphorylation of serine or threonine in the cytoplasmic segment of BK receptors may relate to the desensitization of BK (Roberts and Gullick, 1989).

Interactions of Kallikrein-Kinin with Islet Hormones

The whole pancreas is an integrated organ, in which the exocrine and endocrine tissues are closely interrelated for optimal functional activities. Although acinar cells and islet cells secrete different compounds into different locations, there is documented interaction between these two types of cells. The dispersion of the small islets throughout the exocrine tissue may provide a considerable contact between exocrine and endocrine pancreas for promoting interaction.

As mention previously, islet cells secrete insulin, glucagon, somatostatin and other hormones (Hazelwood, 1989) to control glucose homeostasis while pancreatic acinar cells secrete more than 20 different enzymes including kallikrein for processing precursor proteins (Seidah et al., 1988). Tissue kallikrein exists in pancreatic islets and acini with the majority of the enzyme being in the acini (Pinkus et al., 1983). In fact, the pancreas is one of the richest sources of tissue kallikrein (Bhoola, 1992) and the substrate, kininogens, are abundant in the pancreas (Barbe et al., 1988). In addition, circulatory HWK binds to vascular endothelium and plasma kallikrein can release BK from the endothelial-bound HWK (Nishikawa et al., 1992).

However, the physiological function of kallikreins and kinins in the pancreas is still not well-understood, except that tissue kallikrein may be involved in the formation of insulin from proinsulin (Ole-Moiyoi et al., 1979), and BK may aggravate acute pancreatitis induced by cerulein in rabbits (Yotsumoto et al., 1993). In diabetic patients, the tissue kallikrein-kinin system is suppressed, but the plasma kallikrein-kinin system is activated depending on the degree of the impairment of glucose metabolism (Uehara et al., 1988).

Influences of Islet Hormones on Glucose Homeostasis

Glucose is the major energy source for cellular metabolism. The concentration of glucose in the blood is controlled by the actions of insulin and glucagon, and the secretion of these two hormones is regulated by the presence of somatostatin. Insulin increases glucose transport in muscle and adipocytes, and glycogen synthesis in liver and muscle. In addition, it also stimulates the expression of a variety of intracellular enzymes and the transport systems for glucose (White and Kahn, 1994).

Glucagon is responsible for the breaking down of glycogen to release glucose from liver and adipose tissue by activating cAMP and initiating phosphoryation of glycogen. In addition, glucagon regulates glucose homeostasis by stimulating the release of insulin and somatostatin, and insulin inhibits glucagon secretion (Samols and Harrison, 1976).

Pancreatic islets synthesize and release both SS-14 and SS-28 which lower the levels of cAMP and inhibit arginine-induced release of insulin and glucagon, and regulate the ratios of insulin/glucagon (Hazelwood, 1989).

Influences of Kallikrein-Kinin on Glucose Homeostasis

A hypoglycemic effect caused by kallikrein was first reported by Frey in 1932. Administration of kallikrein into human patients with non-insulin-dependent diabetes mellitus (NIDDM; type II diabetes) (Wicklmayr and Dietze, 1977) or into pancreatectomized dogs (Bhoola et al., 1992) results in a decrease in the plasma glucose concentration. In addition, BK reduces the blood glucose concentrations in postoperative patients by increasing whole body glucose uptake (Jauch et al., 1986).

Kinins also improve blood reperfusion, oxygenation, and the transport of glucose in isolated ischemic hearts (Needleman et al., 1975) and in isolated perfused hearts (Rosen et al., 1983). In alloxan-induced diabetic rats, captopril, an inhibitor of angiotensin-converting enzyme, protects the breakdown of kinins and potentiates the BK-induced glucose-lowering activity and markedly decreases the blood glucose concentration (Jaffa et al., 1986).

In isolated rat adipocytes, BK increases hexose transport under the presence of insulin in a dose-dependent manner. In obese Zucker rats, captopril also enhances insulin-induced glucose transport in skeletal muscle by improving the disposal of glucose. The infusion of BK improves insulin activity in patients with postoperative stress syndrome, in which insulin resistance is due to a postoperative receptor defect in skeletal muscle (Dietze, 1982). The modulatory action of BK on insulin may occur at post-insulin binding sites (Goldman et al., 1987).

BK reduces the production of endogenous glucose, and is accompanied by a significant reduction of glucose, lactate, pyruvate, and alanine in arteries of surgical stressed patients (Jauch et al., 1988). The infusion of BK does not change the glucagon-induced rise in hepatic glucose and glucose cycling, but in the later stage, BK decreases the production of glucose induced by glucagon (Hartl et al., 1989). These results suggest that BK regulates the availability and utilization of glucose in the target tissues by enhancing the uptake of glucose, improving insulin-induced glucose transport, decreasing the glucagon-induced rise of glucose or reducing gluconeogenesis (Jauch et al., 1989).

The infusion of BK doubles the muscle glucose uptake without a change in the formation of lactate (Jauch et al., 1989) and BK fails to stimulate glucose metabolism in the isolated soleus muscle of rat (Shimojo et al.,1987). In addition, BK increases glucose oxidation only in the presence of insulin in rat adipocytes (Symington et al., 1988). Therefore, it seems that kinins do not have direct actions to increase glucose uptake and utilization.

References

Almenoff, J., and M. Orlowski. Membrane-bound kidney neutral endopeptidase: interaction with synthetic substrates, natural peptides and inhibitors. Biochemistry 22: 590- 599, 1983.

Altiok, N., and B. B. Fredholm. Bradykinin inhibits cyclic AMP accumulation in D384-human astrocytoma cells via a calcium-dependent inhibition of adenyl cyclase. Cell Signal 5: 279-288, 1993.

Ammon, H. P. T., C. Reber, and E. J. Verpohl. Indirect evidence for short-loop negative feedback of insulin secretion in the rat. J. Endocrinol. 128: 27-34, 1991..

Baertsci, A.J., H. H. Zingg, and J. J. Dreifuss. Enkephalins, substance P, bradykinin, and angiotensin II: differential sites of action on the hypothalmo-neurohypophysial system. Brain Res. 220: 107-119, 1981.

Barabe, J., C. Babiuk, and D. Regoli. Binding of [³H]des-Arg⁹-BK to rabbit anterior mesenteric vein. Can. J. Physiol. Pharmacol. 60: 1551-1555, 1982.

Barabe, J., D. Huberdean, and A. Bernoussi. Influences of sodium balance on urinary excretion of immunoreactive kinin in rats. Am. J. Physiol. 254: F484-F491, 1988.

Basabe, J. C., J. C. Cresto, and N. Aparicio. Studies on the mode of action of somatostatin on insulin secretion. Endocrinology 101; 1436-1443, 1977.

Baumgarten, L., and M. Villereal. Bradykinin stimulates Ca^2 entry via nitrendipine-sensitive Ca^{2+} channels in cultured human fibroblasts. Agents Actions Suppl. 38: 1-8, 1992.

Baussant, T., A. Michaud, J. Bouhnik, F. Savoie, F. Alhenc-Gelas, and P. Corvol. Effect of dexamethasone on kininogen production by a rat hepatoma cell line. Biochem. Biophys. Res. Commun. 154: 1160-1166, 1988.

Beierwaltes, W. H., L. Prado, and O. A. Carretero. Kinin stimulation of renin release in isolated rat glomeruli. Am. J. Physiol. 248: F757-F761, 1985.

Bedi, G. S., J. Balwierczak, and N. Back. Rodent kinin-forming enzyme systems. II. Purification and characterization of an acid protease from Murphy-Storm lymphosarcoma. Biochem. Pharmacol. 32: 2071-2077, 1983.

Bendayan, M. Anatomic basis of islet-acinar interaction in the pancreas: A quarterly clinical review. Regul. pept. lett. 11: 1-7, 1990.

Bendayan, M. Pathway of insulin in pancreatic tissue on its release by the B-cell. Am. J. Physiol. 64: G187-194, 1993.

Berridge, M. J. and R. F. Irvine. Inositol phosphate and cell signalling. Nature 341: 197-205, 1989.

Bhathena, S. J., S. Awoke, N. R. Voyles, S. D. Wilkins, L. Recant, H. K. Oie, and A. F. Gazdar. Insulin, glucagon, and somatostatin secretion by cultured rat islet cell tumor and its clones. Proc. Natl. Acad. Sci. USA. 175: 35-38, 1984.

Bhoola, K. D. Properties of kallikrein, kallidin and related substances, Ph. D. thesis,University of London, London, England, 1961.

Bhoola, K. D., J. Bewley, D. M. Crothers, M. I. Cingi, and C. D. Figueroa. Kinin receptors on epithelial cells and smooth muscle of the trachea. Adv. Exp. Med. Biol. 247A: 421-427, 1989.

Bhoola, K. D., C. D. Figueroa, and K. Worthy. Bioregulation of kinin: kallikrein, kininogen, and kininases. Pharmacol. Rev. 44: 1-80, 1992.

Blatter, L. A., Z. Taha, S. Mesaros, P. S. Shacklock, W. G. Wier, and T. Malinski. Simultaneous measurements of Ca^{2+} and nitric oxide in bradykinin-stimulated vascular endothelial cells. Circ. Res. 76: 922-924, 1995.

Bönner, G., J. Horn, U. Buchsler, and C. Kapp. Measurement of plasma prekallikrein independent of inhibitors and interfering enzymes. Adv. Exp. Med. Biol. 198: 87-98, 1986.

Bothwell, M. A., W. H. Wilson, and E. M. Sdshooter. The relationship between glandular kallikrein and growth factor-processing proteases of mice submaxillary gland. J. Biol. Chem. 254: 7287-7294, 1979.

Braas, K. M., D. C. Manning, D. C. Perry, and S. H. Snyder. Bradykinin analogues: differential agonist and antagonist activities suggesting multiple receptors. Br. J. Pharmacol. 94: 3-5, 1988.

Brenner, N. J., G. Y. Stonesifer, and K. A. Schneck. <125I>PIP HOE140, a high affinity radioligand for bradykinin B2 receptors. Life Sci. 53: 1879-86, 1993.

Brock, T. A., C. Brugnara, and M. Canessa. Bradykinin and vasopressin stimulate Na^+-K^+-Cl^- cotransport in cultured endothelial cells. Am. J. Physiol. 250: C888-895, 1986.

Buchan, K. W., and W. Martin. Bradykinin induces elevations of cytosolic calcium through mobilisation of intracellular and extracellular pools in bovine aortic endothelial cells. Br. J. Pharmacol. 102: 35-40, 1991.

Bunzli, H. F., B. Glatthaar, P. Kunz, E. Mulhaupt, and R. E. Humbel. Amino acid sequence of the two insulins from mouse (Maus musculus). Hoppe Seyers Z. Physiol. Chem. 353: 451-458, 1972.

Burch, R.M., and J. Axelrod. Dissociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 fibroblasts. Evidence for a G protein regulation of phospholipase A_2. Proc. Natl. Acad. Sci. USA 84 :6347-6378, 1987.

Burch, R. M. Diacylglycerol stimulates phospholipase A_2 from Swiss 3T3 fibroblast. FEBS lett. 234: 283-286, 1988.

Burch, R. M., Ma, A. L., and Axelrod, J. Phorbol esters and diacylglycerols amplify bradykinin-stimulated prostaglandin synthesis in Swiss 3T3 fibroblasts. possible independence from protein kinase C. J. Biol. Chem. 263: 4764-4767, 1988.

Burch, R. M., J. R. Connor, and J. Axelrod. Interleukin-1 amplifies receptor-mediated activation of phospholipase A_2 in 3T3 fibroblasts. Proc. Natl. Acad. Sci. USA 85:6306-6309, 1988.

Cabello, O. A., and W. P. Schilling. Vectorial Ca^{2+} flux from the extracellular space to the endoplasmic reticulum via a restricted cytoplasmic compartment regulates inositol 1,4,5-trisphosphate-stimulated Ca^{2+} release from internal stores in vascular endothelial cells. Biochem. J. 295: 357-366, 1993.

Cahill, M., J. B. Fishman, and P. Polgar. Effect of des arginine9-bradykinin and other bradykinin fragments on the synthesis of prosyacyclin and the binding of bradykinin by vascular cells in culture. Agents-Actions 24: 224-31, 1988.

Campell, D. J., A. Kladis, and A. M. Duncan. Bradykinin peptide in kidney, blood, and other tissues of the rat. Hypertension. 21: 155-165, 1993.

Carter, R. D., W. L. Joyner, and E. M. Renkin. Effect of histamine and some other substances on molecular selectivity of the capillary wall to plasma proteins and dextran. Microvasc. Res. 7: 31-48, 1974.

Chick, W. L., S. Warren, R. N. Chute, A. A. Like, V. Lauris, and K. C. A. Kitchen. Transplantable insulinoma in the rat. Proc. Natl. Acad. Sci. USA 74: 628-632, 1977.

Churchill, L., and P. E. Ward. Relaxation of isolated mesenteric arteries by des-Arg[9]-bradykinin stimulation of B1 receptors. Eur. J. Pharmacol. 130: 11-18, 1986.

Churchill, L., and P. E. Ward. Conversion of B1 kinin receptor-mediated vascular relaxation to contraction. Hypertension 9: 1, 1987.

Clementi, E., I. Vecchio, C. Sciorati, and G. Nistico. Nitric oxide modulation of agonist-evoked intracellular Ca^{2+} release in neurosecretory PC-12 cells: inhibition of phospholipase C activity via cyclic GMP-dependent protein kinase I. Mol. Pharmacol. 47: 517-524, 1995.

Collier, H. O., J. A. Holgate, M. Schachter, and P. G. Shorley. The bronchoconstrictor action of bradykinin in the Guinea pig. Br. J. Pharmacol. 15: 290-297, 1962.

Cuthbert, A W., and H. S. Margolius. Kinins stimulate net chloride secretion by the rat colon. Br. J. Pharmacol. 75: 587-598, 1982.

Damas, J., and A. Adam. The relationship between kininogens and kallikreins in Brown Norway deficient rat plasma. Mol. Physiol. 8;307-316, 1985.

DeBlois, D., J. Bouthillier, and F. Marceau. Pulse exposure to protein synthesis inhibitors enhances vascular responses to des-Arg[9]-bradykinin: possible role of interleukin-I. Br. J. Pharmacol. 103: 1957-1066, 1991.

Demolle, D., M. Lecomte, and J. M. Boeynaems. Pattern of protein phosphorylation in aortic endothelial cells. Modulation by adenine nucleotides and bradykinin. J. Biol. Chem. 263: 18459-18465, 1988.

Dietze, G. J. Modification of the action of insulin in relation to the energy state in skeletal muscle tissue: possible involvement of kinin and prostaglandins. Mol. Cell Endocrinol. 25: 127-149, 1982.

Dixon, B. S. Cyclic AMP selectively enhances bradykinin receptor synthesis and expression in cultured arterial smooth muscle. Inhibition of angiotensin II and vasopressin response. J. Clin. Invest. 93: 2535-2544, 1994.

Erdös, E. G. Kininases. pp. 427-487 in E. G. Erdös, eds. Bradykinin, kallidin, and kallikrein. Springer-Verlag, Berlin, Heidelberg, New York, 1979.

Erdös, E. G. Some old and some new ideas on kinin metabolism. J. Cardiovasc. Pharmacol. 15: 520-524, 1990.

Etscheid, B. G., K. A. Albert, M. L. Villereal, and H. C. Palfrey. Transduction of the bradykinin response in human fibroblasts: prolonged elevation of diacylglycerol level and its correlation with protein kinase C activation. Cell Regul. 2: 229-239, 1991.

Farmer, S. G., R. M. Burch, S. A. Meeker, and D. E. Wilkins. Evidence for a pulmonary B3 bradykinin receptor. Mol. Pharmacol. 36: 1-8, 1989.

Fasolato, C., A. Pandiella, J. Meldolesi, and T. Pozzan. Generation of inositol phosphates, cytosolic Ca^{2+}, and ionic fluxes in PC12 cells treated with bradykinin. J. Biol. Chem. 263: 17350-17359, 1988.

Fasolato, C., B. Innocenti, and T. Pozzan. Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels. Trends Pharmacol. Sci. 15: 77-83, 1994.

Ferreira, S. H. and J. R. Vane. The disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat. Br. J. Pharmacol. 30: 417-424, 1967.

Fiedler, F. Enzymology of glandular kallikreins. pp.103-106 in E. G. Erdös, eds. Bradykinin, kallidin and kallikrein. Springer-Verlag, Berlin, Heidelberg, New York, 1979.

Figueiredo, A. F., A. H. Salgado, G. R. Siqueira, C. R. Velloso, and W. T. Beraldo. Rat uterine contraction by kallikrein and its dependence on uterine kininogen. Biochem. Pharmacol. 15: 763-767, 1990.

Figueroa, C. D., and K. D. Bhoola. Leukocyte tissue kallikrein: an acute phase signal for inflammation. pp.311-320 in H. Fritz, I. Schmidt, and G. Dietz, eds. Kallikrein system in health and disease. Limbach-Verlag, Braunschweug, Germany, 1989.

Figueroa, C. D., A. G. Maciver, and K. D. Bhoola. Identification of a tissue kallikrein in human polymorphonuclear leucocytes. Br. J. Haemotol. 72: 321-328, 1989.

Figueroa, C. D., L. M. Henderson, R. W. Colman, R. A. De La Cadena, W. Müller-Esterl, and K. D. Bhoola. Immunoreactive L- and H-kininogen in human neutrophils. J. Physiol. (Lond.) 425: 65, 1990.

Fleming, I., B. Fisslthaler, and R. Busse. Calcium signaling in endothelial cells involves activation tyrosine kinases and leads to activation of mitogen-activated protein kinases. Circ. Res. 76: 522-529, 1995.

Ford, S., and M. J. Lewis. A factor released from coronary vascular endothelium inhibits myocardial contractile performance. Am. J. Physiol. 264: H830-836, 1993.

Frey, E. K., H. Krout, E. Werle. Kallikrein (Padutin). Stuttgart, Enke Verlag, 1950.

Furchgott, R. F. The requirement for endothelial cells in the relaxation of the arteries by acetylcholine and some other vasodilators. Trends Pharmacol. Sci. 3: 175, 1981.

Gadd, A., and K. D. Bhoola. Modulation of guinea pig lung adenylate cyclase by ovalbumin sensitization. Biochem. Pharmacol. 37: 2027-2034, 1988.

Gazdar, A. F., W. L. Chick, H. K. Oie, H. L. Sim, D. L. King, G. C. Weir, and V. Lauris, V. Continuous, clonal, insulin-and somatostatin-secreting cell lines established from a transplantable rat islet cell tumor. Proc. Natl. Acad. Sci. USA. 77: 3519-3523, 1980.

Gaginella, T. S., and J. F. Kachur. Kinins as mediators of intestinal secretion. Am. J. Physiol. 256: G1-G15, 1989.

Girolami, J. P., F. Alhenc-Gelas, M. L. Dos Reis, J. L. Bascands, J. M., Suc, P. Corvol, and J. Menard. Hydrolysis of high molecular weight kininogen by purified rat urinary kallikrein: identification of bradykinin as the kinin formed. Adv. Exp. Med. Biol. 198A: 137-145, 1986.

Gishizky, M. L. and G. M. Grodsky. Differential kinetics of rat insulin I and II processing in rat islets of langerhans. FEBS. Lett. 223: 227-231, 1987.

Gadd, A., and K. D. Bhoola. Modulation of guinea-pig lung adenylate cyclase by ovalbumin sensitization. Biochem. Pharmacol. 37: 2027-2034, 1988.

Goldman, J., D. Pfister, and R. Vukmirovich. Potentiation of insulin stimulation of hexose transport by kallikrein and bradykinin in isolated adipocytes. Mol. Cell Endocrinol. 50: 183-191, 1987.

Goldstein, R. H., M. Wall, F. G. Graeff, A. P. Corrado, I. R. Pela, and R. Capek, R. Activation of protein formation and cell division by bradykinin and Des-Arg^9-bradykinin. J. Biol. Chem. 259: 9263-9268, 1984.

Greenbaum, C. J., P. J. Havel, G. J. Jr. Taborsky, and L. J. Klaff. Intra-islet insulin permits glucose to directly suppress pancreatic A cell function. J. Clin. Invest. 88: 767-773, 1991.

Gross, D., A. Skvorak, G. Hendrick, G. Weir, L. Villa-Komaroff, and P. Halban. Oxidation of rat insulin II, but not I, leads to anomalous elution profiles upon HPLC analysis of insulin-related peptides. FEBS. Lett. 241: 205-208, 1988.

Gustafson, E. J., D. Schutsky, L. C. Knight, and A. Schmaier. High molecular weight kininogen binds to unstimulated platelets. J. Clin. Invest. 78:310-318, 1986.

Gustafson, E. J., A. Schmaier, Y. T. Wachfogel, N. Kaufman, U. Kulich, and R. W. Colman. Human neutrophils contain and bind high molecular weight kininogen. J. Clin. Invest. 84: 28-35, 1989.

Halban, P. A., G. A. Praz, and C. B. Wollheim. Abnormal glucose metabolism accompanies failure of glucose to stimulate insulin release from a rat pancreatic cell line (RINm5F). Biochem. J. 212: 439-443, 1983.

Hartl, W. H., H. Miyoshi, D. Elahi, and R. R. Wolfe. Glucagon and hepatic glucose production: modulation by low-dose bradykinin infusion. Metabolism. 38: 878-882, 1989.

Hasegawa, H., Y. Okabayshi, M. Koide, Y. Kido, T. Okutani, K. Matauahita, M. Otsuki, and M. Kasuga. Effect of islet hormones on secretion-stimulated exocrine secretion in isolated perfused rat pancreas. Dig. Dis. Sci. 38: 1278-1283, 1993.

Hazelwood, R. L. The endocrine pancreas. Prentice Hall, Englewood Cliffs, New Jersey. pp. 9-83, 1989.

Hellman, B., E. Gylfe, P. Bergsten, E. Grapengiesser, P. E. Lund, A. Bert, S. Dryselius, A. Tengholm, Y. J. Liu, M. Eberhardson, et al. The role of Ca^{2+} in the release of pancreatic islet hormones. Diabetologia 20: 123-131, 1994.

Helmstaedter, V., G. E. Feurle, and W. G. Forssmann. Insulin-, glucagon-, and somatostatin-immunoreactive endocrine cells in the equine pancreas. Cell Tissue Res. 172: 447, 1976.

Hess, J. F., J. A. Borkowski, G. S. Young, C. D. Stader, and R. W. Ransom. Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. Biochem. Biophys. Res. Commun. 184: 260-268, 1992.

Hess, J. F., J. A. Borkowski, G. Y. Stonesifer, T. Macneil, C. D. Strader, and R. W. Ransom. Cloning and pharmacological characterization of bradykinin receptors. Braz. J. Med. Biol. Res. 27: 1725-1731, 1994.

Hial, V., H. R. Keiser, and J. J. Pisano. Origin and content of methionyl-lysyl-bradykinin, lysyl-bradykinin, and bradykinin in human urine. Biochem. Pharmacol. 25: 2499-2503, 1976.

Higashida, H., and D. A. Brown. Two polyphosphatidylinositide metabolites control two K^+ currents in a neuronal cell. Nature 323: 333-335, 1986.

Higashida, H., and D. A. Brown. Bradykinin inhibits potassium (M) currents in N1E-115 neuroblastoma cells. Response resemble those in NG108-115 neuroblastoma X Glioma hybrid cells. FEBS. Lett. 220:302-306, 1987.

Higashida, H. Acetylcholine release by bradykinin, inositol 1,4,5,-triphosphate and phorbol dibutyrate in rodent neuroblastoma cells. J. Physiol. 397: 209-22, 1988.

Higashiyama, S., I. Ohkibo, H. Ishguro, M. Kunimatsu, K. Swaki, and M. Sasaki. Human high molecular weight kininogen as a thiol protease inhibitor: presence of the entire inhibition capacity in the native form of heavy chain. Biochemistry 25: 1669-1675, 1986.

Hock, F. L., K. Wirth, U. Albus, W. Linz, H. J. Gerhards, G. Wiemer, S. Henke, G. Breipohl, W. Konig, J. Wnolle, and B. A. Scholkens. HOE140, a new potent and long acting bradykinin-antagonist: in vitro studies. Br. J. Pharmacol. 102: 769-773, 1991.

Hojima, Y., C. Moriwaki, and H. Moriya. Isolation of dog, rat and hog pancreatic kallikreins by preparative disc electrophoresis and their properties. Chem. Pharmacol. Bull. (Tokyo) 23:1128-1136, 1975.

Hoyer, D., H. Lubbert, and C. Bruns. Molecular pharmacology of somatostatin receptors. Naunyn-Schmiedebergs Arch. Pharmacol. 350: 441-453, 1994.

Inoue, H., K. Fukui, and Y. Miyake. Identification and structure of the rat tissue kallikrein gene expression in the kidney. J. Biochem. (Tokyo) 105: 834-840, 1989.

Ishida, H., A. G. Scicli, and O. A. Carretero. Contributions of various rat plasma peptidases to kinin hydrolysis. J. Pharmacol. Exp. Ther. 251: 817-820, 1989.

Islam, M. S., P. Rorsman, and P. O. Berggren. Ca^{2+}-induced Ca^{2+} release in insulin-secreting cells. Fed. Eur. Biochem. Soc. 296; 287-291, 1992.

Iwanaga, S., Y. N. Han, H. Kato, and T. Suzuki. Actions of various kallikrein on HMW kininogen and its derivatives. pp.79-90 in G. L. Haberland, J. W. Rohen, and T. Suzuki, 4 eds. Kininogenase kallikrein. Schattarur-Verlag, Stuttgart, New York, 1977.

Iwai, N., M. Matsunaga, T. Kita, M. Tei, and C. Kawai, C. Detection of low molecular kininogen messenger RNA in human kidney. J. Hypertens. 6(suppl.):399-400, 1988.

Jacobsen, S. Separation of the two different substrates for plasma kinin-forming enzymes. Nature 210: 98-99, 1966.

Jaffa, A. A., J. Pratt, A. Ashford, and G. S. Bailey. Studies of the effects of insulin, bradykinin, and captopril on blood glucose levels of alloxan-diabetic rats. Adv. Exp.Med. Biol. 198: 373-378, 1986.

Jaffa, A. A., P. F. Rust, and R. K. Mayfield. Kinin, a mediator of diabetic-induced glomerular hyperfiltration. Diabetes 44: 156-160, 1995.

Jauch, K. W., B. Gunther, W. Hartl, K. Rett, M. Wicklmayr, and G. Dietze. Improvement of impaired postoperative insulin action by bradykinin. Biol-Chem-Hoppe-Seyler. 367: 207-210, 1986.

Jauch, K. W., W. Hartl, M. Georgieff, R. R. Wolfe, G. J. Dietze, and B. Gunther. Low-dose bradykinin infusion reduces endogenous glucose production in surgical patients. Metabolism 38: 878-882, 1988.

Jauch, K. W.,B. Gunther, K. Bett, W. Hartl, M. Wicklmayr, G. Dietze, E. Fink, M. Georgieff, and D. Neumeier. Dose-dependent effects of bradykinin infusion in post- operative patients. Adv. Exp. Med. Biol. 247A: 603-608, 1989.

Jin, W., T. M. Lo, H. H. Loh, and S. A. Thayer. U73122 inhibits phospholipase C-dependent calcium mobilization in neuronal cells. Brain Res. 642: 237-243, 1994.

Kaplan, A. P. Initiation of the intrinsic coagulation and fibrinolytic pathways in man: the role of surfaces, Hageman factor, prekallikrein, high molecular weight kininogen and factor XI. Prog. Hemost. Thromb. 4:127-175, 1978.

Kato, H., Y. N. Han, S. Iwanaga, T. Suzuki, and M. Komiya. Bovine plasma HMW and LMW kininogens. Structural difference between heavy and light chains derived from the kinin-free proteins. J. Biochem. (Tokyo) 80:1299-1311, 1976.

Kato, H., K. Enjyoji, T. Miyata, I. Hayashi, S. Oh-Ishi, and S. Iwanaga. Demonstration of arginyl-bradykinin moiety in rat HMW kininogen: direct evidence for liberation of bradykinin by rat glandular kallikreins. Biochem. Biophys. Res. Commun. 127: 289-295, 1985.

Kaya, H., G. M. Patton, and S. L.Hong. Bradykinin-induced activation of phospholipase A_2 is independently of the activation of polyphosphoinositide-hydrolyzing phospholipase C. J. Biol. Chem. 264: 4972-4977, 1989.

Kellermann, J., C. Thelen, F. Lottspeich, A. Henschen, R. Vogei, and M. Müller-Esterl. Arrangement of the disulfide bridges in human low-M kininogen. Biochem. J. 247:15-21, 1987.

Kellett, G. L., and E. D. Barker. The stimulation of glucose absorption and metabolism in rat jejunum by bradykinin: dependence on the composition of commercial diets. Biochem. Biophys. Acta. 21: 128-130, 1989.

Kimball, H. R., K. L. Melmon, and S. M. Wolff. Endotoxin-induced kinin production in man. Proc. Soc. Exp. Biol. Med. 139:1078-1082, 1972.

Kitamura, N., Y. Tagagaki, S. Furuto, T. Tanaka, H. Nawa, and S. A. Nakanishi. A single gene for bovine high molecular weight and low molecular weight kininogens. Nature 305:545-549, 1983.

Kortmann, H., E. Flink, and G. Bönner. The influence of the kallikrein-kinin system in the development of the pancreatic shock. Adv. Exp. Med. Biol. 167:495, 1984.

Kutzbach, C., and G. Schmidt-Kastner. Kallikrein from pig pancreas. Purification, separation of components A and B, and crystallization. Hoppe-Seylers-Z-Physiol-Chem. 353:1099-1106, 1972.

Kyle, D. J., S. Chakravarty, J. A. Sinsko, and T. M. Stormann. A proposed model of bradykinin bound to the rat B2 receptor and its utility for drug design. J. Med. Chem. 37:1347-1354, 1994.

Laychock, S. G., M. E. Maria, and C. T. Cavanaugh. L-arginine stimulates cyclic guanosine 3',5'-monophosphate formation in rat islets of Langerhans and RINm5F insulinoma cells: Evidence for L-arginine:nitric oxide synthase. Endocrinology 129: 3043-3052, 1991.

Leclercq-Meyer, V., M. H. Giroix, A. Sener, J. Marchand, and W. J. Malaisse. Dynamic insulin release by perifused insulin-producing tumoral cells: effects of glucose, forskolin, leucine, barium and theophylline. Int. J. Pancreatol. 3: 17-31, 1988.

Leeb-Lundberg, L. M., X. H. Song, and S. A. Mathis. Focal adhesion-associated proteins p125FAK and paxillin are substrates for bradykinin-stimulated tyrosine phosphorylation in Swiss 3T3 cells. J. Biol. Chem. 269: 24328-24334, 1994.

Leis, H. J., D. Zach, E. Huber, L. Ziermann, H. Gleispacg, and W. Windischhofer. On the inhibition of prostanoid formation by SK&F 96365, a blocker of receptor-operated calcium entry. Br. J. Pharmacol. 114: 598-601, 1995.

Lenzen, S., M. Tiedge, and U. Panten. Glucokinase in pancreatic B-cells and its inhibition by alloxan. Acta. Endocrinol. 115: 21-29, 1987a.

Lenzen, S., M. Tiedge, P. R. Flatt, C. J. Bailey, and U. Panten. Defective regulation of glucokinase in rat pancreatic islet cell tumours. Acta. Endocrinol. 115: 514-520, 1987b.

Lewis, R., S. R. Childers, and M. I. Phillips. [^{125}I]Try-bradykinin binding in primary rat brain cultures. Brain Res. 346: 263-272, 1985.

Liao, J. K. and C. J. Homcy. The G proteins of the G alpha i and G alpha q family couple the bradykinin receptor to the release of endothelium-derived relaxing factor. J. Clin. Invest. 92(5): 2168-2172, 1993.

Linz, W., B. A. Schölkens, and Y. Han. Beneficial effects of the converting enzyme inhibitors, ramipril, in ischaemic rat heart. J. cardiovasc. Pharmacol. 8: S91- S99, 1986.

Linz, W., P. A. Martorana, and B. A. Schölkens. Local inhibition of bradykinin degradation in ischemic hearts. J. Cardiovasc. Pharmacol. 15: S99-S109, 1990.

Liona, I., X. Galleguillos, and J. Belmar. Bradykinin modulates the release of noradrenaline from vas deferens nerve terminals. Life Sci. 48: 2585-2592, 1991.

Lo, T. M. and S. A. Thayer. Refilling the inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store in neuroblastoma x glioma hybrid NG108-15m cells. Am. J. Physiol. 264: C641-653, 1993.

Ma, J. X., D. J. Wang, D. C. Ward, L. Chen, T. Dessai, J. Chao, and L. Chao. Structure and chromosomal localization of the gene (BDKRB2) encoding human bradykinin B2 receptor. Genomics 23: 362-369, 1994.

Mandle, R. J. Jr., and A. P. Kaplan. Hageman factor substrates. II. Human plasma prekallikrein. Mechanism of activation by hageman factor and participation in hageman factor dependent fibrinolysis. J. Biol. Chem. 252:6097-6104, 1977.

Marceau, F., and B. Tremblay. Mitogenic effect of bradykinin and Des-Arg^9-bradykinin on cultured fibroblast. Life Sci. 39: 2351-2358, 1986.

Mason, A. J., B. A. Evans, D. R. Cox, J. Shine, and R. I. Richards. Structure of mouse kallikrein gene family suggests a role in specific processing of biological active peptides. Nature 303: 300-307, 1983.

McCarthy, D. A., D. E. Potter, and E. D. Nicolaides. An in vivo estimation of the potencies and half-lives of synthetic bradykinin and kallidin. J. Pharmacol. Exp.Ther. 148: 117-122, 1965.

McEachern A. E., E. R. Shelton, S. Bhakta, R. Obernolte, C. Bach, P. Zuppan, J. Fujisaki, R. W. Aldrich, and K. Jarnagin. Expression cloning of a rat B2 bradykinin receptor. Proc. Natl. Acad. Sci. USA 88: 7724-7728, 1991.

Menke, J. G., J. A. Borkowski, K. K. Bierilo, T. MacNeil, A. W. Derrick, K. A. Schneck, R. W. Ransom, C. D. Stader, D. L. Linemeyer, and J. F. He's. Expression cloning of a human B1 bradykinin receptor. J. Biol. Chem. 269: 21583-21586, 1994.

Meldolesi, J., and T. Pozzan. Pathways of Ca^{2+} influx at the plasma membrane: voltage-, receptor-, and second messenger-operated channels. Exp. Cell. Res. 171: 271-283, 1987.

Miller, R. E. Pancreatic neuroendocrinology; Peripheral neural mechanisms in the regulation of the islets of Langerhans. Endocrinol. Rev. 2: 471-494, 1981.

Mindroiu, T., O. A. Carretero, and A. G. Scicli. T-kininogen in rat plasma after nephrectomy and other surgical trauma. Agents Actions Suppl. 22: 265-276, 1987.

Moncada, S., M. W. Radomski, and R. M. Palmer. Endothelium-derived relaxing factor. Identification as nitric oxide and role in the control of vascular tone and platelet function. Biochem. Pharmacol. 37: 2495-2501, 1988.

Moller, N., J. P. Bagger, O. Schmita, J. O. Jorgensen, P. Ovesen, J. Moller, K. G. Alberti, and H. Orskov. Somatostatin enhances insulin-stimulated glucose uptake in the perfused human forearm. J. Clin. Endocrinol. Metab. 80: 1789-1793, 1995.

Müller-Esterl, W., S. Iwanaga, and S. Nakanishi. Kininogens revisited. Trends Biochem. Sci. 11: 336-339, 1986.

Müller-Esterl, W. Kininogen, kinin and kinships. Thromb. Haemost. 61: 2-6, 1989.

Muschel, R., G. Khouryl, and L. M. Reid. Regulation of insulin mRNA abundance and adenylation dependence on hormones and matrix substrata. Mol Cell Biol. 6: 337-341, 1986.

Nakagawa, A., J. I. Stagner, and E. Samols. In situ binding of islet hormones in the isolated perfused rat pancreas: evidence for local high concentrations of islet hormones via the islet-acinar axis. Diabetologia 38: 262-268, 1995.

Needleman, P., S. L. Key, S. E. Denny, P. C. Isakson, and G. R. Marshall. The mechanism and modification of bradykinin-induced coronary vasodilation. Proc. Natl.Acad. Sci. USA 72:2060-2063, 1975.

Nishikawa, K., Y. Shibayama, P. Kuna, E. Calcaterra,A. P. Kaplan, and S. R. Reddigari. Generation of vasoactive peptide bradykinin from human umbilical vein endothelium-bound high molecular weight kininogen by plasma kallikrein. Blood 80: 1980-1988, 1992.

Noe, B. D., D. J. Fletcher, and G. E. Baver. Biosynthesis of glucagon and somatostatin. In the islets of Langerhans, eds. S. J. Cooperstein and D. Watkins, chap. 9. New York: Academic Press, 1981.

Nolly, H., and M. C. Lama,. "Vascular kallikrein": a kallikrein-like enzyme present in vascular tissue of rat. Clin. Sci. 63: 249-251, 1982.

Nolly, H., M. C. Lama, O. A. Carretero, and A. G. Scicli. The Kallikrein--kinin system in blood vessels. Agents Actions suppl. 38: 1-9, 1992.

Nwator, I. A. A. and E. T. Whalley. Angiotension converting enzyme inhibitors and expression of des-Arg^9-BK (kinin B$_1$) receptors in vivo. Eur. J. Pharmacol. 160: 125-132, 1989.

Oie, H. K., A. F. Gazdar, J. D. Minna, G. C. Weir, and S. B. Baylin. Clonal analysis of insulin and somatostatin secretion and L-dopa decarboxylase expression by a rat islet cell tumor. Endocrinol. 112: 1070-1075, 1983.

Okamoto, H., and L. M. Greenbaum. Isolation and structure of T-kinin. Biochem. Biophys. Res. Commun. 112:701-708, 1983.

Ole-Moiyoi, O., D. C. Seldin, J. Spragg, G. Pinkus, and K. F. Austein. Sequential cleavage of proinsulin by human pancreatic kallikrein and a human pancreatic kinase. Proc. Natl. Acad. Sci. USA 76: 3612-3616, 1979.

Ørstavik, T. B., K. Nustad, and P. Brandtzaeg. Localization of glandular kallikreins in rat and man. pp. 137-149 in F. Gross and H. G. Vogel, eds. Enzymatic release of vasoactive peptides. Raven Press, New York, 1976.

Ørstavik, T. B., I. B. Brekke, J. Alumets, and O. A. Carretero. Kallikrein in rat pancreatic tissue after beta cell destruction or acinar cell atrophy. J. Histochem. Cytochem. 29: 1431-1436, 1981.

Ørstavik, T. B. The kallikrein-kinin system in exocrine organs. J. Histochem. Cytochem. 28: 881-889, 1990.

Oza, N. B., J. H. Schwartz, M. D. Goud, and N. G. Levinsky. Rat aortic smooth muscle cells in culture express kallikrein, kininogen and bradykininase activity. J. Clin. Invest. 85:597-600, 1990.

Palov, O. G., and V. I. Ovsiannikov. Role of cholinergic and adrenergic mechanisms in the development of contractile responses of the small intestines to bradykinin. Fiziol. Zh. SSSR. 74: 404-413, 1988.

Pan, H. L., C. L. Stebbins, and J. C. Longhurst. Bradykinin contributes the exercise pressor reflex: mechanism of action. J. Appl. Physiol. 75: 2261-2268, 1993.

Perkins, M. N. and D. Kelly. Interleukin-Iβ (IL-1β) but not tumor necrosis factor-alpha (TNF-α) can induce bradykinin B1 receptor-mediated thermal hyperalgesia in the rat. Br. J. Pharmacol. 108: 189, 1993.

Pearse, A. G. The APUD concept and hormone production. Clin. Endocrinol. Metab. 9: 211-222, 1980.

Pinkus, G. S., M. Maier, D. C. Seldin, O. Ole-Moiyoi, K. F. Austen, and J. Spragg. Immunohistochemical localization of glandular kallikrein in the endocrine and exocrine pancreas. J. Histochem. Cytochem. 31: 1279-1288, 1983.

Pisano, J. J. and K. F. Austen. Chemistry and biology of the Kallikrein-kinin system in health and disease. National Institutes of health, bethesda, Maryland, Oct.20-23, DHEW publication No.(NIH) 76-791, 1974.

Pisano, J. J. Chemistry and biology of the kallikrein-kinin system. pp. 199-222 in E. Reich, D. B. Rifkin, and E. Shaw, eds. Proteases and biological control. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1975.

Pisano, J. J. Observation on the kallikrein-kinin system in the kidney. p.929. In Fritz, H., Back, N., Dietze, G., and Haberland, G. L. eds. Kinins-III, par A, Plenum, New York and London, 1981.

Potempa, J., A. Dubin, G. Korzus, and J. Travis. Degradation of elastin by a cysteine protease from Staphylococcus aureus. J. Biol. Chem. 263:2644, 1988.

Praz, G. A., P. A. Halban, C. B. Wollheim, B. Blondel, A. J. Strauss. and A. E. Renold. Regulation of immunoreactive-insulin release from a rat cell line)RINm5F). Biochem. J. 210: 345-352, 1983.

Proud, D. Kinins in the pathogenesis of human airway disease. Braz. J. med. Biol. Res. 27: 2021-2031, 1994.

Puri, R. N., E. J. Gustafson, F. X. Zhou, H. Bradford, R. F. Colman, and R. W. Colman. Inhibition of thrombin-induced platelet aggregation by high molecular weight kininogen. Trans. Assoc. Am. Physicians. 100: 232-240, 1987.

Qin, H., J. Kemp, M. Yip, P. R. Lam-Po-Tang, and B. J. Morris. Localization of human glandular kallikrein-1 gene to chromosome 19q13.3-13.4 by *in situ* hybridization. Hum. Hered. 41: 222-226, 1991.

Reddigari, S. R., P. Kuna, G. Miragliotta, Y. Shibayama, K. Nishikawa, and A. P. Kaplan. Human high molecular weight kininogen binds to human umbilical vein endothelial cells via its heavy and light chains. Blood 81: 593-603, 1993.

Regoli, D., J. Barabe, and W. K. Park. Receptors for bradykinin in rabbit aorta. Can. J. Physiol. Pharmacol. 55: 855-867, 1977.

Regoli, D., F. Marceau, and J. Lavigne. Induction of B1 receptors for kinin in the rabbit by a bacterial lipopolysaccharide. Eur. J. Pharmacol. 71: 105-115, 1981.

Regoli, D., G. Drapeau, P. Rovero, S. Dion, P. D'Orleans-Juste, and J. Barabe. The actions of kinin antagonists on B_1 and B_2 receptor systems. Eur. J. Pharmacol. 123: 61-66, 1986.

Regoli, D., G. Drapeau, P. Rovero, S. Dion, N. E. Rhaleb, J. Barabe, P. D'Orleans Juste, and P. Ward. Conversion of kinins and their antagonists into B1 receptor activators and blockers in isolated vessels. Eur. J. Pharmacol. 127:219-224, 1986.

Regoli, D., N. E. Rhalab, G. Drapeau,and S. Dion, S. Kinin receptor subtypes. J. Cardiovasc. Pharmacol. 15: S30-S38, 1990.

Regoli, D., D. Jukic, F. Gobeil, and N. E. Rhaleb. Receptors for bradykinin and related kinin: a critical analysis. Can. J. Physiol. Pharmacol. 71(8): 556-67, 1993.

Regoli, D., F. Gobeil, Q. T. Nguyen, D. Jukic, P. R. Seoane, J. M. Salvino, and D. G. Sawutz. Bradykinin receptor types and B2 subtypes. Life Sci. 55: 735-749, 1994.

Reis, M. L., L. Okino, and M. Rocha e Silva. Comparative pharmacological actions of bradykinin and related kinins of larger molecular weight. Biochem. Pharmacol. 30:2935, 1971.

Rhodes, C. J., and P. A. Halban. Newly synthesized proinsulin/insulin and stored insulin released from pancreatic B cells predominantly via a regulated, rather than a constitutive, pathway. J. Cell Biol. 105: 145-153, 1987.

Ribuot, C., N. Yamaguchi, D. Godin, L. Jette, A. Adam, and R. Nadeau. Intracoronary infusion of bradykinin: effects on noradrenaline overflow following reperfusion of ischemic myocardium in the anesthetized dog. Fund. Clin. Pharmacol. 8: 532-538, 1994.

Roberts, R. A. Bradykinin receptors: Characterization, distribution and mechanisms of signal transduction. Prog. Growth Factor Res. 1:237-252, 1989.

Roberts, R. A., and W. J. Gullick. Bradykinin receptor number and sensitivity to ligand stimulation of mitogenesis is increased by expression of a mutant ras oncogene. J. Cell. Sci. 94: 527-535, 1989.

Rocha e Silva, M. Biochemistry and pharmacology of bradykinin. pp. 210-231 in M. Schachter, eds. Polypeptides which affect smooth muscles and blood vessels. Oxford, Pergamon, 1960.

Rosen, P., J. Eckel, and H. Reinauer. Influence of bradykinin on glucose uptake and metabolism studied in isolated cardiac myocytes and isolated perfused rat heart. Hoppe-Seyler Z. Physiol. Chem. 364: 1431-1438, 1983.

Salvemini, D., G. De Nucci, R. J. Gryglewski, and J. R. Vane. Human neutrophils and mononuclear cells inhibit platelet aggregation by releasing a nitric oxide-like factor. Proc. Natl. Acad. Sci. USA 86: 6328-6332, 1989.

Samols, E., and J. Harrison. Intraislet negative insulin-glucagon feedback. Metabolism 25: 1443-1447, 1976.

Samols, E., and J. I. Stagner. Islet somatostatin--microvascular, paracrine, and pulsatile regulation. Metabolism 39: 55-60, 1990.

Sato, F., and S. Nagasawa. Mechanism of kinin release from human low-molecular-mass-kininogen by the synergistic action of human plasma kallikrein and leukocyte elastase. Biol. Chem. Hoppe-Seylers 369: 1009-1017, 1988.

Schmaier, A. H., A. Zuckerberg, C. Silverman, J. Kuchibothla, G. P. Tuszynski, and R. W. Colman. High molecular weight kininogen: a secreted platelet protein. J. Clin. Invest. 71:1477-1489, 1983.

Schmaier, A. H., D. Schutsky, A. Farber, L. D. Silver, H. N. Bradford, and R. W. Colman. Determination of bifunctional properties of high molecular weight kininogen by studies with monoclonal antibodies directed to each of its chains. J. Biol. Chem 262: 1405-1411, 1987.

Schmaier, A. H., A. Kuo, D. Lundberg, S. Murray, and D. B. Cines. The expression of high molecular weight kininogen on human umbilical vein endothelial cells. J. Biol. Chem. 263:16327-16333, 1988.

Scicli, A. G., T. Mindroiu, G. Scicli, and O. A. Carretero. Blood kinins, their concentration in normal subjects and in patients with congenital deficiency in plasma prekallikrein and kininogen. J. Lab. Clin. Med. 100: 81-93, 1982.

Sealey, J. E., S. A. Atlas, and J. H. Laragh. Linking the kallikrein and renin systems via activation of inactive renin. Am. J. Med. 65:994-1000, 1978.

Seidah, N. G., J. Paquin, J. Hamelin, S. Benjannet, and M. Chretien. Structure and immunological homology of human and porcine pituitary and plasma IRCM-serine protease 1 to plasma kallikrein: marked selectivity for pairs of basic residues suggests a widespread role in pro-hormone and pro-enzyme processing. Biochemistry 70:33-46, 1988.

Seidah, N. G., R. Ladenheim, M. Mbikay, J. Hamelin, G. Lutfalla, F. Rougeon, C. Lazure, and M. Chretien. The cDNA structure of rat plasma kallikrein. DNA 8:563-574, 1989.

Shai, S., C. Woodly-Miller, L. Chao, L., and L. Chao. Characterisation of genes encoding rat tonin and a kallikrein-like serine protease. Biochemistry 28:5334-5343, 1989.

Sharp, P. A., and E. S. Debnam. Rapid stimulatory effect of bradykinin on glucose transport across the brush-border and basolateral membranes of rat jejunal enterocytes. Exp. Physiol. 77: 913-916, 1992.

Shimojo, N., T. G. Pickens, H. S. Margolius, and R. K. Mayfield. Tissue kallikrein and bradykinin do not have direct insulin-like actions on skeletal muscle glucose utilization. Biol. Chem. Hoppe-Seyler 368: 1355-1361, 1987.

Simeone, D. M., D. I. Yule, C. D. Logsdon, and J. A. Williams. Ca^{2+} signaling through secretagogue and growth factor receptors on pancreatic AR42J cells. Regul. Pept. 55: 197-206, 1995.

Skidgel, R. A., R. M. Davis, and E. G. Erdös. Purification of a human urinary carboxypeptidase (kininase) distinct form carboxypeptidase A, B. or N. Anal. Biochem. 140:520-531, 1984.

Smith, J. A., C. Webb, J. Holford, and G. M. Burgess. Signal transduction pathways for B1 and B2 bradykinin receptors in bovine pulmonary artery endothelial cells. Mol. Pharmacol. 47: 525-534, 1995.

Sopwith, A. M, J. C. Hutton, S. P. Naber, W. L. Chick, and C. N. Hales. Insulin secretion by a transplantable rat islet cell tumour. Diabetologia 21: 224-229, 1981.

Steranka, L. R., D. C, Manning, C. J. Dehaas, J. W. Ferkany, S. A. Borosky, J. R. Connor, R. J, Vavrex, J. M. Sreward, and S. H. Snyder. Bradykinin as a pain mediators: receptors are localized to sensory neurons and antagonists have analgesic action. Proc. Natl. Acad. Sci. USA 85: 3245-3249, 1988.

Steiner, D. F. Evidence for a precursor in the biosynthesis of insulin. Trans. N. Y. Acad. Sci. 30: 60-68, 1967.

Suidan, H. S., R. D. Murrell, and A. M. Tolkovsky. Carbachol and bradykinin elevated cyclic AMP and rapidly deplete ATP in cultured rat sympathetic neurons. Cell Regul. 2: 13-25, 1991.

Swift, G. H., J. C. Dargorn, P. L. Ashley, S. W. Cummings, and R. J. MacDonald. Rat pancreatic kallikrein m RNA: nucleotide sequence and amino acid sequence of the encoded preproenzyme. Proc. Natl. Acad. Sci. USA 79: 7263-7267, 1982.

Symington, R. P., A. Ashford, and G. Bailey. Effect of bradykinin and insulin on glucose oxidation in adipocytes from normal and streptozotocin diabetic rats. Biochem. Soc. Trans. 16: 1012-1013, 1988.

Tait, J., and K. Fijikawa. Primary structure requirements for the binding of human high molecular weight kininogen to plasma prekallikrein and factor XI. J. Biol. Chem. 262: 11651-11656, 1987, .

Takagaki, Y., N. Kitamura, and S. Nakanishi. Cloning and sequence analysis of cDNAs for human high molecular weight and low molecular weight prekininogens. Primary structures of two human prekininogens. J. Biol. Chem. 260: 8601-8609, 1985.

Takeuchi, K., K. Abe, K. Maeyama, M. Sato, M. Yasujimam, K. Omata, Y. Kassai, T. Watanabe, and K. Yoshinaga. Relationship between bradykinin induced rise in cytosolic free calcium and prostacyclin synthesis in cultured rat vascular smooth muscle cells. pp. 117-122 in O. Iimora and H. S. Margolius eds. Renal function, hypertension and kallikrein-kinin system. University of Tokyo Press. Tokyo, Japan, 1988.

Talamo, R. C., E. Haber, and K. F. Austen. A radioimmunoassay for bradykinin in plasma and synovial fluid. J. Lab. Clin. Med. 74: 816-827, 1969.

Thermos, K., M. D. Meglasson, J. Nelson, K. M. Lounsbury, and T. Reisine. Pancreatic beta-cell somatostatin receptors, Am. J. Physiol. 259: E216-224, 1990.

Thomas, T. P., T. R. Ellis, and S. B. Pek. Insulin release in RINm5F cells and glyceraldehyde activation of protein kinase C. Diabetes 38: 1371-1376, 1989.

Tiffany, C. W., and R. M. Burch. Bradykinin stimulates tumor necrosis factor and interleukin-1 release from macrophages. FEBS. Lett. 247: 189-192, 1989.

Uehara, S., S. Isogawa, W. Sakamoto, and A. Hirayama. Kallikrein-kinin system in diabetic patients. Arzneimittelforschung 38: 721-723, 1988.

Unger, R. H., and L. Orci. Possible roles of the pancreatic D-cell in the normal and diabetic states. Diabetes 26: 241-244, 1977.

Van Blitterswijk W. J., H. Hilkmann, J. de Widt, and R. L. van der Bend. Phospholipid metabolism in bradykinin-stimulated human fibroblasts. I. Biphasic formation of diacylglycerol from phosphotidylinositol and phosphotidylcholine, controlled by protein kininase C. J. Biol. Chem. 266: 10337, 1991.

Vane,J. R. The release and fate of vaso-active hormones in the circulation. Br. J. Pharmacol. 35: 209-242, 1969.

Vavrek, R. J., and J. M. Stewart. Competitive antagonists of bradykinin. Peptides 6: 161-164, 1985.

Veloso, D., L. D. Silver, S. Hahn, and R. W. Colman. A monoclonal antihuman plasma prekallikrein antibody that inhibits activation of prekallikrein by factor XII on a surface. Blood 70:1053-1062, 1987.

Vieira, M. A., M. F. Moreira, T. Maack. and J. A. Guimaraes. Conversion of T-kinin to bradykinin by the rat kidney. Biochem. Pharmacol, 47: 1693-1699, 1994.

Webb, M., P. McIntyre, and E. Phillips. B1 and B2 bradykinin receptors encoded by distinct mRNAs. J. Neurochem. 62(4): 1247-53, 1994.

Whalley, E. T., S. Clegg, J. M. Stewart, and R. J. Vavrek. The effect of kinin agonists and antagonists on the pain response of the human blister base. Naunyn Schmiedebergs Arch. Pharmacol. 336: 652-655, 1987.

Whalley, E. T., Y. O. Amure, and R. H. Lye. Analysis of the mechanism of action of bradykinin on human basilar artery in vitro. Naunyn Schmiedebergs Arch. Pharmacol. 335: 433-437, 1987.

White, D. M. and M. Zimmermann. The bradykinin-induced release of substance P from nerve fiber endings in the rat saphenous nerve neuroma is not related to electrophysiological excitation. Neurosci. Lett. 92: 108-13, 1988.

White, M. F., and C. R. Kahn. The insulin signaling system. J. Biol. Chem. 269: 1-4, 1994.

Wicklmayr, M., and G. Dietze. Effect of oral kallikrein and intrabrachial-arterial bradykinin on forearm-metabolism in maturity onset diabetes. pp. 299-308 in G. L. Haberland, J. W. Rohen, and T. Suzuki, 4 eds. Kininogen, kallikrein. Schattauer Verlag, Stuttgart, New York, 1977.

Williams, J. A., and I. D. Goldfine. The insulin-pancreatic acinar axis. Diabetes 34: 980-986, 1985.

Williams, J. A. Physiological evidence for islet-acinar interaction. Dept. of Physiol. University of Michigan. Ann. Arbor, 1994.

Willingham, M. C. CyclicAMP and cell behaviour in cultured cells. Int. Rev. cytosol. 44: 319-363, 1976.

Wirth, K., F. J. Hock, U. Albus, W. Linz, H. G. Aipermann, H. Anagnostopoulos, S. Henk, G. Bteipohl, W. Konig, J. Knolle, and et al. HOE 140 a new potent and long acting bradykinin-antagonist: in vivo studies. Br. J. Pharmacol. 102: 774-777, 1991.

Wong, P., R. W. Colman, and R. C. Talamo. Kallikrein-bradykinin system in chronic alcoholic liver disease. Ann. Int. Med. 77:205-209, 1972.

Yang, C. M., H. C. Hsia, J. T. Hsieh, R. Ong, and S. F. Luo. Bradykinin-stimulated calcium mobilization in cultured canine tracheal smooth muscle cells. Cell Calcium 16: 59-70, 1994.

Yamamoto, T. Characterization of guinea pig high molecular weight kininogen as a multifunctional molecule. Biochem. Biophys. Acta. 914: 259-274, 1987.

Yotsumoto, F., T. Manabe, and G. Ohshio. Bradykinin involvement in the aggravation of acute pancreatitis in rabbits. Digestion 54: 224-230, 1993.

ZhuGe, R., S. Li, T. H. Chen, and W. H. Hsu. Oxytocin induced a biphasic increase in the intracellular Ca^{2+} concentration of porcine myometrial cells:participation of a pertussis toxin-insensitive G-protein, inositol 1, 4, 5-trisphosphate-sensitive Ca^{2+} pool and Ca^{2+} channels. Mol. Reprod. Dev. 41: 20-28, 1995.

CHAPTER 2. STIMULATORY EFFECT OF BRADYKININ ON INSULIN RELEASE FROM THE PERFUSED RAT PANCREAS

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Abstract

Rat pancreas perfusion was performed to study the effect of bradykinin (BK) on insulin release. At the perfusate glucose concentration of 6 mM, BK (0.01 - 1 μM) increased insulin release in a concentration-dependent manner. In addition, BK (1 μM) increased the glucose (10 mM)-induced insulin release. HOE140 (0.1 μM), a BK_2-receptor antagonist, decreased the baseline insulin release and abolished the BK (1 μM)-induced increase in insulin release. In addition, HOE140 (0.1 μM) attenuated the glucose (10 mM)-induced increase in insulin release. Because the blockade of BK receptors by HOE140 attenuated the glucose-induced increased insulin release, our present findings suggest that BK could play a physiological role in the regulation of insulin release.

Introduction

Kinins including bradykinin (BK) display an extraordinarily high degree of physiological activity. They are the most potent vasodilator autacoids of mammals and they increase capillary permeability, produce edema, influence blood pressure and local blood flow, evoke pain and reflexes by acting on nerve endings, increase chloride and glucose transport, cell proliferation and other cellular functions (Bhoola et al., 1992). Kinins stimulate the release of hormones, e.g., renin from the kidney (Beierwaltes et al., 1985), vasopressin from the posterior pituitary gland (Baertschi et al., 1981), growth hormone and prolactin from the anterior pituitary cells (Drouhault et al., 1987; Jones et al., 1988), and catecholamines from the adrenal medulla (Staszewska-Barczak and Vane, 1967).

Activation of the kallikrein-kinin system induces hypoglycemia (Frey et al., 1932; Wicklmayr and Dietze, 1977). The administrations of kallikrein, the enzyme which catalyzes the formation of kallidin and BK, to human patients with non-insulin-dependent diabetes mellitus (NIDDM) (Wicklmayr and Dietze, 1977) or pancreatectomized dogs (Frey et al., 1932) result in a decrease in the plasma glucose concentration. The infusion of BK improves insulin activity in human patients with postoperative stress syndrome (Dietze et al., 1978; Dietze, 1982). In addition, BK increases glucose oxidation in adipocytes isolated from both normal and streptozotocin-induced diabetic rats (Symington et al., 1988). These findings indicate that BK directly promotes glucose utilization by cells.

The pancreas is one of the richest sources of tissue kallikrein (Bhoola et al., 1992) that is found in both islets and acini with most present in acini (Ørstavik et al., 1980; Pinkus et al., 1983). However, the function of the kinin system in the pancreas is not well-understood, except that tissue kallikrein could be involved in the formation of insulin from proinsulin (Ole-Moiyoi et al., 1979) and that BK may aggravate acute pancreatitis induced by cerulein in rabbits (Yotsumoto et al., 1993).

In a preliminary study, we found that two kinins, BK and kallidin, increased insulin release in the perfused canine and rat pancreata (unpublished results). Since BK, but not kallidin, is the physiological kinin in rats (Bhoola et al., 1992), the present study was undertaken to further investigate the stimulatory effect of BK on insulin release in the perfused rat pancreas.

Materials and Methods

Male Sprague-Dawley rats (320-400 g) were used in these experiments. The in situ perfused rat pancreas preparation with an open system was performed at 37°C using a modified method of Grodsky and Fanska (1975). The rats were anesthetized with pentobarbital Na (60 mg/kg, i.p., Fort Dodge Labs, Fort Dodge, IA) and their celiac arteries and portal veins were cannulated with polyvinyl tubings (0.625 mm, i.d.). The rats were maintained at 37° C during the experiment. The Krebs-Ringer bicarbonate buffer (KRB), supplemented with 10 mM HEPES, 6 mM glucose, and 0.2% bovine serum albumin (pH 7.4), was used as the perfusate (basal medium). This solution was continuously aerated with 95% O_2-5% CO_2. In our preliminary experiments, this medium with a flow rate of 1 ml/min did not cause noticeable edema or impair insulin release during the 60 min of perfusion.

In the present study, the first 10 min of perfusion was considered as an equilibration period. Subsequently, a fraction of the effluent fluid was collected every min from the cannula of the portal vein. In experiment 1, after a baseline period of 10 min, the medium containing BK (Sigma Chemical, St. Louis, MO) was administered for 5 min followed by the basal medium. In experiment 2, after a baseline period of 5 min, the medium containing 10 mM glucose was administered for 5 min followed by the basal medium for another 5 min for the washout. Subsequently, glucose (10 mM) with or without BK (1 μM) was administered for 5 min and followed by the basal medium for the washout. In experiment 3, after a baseline period of 5 min, the medium containing a BK_2-receptor antagonist HOE140 (0.1 μM) (D-Arg[Hyp³,Thf,D-Tic⁷,Oic⁸]BK, donated by Hoechst-Roussel Pharmaceuticals, Somerville, NJ) was administered for 5 min followed by HOE140/1 μM BK for another 5 min and the basal medium for the last 5 min for the washout. In experiment 4, after a baseline period of 5 min, the medium containing 10 mM glucose was administered for 5 min followed by the basal medium for the washout. Subsequently, 0.1 μM HOE140 was administered for 5 min and was followed by HOE140/glucose for another 5 min and the basal medium for the last 5 min for the washout. In all experiments except for the ones having 10 mM glucose, 15 mM glucose was perfused at the end of the experiment to confirm that the tissue retained normal secretory capacity under the experimental conditions. The data were excluded from those experiments in which the pancreas did not respond to 15 mM glucose. The effluent fractions were kept at 4°C and subsequently assayed within 12 h for insulin using radioimmunoassay (RIA) as previously described (Hale and Randle, 1963). Rat insulin was used as standards for the RIA.

Data expression and statistical analysis

Data of effluent concentrations of insulin were expressed as a percentage of baseline (mean of last five baseline values) and analyzed using analysis of variance (ANOVA) to determine the effects of pancreas, treatment, and time. The treatment x time interaction was used as an error term to determine the effect of treatment. The conservative F value was used to establish significance for the effect of treatment. Tukey's Highly Significant Difference test was used to determine differences between treatments for which the ANOVA indicated a significant ($P<0.05$) F ratio.

Results

BK (0.01 - 1 μM) increased insulin release from the perfused rat pancreas with the onset time of <1 min, and reached maximum response at ≤3 min (Fig. 1). The BK (0.01, 0.1, 1 μM)-induced increases in the peak effluent concentrations of insulin were 1.5, 2.5, and 3.5 times, respectively, above the basal control group.

Repeated administrations of 10 mM glucose induced a 6.5-fold increase in the peak effluent concentration of insulin with no significant differences between the two glucose administrations (Fig. 2). BK alone (1 μM) caused a 3.5-fold increase in the peak effluent concentration of insulin. BK significantly enhanced the effect of glucose on insulin release by raising the peak effluent concentration of insulin to 11.2 folds above the basal control group.

A BK$_2$-receptor antagonist, HOE140, blocked the BK-induced insulin release (Fig. 3). BK alone (1 μM) significantly increased the effluent concentration of insulin when compared with the basal control group. Pretreatment with HOE140 (0.1 μM) significantly lowered the insulin level when compared with the basal control group. This pretreatment further abolished the BK (1 μM)-induced increase in insulin release. In fact, the HOE140 + BK group had significantly lower effluent insulin concentrations than the basal control group.

The effect of HOE140 on the glucose-induced increase in insulin release is depicted in Fig. 4. Repeated administrations of 10 mM glucose induced a 6.8-fold increase in the peak effluent concentration of insulin with no significant differences between the two glucose administrations. HOE140 (0.1 μM) significantly lowered the baseline insulin level and significantly attenuated the glucose-induced increase in the effluent concentration of insulin by 31%.

Discussion

BK (0.01 - 1 μM) evoked insulin release from the perfused rat pancreas in a dose-dependent manner. Although the pancreatic concentrations of kinins are not known, this effect of bradykinin may be physiologically significant, since in other tissues, e.g., the submaxillary gland (Scicli et al., 1983) and kidney (Barabé et al., 1982), concentrations of BK are detectable in the range of ng/mg protein. The pancreas is one of the richest sources for tissue kallikrein (Bhoola et al., 1992) and thus pancreatic kallikrein should promote the formation of kinins in this organ.
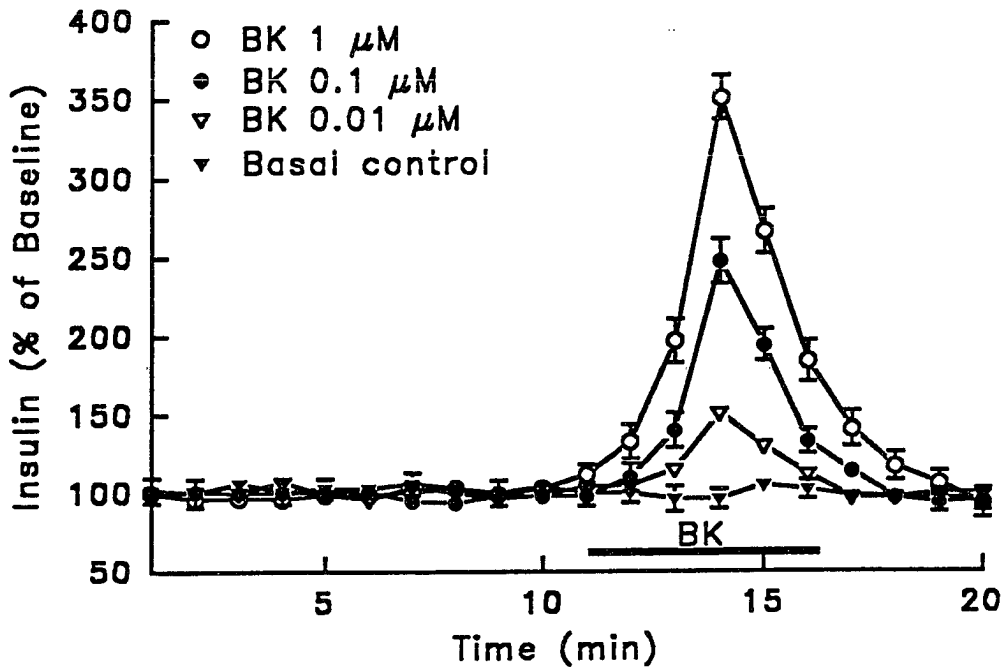
Fig. 1. Effect of bradykinin (BK) on insulin release from the perfused rat pancreas. In this and subsequent figures, a 10-min equilibration period preceded time 0. after a baseline period of 10 min, BK (shown in the heavy line) was administered for 5 min followed by the basal medium. Values are shown as mean ± SE (n=6). The baseline effluent concentrations of insulin were 6.6 ± 0.5, 7.0 ± 0.6, 8.3 ± 0.7, and 7.0 ± 0.6 ng/ml for the control, 0.01 μM, 0.1 μM, and 1 μM BK groups, respectively.
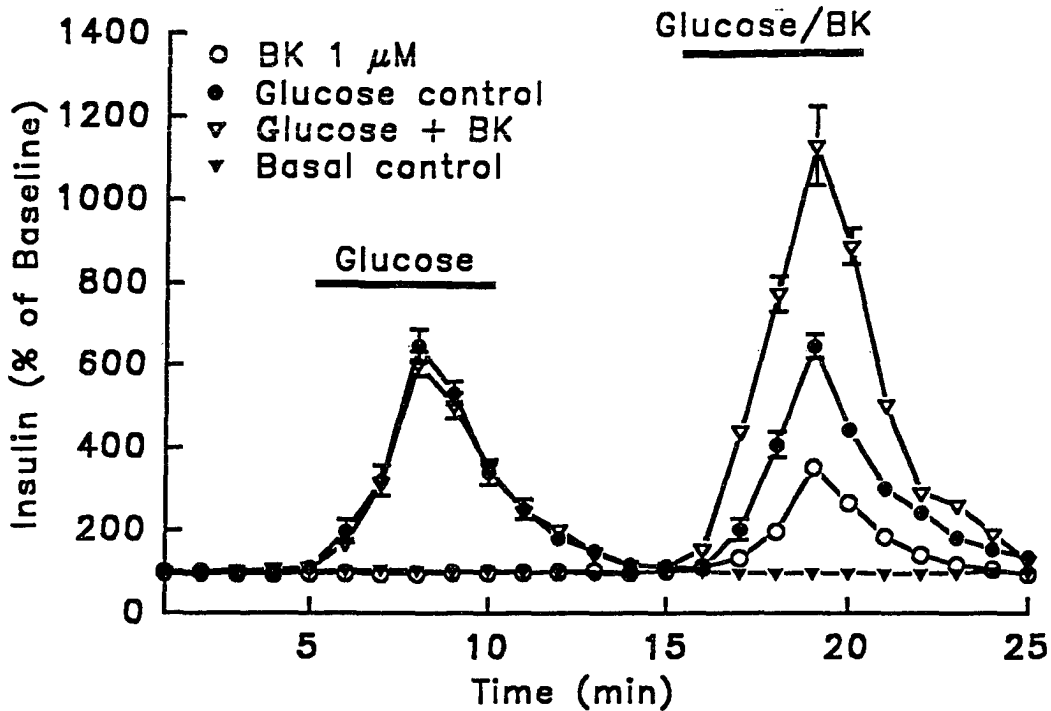
Fig. 2. Enhancement by bradykinin (BK) of the glucose-induced insulin release in the perfused rat pancreas. After a baseline period of 5 min, 10 mM glucose was administered for 5 min followed by the washout for another 5 min. Subsequently, glucose (10 mM) with or without BK (1 μM) was administered for 5 min. The presences of treatments are indicated by heavy lines. Values are shown as mean ± SE (n=6). The baseline effluent concentrations of insulin were 6.6 ± 0.5, 6.3 ± 0.7, 7.1 ± 0.6, and 5.2 ± 0.4 ng/ml for the basal control, glucose control, BK, and glucose + BK groups, respectively.
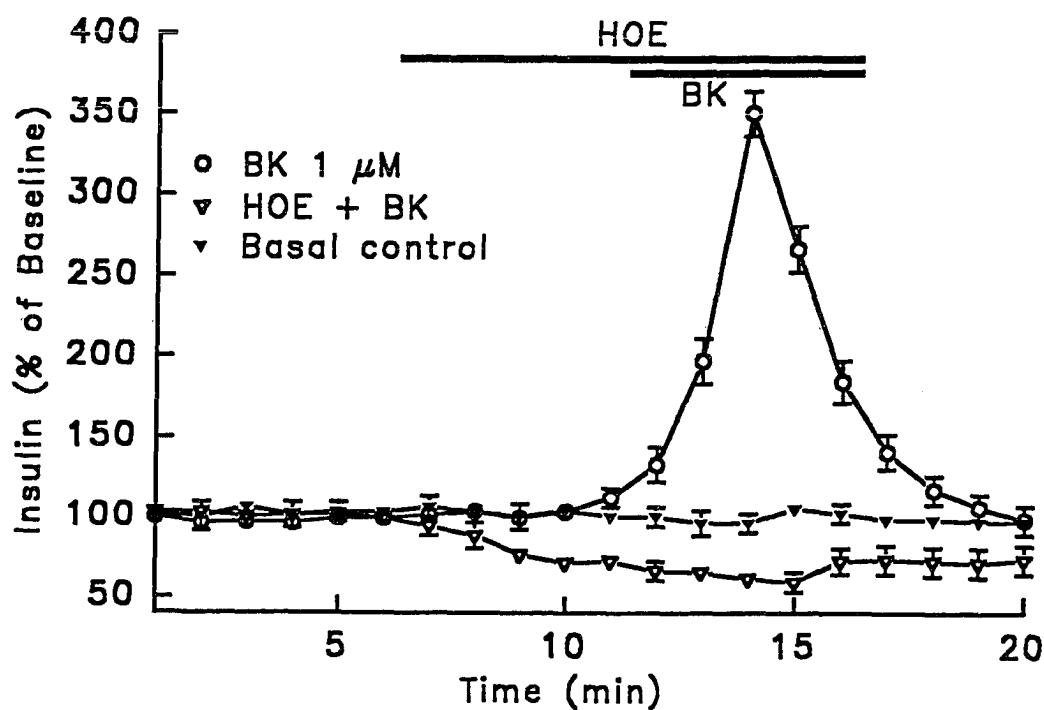
Fig. 3. Effect of HOE140 on the basal and bradykinin (BK)-induced insulin release in the perfused rat pancreas. After a baseline period of 5 min, 0.1 μM HOE was administered for 5 min followed by HOE/1 μM BK for another 5 min. The presences of treatments are indicated by heavy lines. Values are shown as mean ± SE (n=6). The baseline effluent concentrations of insulin were 6.6 ± 0.5, 7.1 ± 0.6, and 7.7 ± 0.3 ng/ml for the basal control, BK, and HOE140 + BK groups, respectively.
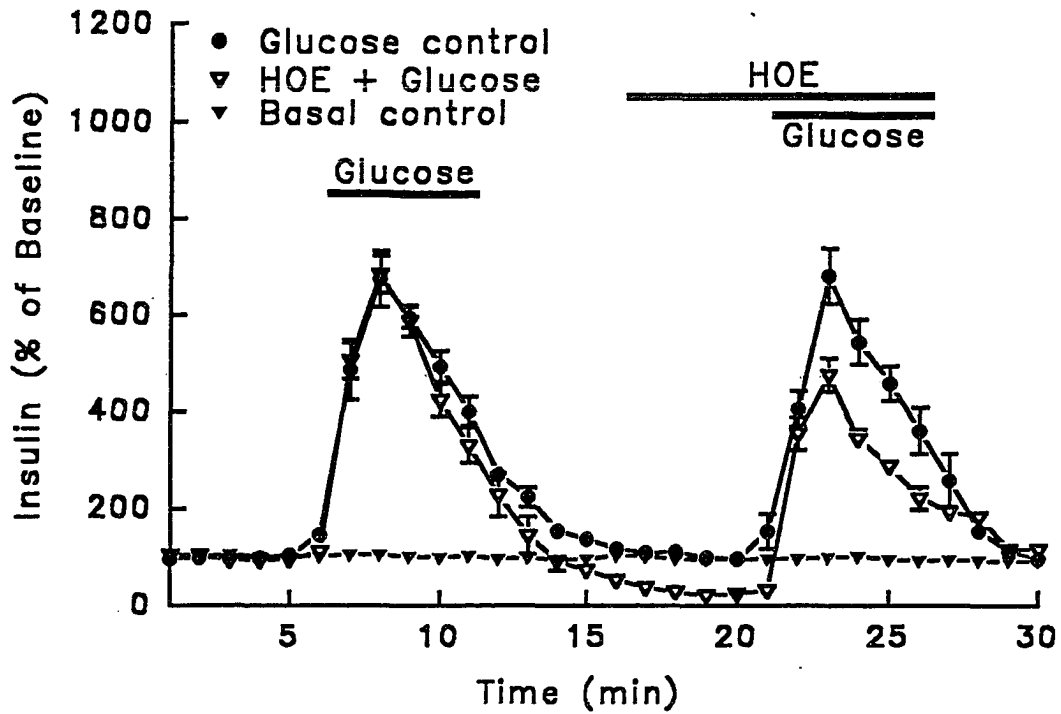
Fig. 4. Effect of HOE140 on the basal and glucose-induced insulin release in the perfused rat pancreas. After a baseline period of 5 min, 10 mM glucose was administered for 5 min followed by a washout. Subsequently, 0.1 µM HOE was administered for 5 min and was followed by HOE/glucose for another 5 min. The presences of treatments are indicated by heavy lines. Values are shown as mean ± SE. The baseline effluent concentrations of insulin were 6.6 ± 0.5, 8.0 ± 3.2, and 6.6 ± 0.7 ng/ml for the basal control (n=6), glucose control (n=5), and HOE140 + glucose (n=4) groups, respectively.

Further studies are needed to determine the pancreatic concentrations of BK in rats under different experimental conditions. This information will help to determine the physiological significance of BK in stimulating insulin release.

Most of the kinin action is mediated through BK_2-receptors (Bhoola et al., 1992), while BK_1-receptors seem to be absent normally, with expression in smooth muscle cells, fibroblasts, and pain fibers evident only in pathological states, e.g., inflammation (Bhoola et al., 1992) and in the rabbit isolated vascular smooth muscle de novo in a time-dependent manner (Barabé et al., 1982). The stimulatory effect of BK on insulin release may be mediated by BK_2-receptors, since a specific BK_2-receptor antagonist, HOE140 (0.1 μM), abolished this effect of BK. Also, 0.1 μM of HOE140 has been shown to block maximally the effects of kinins mediating through BK_2-receptors in other tissues (14). In addition, we found that HOE140, but not a BK_1-receptor antagonist des-Arg^9,[Leu^8]-BK (1 μM), antagonized the BK-induced insulin release from a clonal β-cell line RINm5F (unpublished results).

In the present study, HOE140 not only abolished the BK-induced insulin release, but it even lowered the baseline insulin release and attenuated the glucose-induced insulin release. The HOE140-induced inhibition of insulin release probably is due to a specific effect on β-cells which blocks the influence of endogenous BK, since this drug did not inhibit insulin release in vitro from RINm5F cells (unpublished results). Therefore, our findings further suggest that kinins an their receptors could play an important role in the regulation of insulin release. However, more studies are needed to establish the physiological significance of kinins in stimulating insulin release. In particular, the dose-response characteristics of HOE140 on the BK effect, baseline and glucose-stimulated insulin release will help to establish this physiological significance.

Since the acini contain most of the tissue kallikrein found in the pancreas, it is likely that the acinar kinins can alter insulin secretion from the β-cells of the islet. To date, there is no direct evidence that acinar tissues affect islet function, but clinical observations support this premise. Human patients with chronic pancreatitis or acinar atrophy develop glucose intolerance as a result of insufficient insulin release (Bendayan, 1990). This reduction in insulin release may be due to a lack of the acinus-islet interaction, because the reduction of β-cell numbers in these human patients appears too small to explain the impaired insulin secretion in response to various stimuli (Vinik and Jackson, 1980). In addition, pancreatic acinar atrophy is induced with a Cu^{2+}-deficient diet in rats (Fölsch et al., 1988). Both the baseline and glucose-stimulated plasma insulin concentrations of these rats were lower than the controls despite the fact that the insulin content of the islets was

not significantly different between normal rats and rats with acinar atrophy (Fölsch et al., 1988). Future studies using the acinar atrophy model may gain an insight into the physiological role of kinins in the regulation of insulin release.

Acknowledgments

References

Baertschi, A. J., H. H. Zingg, and J. J. Dreifuss. Enkephalins, substance P, bradykinin and angiotensin II: differential sites of action on the hypothalamo-neurohypophysial system. Brain Res. 220:107-119, 1981.

Barabé, J., C. Babiuk, and D. Regoli. Binding of [^{3}H]des-Arg^9-BK to rabbit anterior mesenteric vein. Can. J. Physiol. Pharmacol. 60: 1551-1555, 1982.

Barabé, J., D. Huberdeau, and A. Bernoussi. Influence of sodium balance on urinary excretion of immunoreactive kinins in rats. Am. J. Physiol. 254:F484-F491, 1988.

Beierwaltes, W. H., J. Prado, and O. A. Carretero. Kinin stimulation of renin release in isolated rat glomeruli. Am. J. Physiol. 248: F757-F761, 1985.

Bendayan, M. Anatomic basis of islet-acinar interaction in the pancreas. Regul. Pept. Lett. 2: 8-11, 1990.

Bhoola, K., C. D. Figueroa, and K. Worthy. Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol. Rev. 44: 1-80, 1992.

Dietze, G., M. Wicklmayr, L. Mayer, I. Bottger, and H. Von Funcke. Bradykinin and human forearm metabolism: inhibition of endogenous prostaglandin synthesis. Hoppe-Seylers Z. Physiol. Chem. 359: 369-378, 1978.

Dietze, G. J. Modulation of the action of insulin in relation to the energy state in skeletal muscle tissue: possible involvement of kinins and prostaglandins. Mol. Cell. Endocrinol. 25:127-149, 1982.

Drouhault, R. T., N. Abrous, J. P. David, and B. Dufy. Bradykinin parallels thyrotrophin-releasing hormone actions on prolactin release from rat anterior pituitary cells. Neuroendocrinology 46: 360-364, 1987.

Fölsch, U. R., M. Fussek, R. Ebert, and W. Creutzfeldt. Endocrine pancreatic function during atrophy of the exocrine gland. Pancreas 3: 536-542, 1988.

Frey, E. K., H. Draut, and E. Werle. Uber die blutzuckersenkende Wirkung des Kallikreins (Padutins). Klins. Wochenschr. 11: 846-849, 1932.

Grodsky, G. M., and R. E. Fanska. The in vitro perfused pancreas. In: Methods in Enzymology, Vol.XXXIX, Hormone Action, Pat D. Isolated Cells, Tissues, and Organ Systems, edited by J. G. Hardman and B. W. O'Malley, New York, Academic Press, 1975, p. 364-372.

Hale, C. N., and P. J. Randle. Immunoassay of insulin with insulin antibody precipitate. Biochem. J. 88: 137-146, 1963.

Hock, F.J., K. Wirth, U. Albus, W. Kinz, H. J. Gerhards, G. Wiemer, Henke, St., G. Breipohl, W. König, J. Knolle, B. A. Schölkens. Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. Br. J. Pharmacol. 102: 769-773, 1991.

Jones, T. H., B. L. Brown, and P. R. M. Dobson. Evidence that angiotensin II is a paracrine agent mediating gonadotrophin-releasing hormone, inositol phosphate production and prolactin secretion in the rat. J. Endocrinol. 116:367-371, 1988.

Ole-Moiyoi, O., D. C. Seldin, J. Spragg, G. Pinkus, and K. F. Austen. Sequential cleavage of pro-insulin by human pancreatic kallikrein and a human pancreatic kinase. Proc. Natl. Acad. Sci. USA 76:3612-3616, 1979.

Ørstavik, T. B., P. Brandtzaeg, K. Nustad, and J. V. Pierce. Immunohistochemical localization of kallikrein in human pancreas and salivary glands. J. Histochem. Cytochem. 28: 557-562, 1980.

Pinkus, G. S., M. Maier, D. C. seldin, O. Ole-Moiyoi, K. F. Austen, and J. Spragg. Immunohistochemical localization of glandular kallikrein in the endocrine and exocrine human pancreas. J. Histochem. Cytochem. 31: 1279-1288, 1983.

Scicli, A. G., T. B. Orstavik, S. F. Rabito, F. D. Murray, and O.A. Carretero. Blood kinins after sympathetic nerve stimulation of the rat submaxillary gland. Hypertension 5: I101-I106, 1983.

Staszewska-Barczak, J., and J. R. Vane. The release of catecholamines from the adrenal medulla by peptides. Br. J. Pharmacol. 30: 655-667, 1967.

Symington, R. P., A. Ashford, and G. Bailey. Effect of bradykinin and insulin on glucose oxidation in adipocytes from normal and streptozotocin diabetic rats. Biochem. Soc. Trans. 16: 1012-1013, 1988.

Vinik, A. I., and W. P. U. Jackson. Endocrine secretions in chronic pancreatitis. In: Secondary Diabetes: The Spectrum of the Diabetic Syndrome, edited by S. Podolsky and M. Viswanathan. New York, Raven Press, 1980, p. 165-189.

Wicklmayr, M., and G. Dietze. Effect of oral kallikrein and intrabrachial-arterial bradykinin on forearm-metabolism in maturity onset diabetes. In: Kininogenases, Kallikrein 4, edited by G. L. Haberland, J. W. Rohen, and T. Suzuki, Stuttgart-New York, Schattauer Verlag, 1977, p. 299-308.

Yotsumoto, F., T. Manabe, and G. Ohshio. Bradykinin involvement in the aggravation of acute pancreatitis in rabbits. Digestion 54: 224-230, 1993.

CHAPTER 3. GLUCOSE-DEPENDENCY OF BRADYKININ-INDUCED INSULIN SECRETION FROM THE PERFUSED RAT PANCREAS

A paper to be submitted to the American Journal of Physiology

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Abstract

In order to investigate the glucose dependency of bradykinin (BK)-induced insulin secretion, rat pancreata were perfused in situ with BK (1 µM) for 30 min in the presence of glucose concentrations of 1 - 20 mM. Glucose (6 mM) alone induced a steady basal insulin secretion which was 6.7 ± 0.5 ng/ml. Glucose of 1 mM gradually decreased insulin secretion to ~ 30-40% of the baseline level in which insulin secretion was maintained by 6 mM glucose. Glucose of 10 and 20 mM induced a biphasic insulin secretion, characterized by a transient peak and a sustained phase; the sustained phase was higher than the transient peak. Glucose of 20 mM induced a higher insulin secretion than 10 mM glucose. When BK was administered in 1 mM glucose, it induced a transient insulin release peak which was ~ 2.8-fold of the baseline level, then the insulin concentrations of the effluents decreased gradually to the levels which were slightly higher than that of the 1 mM glucose alone, but lower than the baseline level. After BK was administered in 6 mM glucose, it induced a transient insulin release peak which was ~ 3.6-fold of the baseline, and followed by a sustained insulin secretion phase which was significantly higher than that of the 6 mM glucose control group, but lower than the transient peak. When BK was perfused with 10 mM or 20 mM glucose, it induced a similar insulin secretion pattern as in glucose (10 or 20 mM) alone. However, the combination of glucose and BK induced a higher transient insulin release peak, about 7.8- and 12-fold of the baseline, respectively. Also, the BK-glucose-induced sustained phase was higher than the transient peak. BK induced a greater potentiation of insulin secretion in 20 mM glucose than in 10 mM glucose. Taken together, our findings suggest that BK induced insulin secretion from rat pancreata in a glucose-dose-dependent manner. The higher the glucose concentration, the greater the potentiation in BK-induced insulin secretion. Since the sustained insulin secretion phase is due to increased synthesis of insulin, BK may stimulate both the synthesis and release of insulin.

Introduction

Kinins, including bradykinin (BK), display an extraordinarily high degree of physiological activities (Pisano and Austin, 1974). They influence a number of biological processes, including blood pressure and local blood flow (Nwator and Whalley; 1989; Pan et al., 1993), pain (Steranka et al., 1988) and inflammation (Figueroa,1990), increased vascular permeability and local edema (Carter et al., 1974), contraction of smooth muscle (Collier et al., 1962), decreased blood glucose concentration (Wicklmayr and Dietze, 1977), promotion of cell proliferation (Goldstein et al., 1984; Marceau and Tremble, 1986), increased glucose uptake by intact intestine (Sharp and Debnam, 1992; Kellett and Barker, 1989), and other cellular functions.

Kinins also stimulate the release of hormones, e.g., renin from the kidney (Beierwalres et al., 1985), vasopressin from the posterior pituitary gland (Baertschi et al., 1981), growth hormone and prolactin from the anterior pituitary gland (Drouhault et al., 1987; Jones et al., 1988) and catecholamines from the adrenal medulla (Staszewska-Barczak and Vane, 1967). The effects of BK are mediated by at least two groups of BK receptors, BK_1 and BK_2, but the latter mediates most of the effects of BK (Roberts, 1989).

BK reduces blood glucose concentrations in post-surgical patients (Jauch et al., 1986) and in isolated ischemic hearts (Needleman et al., 1975) by enhancing glucose uptake, improving insulin-induced glucose transport, decreasing glucagon-induced glucose production and reducing gluconeogenesis (Jauch et al., 1989). In alloxan-diabetic rats, captopril, an inhibitor of angiotensin-converting enzyme, inhibits the breakdown of kinins, thereby potentiating BK-induced hypoglycemia (Jaffa et al., 1986). In addition, BK increases glucose oxidation in adipocytes isolated from both normal and streptozotocin-induced diabetic rats (Symington et al., 1988). Thus, BK directly promotes glucose utilization by cells.

BK may regulate glucose metabolism by increasing insulin secretion. In our previous study, administration of BK for 5 min caused a dose-dependent increase in insulin secretion which was mediated by BK_2-receptors (Yang and Hsu, 1995). BK also enhanced glucose-induced insulin release. The specific BK_2-receptors blocker, HOE 140, reduced the basal insulin secretion, abolished the BK-induced insulin secretion, and attenuated the glucose-induced insulin secretion. These results indicated that endogenous bradykinin plays a physiological role in maintaining the basal and glucose-induced insulin secretion (Yang and Hsu, 1995). However, the glucose-dependency of BK-induced insulin secretion remains unknown.

In the present study, the rat pancreas was perfused with BK for 30 minutes in the presence of various glucose concentrations to investigate the effect of BK and if BK-induced insulin secretion was glucose-dependent.

Materials and Methods

Male Sprague-Dawley rats (300-400 g) were anesthetized with pentobarbital sodium (60 mg/kg ip; Fort Dodge Labs, Fort Dodge, IA) and kept on a 37°C hot plate. The rat pancreatic perfusion was performed as previously described (Yang and Hsu, 1995). After celiac arteries were cannulated, the rat pancreata were immediately perfused in situ with oxygenated (95% O_2/5% CO_2) Krebs-Ringer bicarbonate (KRB) solution supplemented with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 6 mM glucose, 0.2% bovine serum albumin and pH 7.4 as the basal medium. The perfusion rate was maintained at 1 ml/min and the effluent was collected every minute from the cannulated portal vein.

After the rat pancreatic preparations were established, the first 10-15 min of perfusion was considered as the equilibration period. After a baseline period of 10 min, the perfusate containing glucose (1, 6, 10, or 20 mM) was administered for 30 min with or without BK (1 μM) (Sigma Chemicals, St. Louis, MO). In our previous study, BK (0.01 - 1 μM) dose-dependently increased insulin release and BK 1 μM induced a 3.7-fold increase of the baseline level of insulin (Yang and Hsu, 1995). Therefore, we used 1 μM BK in these experiments. The effluent fractions were kept at 4°C and subsequently assayed within 12 h for insulin by using radioimmunoassay (RIA) as previously described (Hale and Randle,1963). Rat insulin was used as standards for the RIA.

Data of effluent insulin concentrations are expressed as a percentage of the baseline level (mean of 10 baseline values) in means ± SE. For results in Fig. 5, the areas under the 30 min perfusion curve were calculated with a scanning program (SigmaScan; Jandel, Corte Madera, CA). Data were analyzed by using analysis of variance (ANOVA) to determine the significance of treatment and time. The treatment x time interaction was used as an error term to determine the effect of treatment. The significance of treatment was determined from the conservative F value. Tukey's highly significant different test was used to determine the differences between treatments for which the ANOVA indicated a significant ($P \leq 0.05$) F ratio.

Results

The insulin secretion in the 6 mM glucose control group was maintained at a steady level of 6.7 ± 0.5 ng/ml (n = 4), throughout the perfusion period (Fig. 1). BK (1 μM) induced a biphasic insulin secretion. The onset time of insulin secretion was less than 1 min and reached the peak that was ~ 3.7-fold of the baseline level at ≤ 3 min, then the insulin levels decreased gradually within 5 min to a level which was still significantly higher (~ 50 %) than that of the basal level and maintained at this level to the end of perfusion (Fig. 1). The combination of BK and 6 mM glucose in insulin secretion was significantly higher than that of 6 mM glucose alone.

Glucose of 1 mM alone reduced insulin secretion to a level of 30-40% of the baseline level in which insulin secretion was maintained by 6 mM glucose (Fig. 2). Administration of BK (1 μM) in 1 mM glucose induced a transient insulin release with a peak of 2.8-fold of the baseline level. Subsequently, the insulin levels decreased gradually to a level ~ 50 % of the baseline level, which was still slightly higher than that of the 1 mM glucose alone group (Fig. 2). Although the concentrations of BK plus 1 mM glucose treatment group in insulin secretion was lower than that of baseline level which was perfused with 6 mM glucose alone, the insulin concentrations were still significantly higher than that of 1 mM glucose alone.

Glucose of 10 mM alone also induced a biphasic insulin secretion. The first transient insulin release peak was 7.8-fold of the baseline level at 3 min; subsequently, the insulin levels decreased to 4.5-fold of the baseline level within 4 min, followed by a sustained level of ~ 9-fold of the baseline level (Fig. 3). By comparing with the 10 mM glucose alone treatment group, the combination of BK and 10 mM glucose significantly increased insulin secretion which was higher than that of 10 mM glucose alone. It showed a similar insulin secretion pattern to that induced by 10 mM glucose alone; BK increased insulin secretion with the first peak and sustained level of 12- and 13.5-fold of the baseline level, respectively (Fig. 3).

Glucose of 20 mM alone also caused a similar insulin secretion pattern to that induced by 10 mM glucose; the first transient peak and sustained phase were 12- and 15-fold of the baseline level, respectively (Fig. 4). BK (1 μM) significantly enhanced 20 mM glucose-induced insulin secretion; the first peak and sustained phase were about 18- and 24-fold of the baseline level, respectively (Fig. 4). The insulin secretion of BK plus 20 mM glucose was significantly higher than that of 20 mM glucose alone.
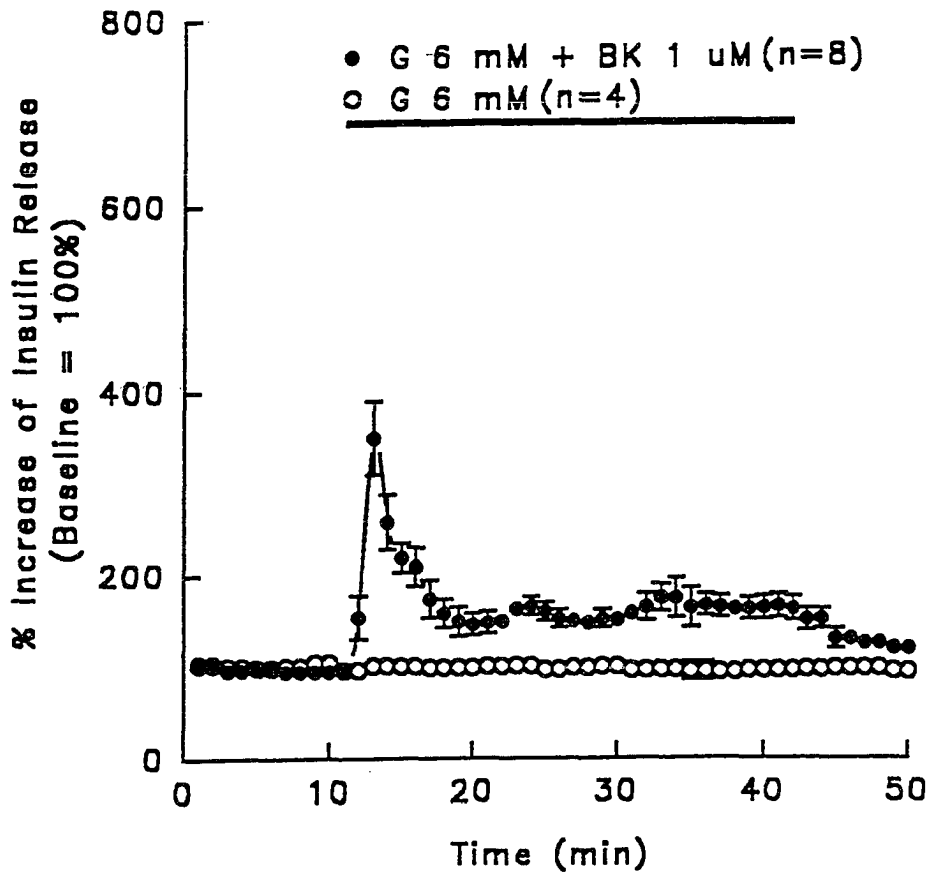
Fig. 1. Effect of 1 μM bradykinin (BK) in the medium containing 6 mM glucose (G) on insulin secretion from the perfused rat pancreas. BK was administrated for 30 min (bar). Values are shown as means ± SE. In this and all other figures, G of 6 mM was administered during the equilibration period of 10 -15 min followed by another 10 min of the baseline period. The baseline concentrations of insulin in the effluents were 6.7 ± 0.5 ng/ml (n = 4 rats) and 6.6 ± 0.5 ng/ml (n = 8 rats) for 6 mM glucose alone and bradykinin + glucose groups, respectively.
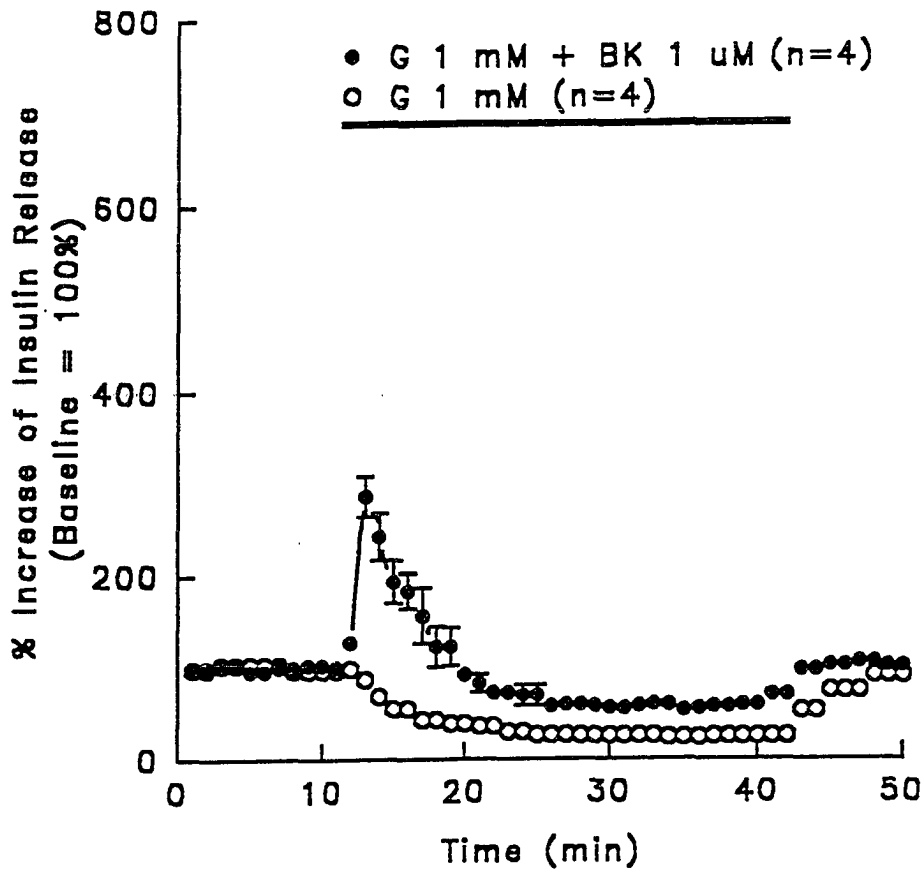
Fig. 2. Effect of 1 μM bradykinin (BK) in the medium containing 1 mM glucose (G) on insulin secretion from the perfused rat pancreas. BK was administrated for 30 min (bar). Values are shown as means ± SE. The baseline concentrations of insulin in the effluents were 6.0 ± 0.5 ng/ml (n = 4 rats) and 5.5 ± 0.6 ng/ml (n = 4 rats) for 1 mM glucose alone and bradykinin + glucose groups, respectively.
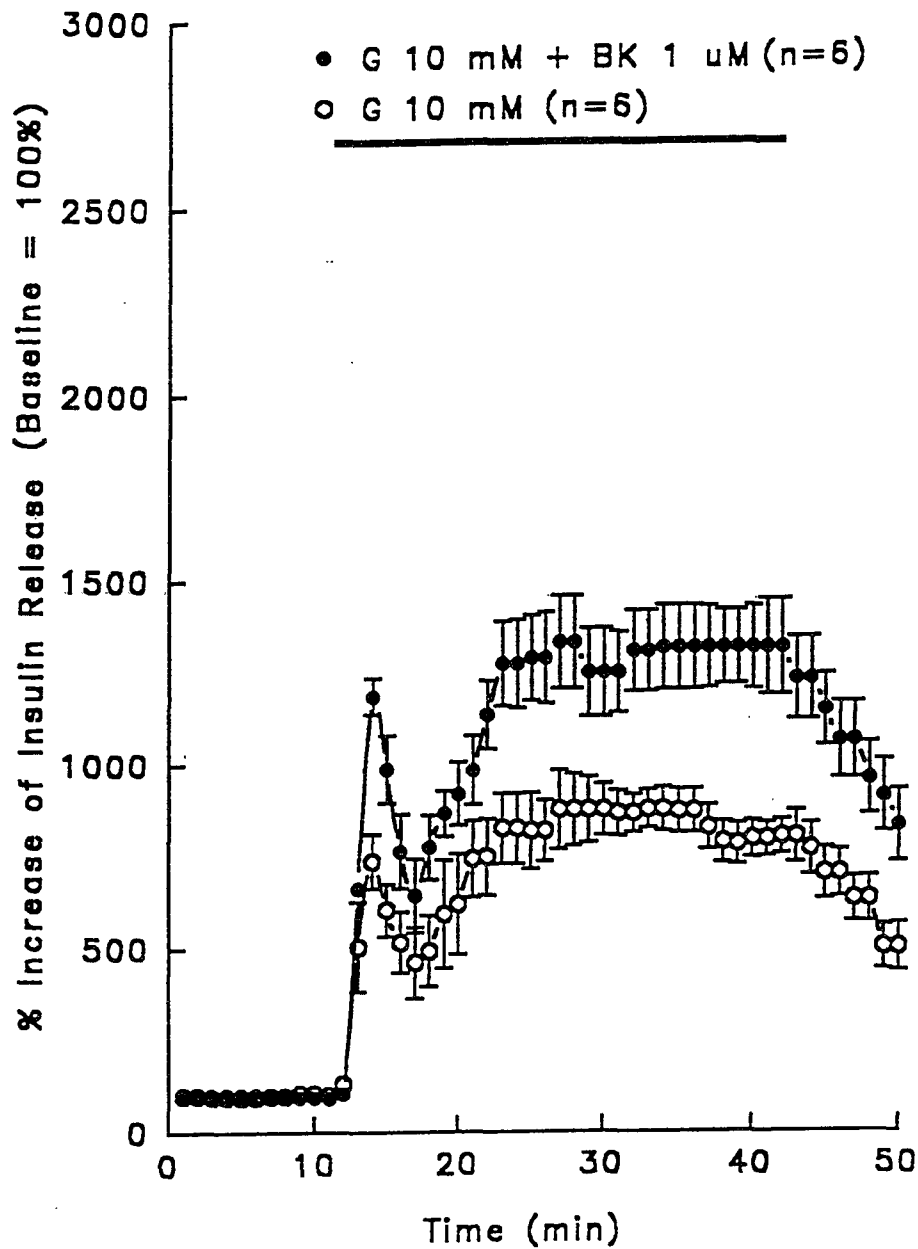
Fig. 3. Effect of 1 μM bradykinin (BK) in the medium containing 10 mM glucose (G) on insulin secretion from the perfused rat pancreas. BK was administrated for 30 min (bar). Values are shown as means ± SE. The baseline concentrations of insulin in the effluents were 6.5 ± 0.6 (n = 6 rats) and 6.8 ± 0.5 ng/ml (n = 6 rats) for 10 mM glucose alone and bradykinin + glucose groups, respectively.
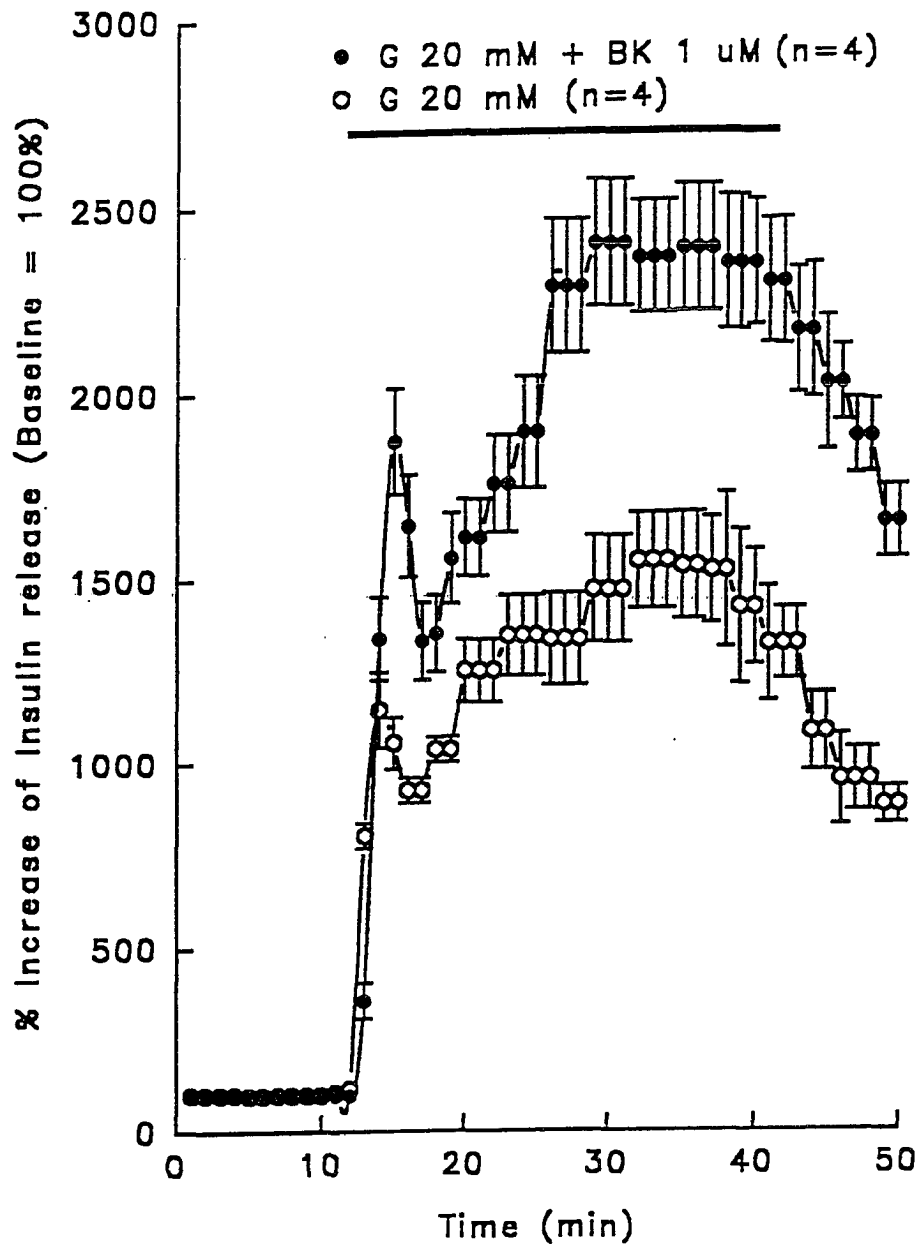
Fig. 4. Effect of 1 μM bradykinin (BK) in the medium containing 20 mM glucose (G) on insulin secretion from the perfused rat pancreas. BK was administrated for 30 min (bar). Values are shown as means ± SE. The baseline concentrations of insulin in the effluents were 5.5 ± 0.5 (n = 4 rats) and 5.8 ± 0.5 ng/ml (n = 4 rats) for 20 mM glucose alone and bradykinin + glucose groups, respectively.

By calculating the areas under the 30 minutes insulin secretion curve and by using the area of 6 mM glucose treatment group as 100%, glucose induced a dose-dependent increase in insulin secretion, and BK significantly potentiated glucose-induced insulin secretion in a glucose-dose-dependent manner (Fig. 5). The higher the glucose concentration, the more potentiation.

Discussion

The major kinin released in rats is BK (Bhoola et al., 1992). The BK concentration of rat pancreas is not known; however, the kininogen precursors are abundant in plasma (55 µg/ml) (Bhoola et al., 1992; Reddigari and Kaplan, 1989) and the circulatory kininogens can bind to the surface receptors of the endothelial cells as a substrate for BK synthesis (Schmaier et al., 1987). In addition, the catalytic enzymes, kallikreins are synthesized and rich in the pancreas (Bhoola et al., 1992; Pinkus et al., 1983). The concentration of BK are in the range of ng/mg protein or µM in the rat submaxillary gland (Scicli et al., 1983) and kidney (Barabe et al., 1988).

BK decreases blood glucose concentration in patients with non-insulin dependent diabetic mellitus (Wicklmayr and Dietze, 1977), and regulates the availability and utilization of glucose in the target tissues (Jauch et al., 1989). In our previous report, we perfused rat pancreata with BK which dose-dependently increased insulin release through activation of BK_2-receptors (Yang and Hsu, 1995). In the present study, BK potentiated glucose-induced insulin release in a glucose dose-dependent manner. Thus, the hypoglycemic effect of BK may be partly mediated through increased insulin release which, in turn, promotes the transport and utilization of glucose.

We also investigated the influence of ultralow glucose of 1 mM (18 mg/dl) on insulin release from the perfused rat pancreas. The abnormally low glucose concentration of 1 mM alone significantly decreased the basal insulin release to a level of 30-40% of the baseline level. Glucose of 6 mM (108 mg/dl) which is close to normal physiological concentration, maintained a steady insulin secretion throughout the perfusion period, whereas, glucose at high concentration of 10 and 20 mM induced a biphasic insulin secretion pattern, characterized by a transient peak and a sustained phase. These results were similar to those found in isolated perfused rat pancreata (Grodsky and Bennett, 1963; Grodsky et al., 1969) and in humans (Cerasi, 1967; Cerasi and Luft, 1967). The transient phase is attribute to the rapid transport and release of pre-existing insulin from the secretory granules, whereas, the sustained phase is attributed to both the increased synthesis of insulin and the release of stored insulin (Lacy, 1970; Lacy et al., 1972).
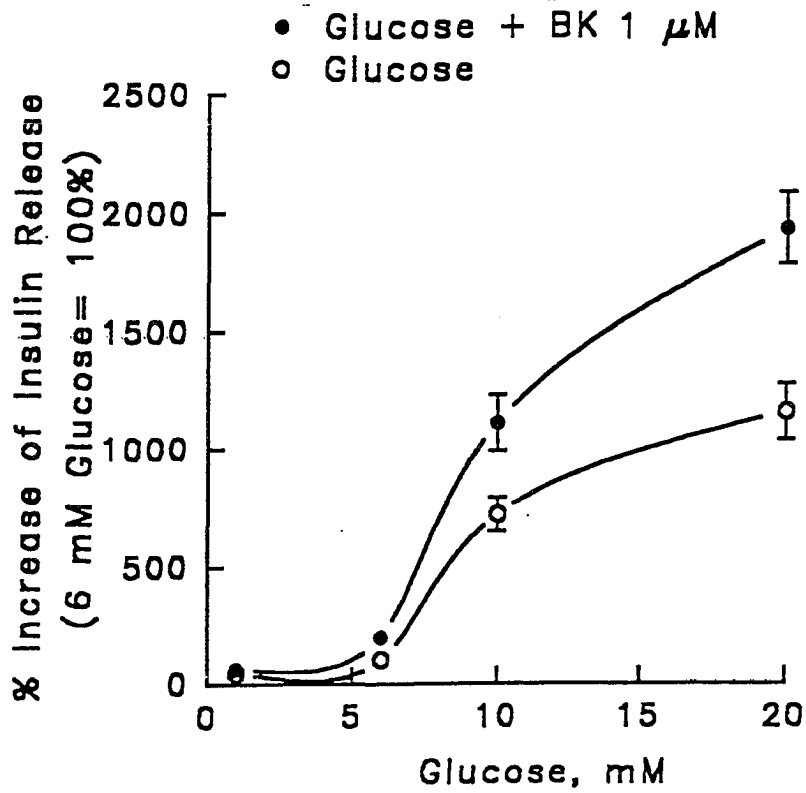
Fig. 5. Effect of glucose on bradykinin (BK)-induced insulin secretion in the perfused rat pancreas. Values were obtained by calculating the areas under the 30-min insulin secretion curve and by using the area induced by the 6 mM glucose alone group as 100%, and are shown as means ± SE.

Perfusion of rat pancreata with BK for 5 min induced only a transient release of insulin (Yang and Hsu, 1995). Thus, in a short-term administration, BK may only stimulate insulin release. However, in the present study, administration of BK for 30 min induced a biphasic insulin secretion. Thus, in the long-term administration of 30 min, BK not only stimulates insulin release, but also increases both the synthesis and the release of insulin.

BK in the presence of 1 mM glucose only had a small and transient stimulating effect on insulin secretion, whereas it induced a greater increase in insulin secretion in high glucose concentrations than that in low glucose concentrations in both phases. Therefore, we suggest that BK induces insulin secretion in a glucose-dependent manner. Glucose dependency also occurs to glucagon-like peptides-1-induced insulin secretion (Hargrove et al., 1995; Goke et al., 1993). In normal rats, GLP-1 (5 pmol/min/kg IV) only induces a small transient increase in plasma insulin in vivo, whereas, at high glucose concentrations of 11 and 17 mM, it induces an incremental increase in insulin (Hargrove et al., 1995). The glucose dependency of cholecystokinin-8-induced insulin secretion also occurs at the high glucose levels of 11.1 and 16.7 mM in rat perfused pancreata and perifused islets (Kimura et al., 1994). These reports were similar to our findings. However, in isolated rat islet, the onset of GLP-1(25 nM)-induced insulin secretion was at 3 mM glucose, whereas, in perfused pancreas, it induces a strong insulin secretion at 5 mM glucose (Goke et al., 1993).

In our previous study, BK enhances 10 mM glucose-induced insulin release (Yang and Hsu, 1995). In addition, the specific BK_2-receptor antagonist, HOE 140 (0.1 μM), reduces the basal and glucose-induced insulin secretion (Yang and Hsu, 1995). Thus, endogenous BK may be involved in glucose-induced insulin secretion under normal physiological conditions. In the present study (Fig. 5), when BK was administered in the presence of glucose concentrations from 1, 6, 10 and 20 mM for 30 min, the potentiation by BK of insulin secretion was increased as the concentration of glucose increased. With high glucose concentration (10 or 20 mM), BK also induced a higher sustained insulin secretion phase than the transient insulin release peak. Further studies are needed to determine the concentration of BK in the pancreas and the mechanisms by which BK potentiates glucose-induced insulin secretion.

References

Baertsci, A. J., H. H. Zingg, and J. J. Dreifuss. Enkephalins, substance P, bradykinin, and angiotensin II: differential sites of action on the hypothalmo-neurohypophysial system. Brain Res. 220: 107-119, 1981.

Barabe, J., D. Huberdean, and A. Bernoussi. Influences of sodium balance on urinary excretion of immunoreactive kinin in rats. Am. J. Physiol. 254: F484-F491, 1988.

Beierwaltes, W. H., L. Prado, and O. A. Carretero. Kinin stimulation of renin release in isolated rat glomeruli. Am. J. Physiol. 248: F757-F761, 1985.

Bhoola, K. D., C. D. Figueroa, and K. Worthy. Bioregulation of kinin: kallikrein, kininogen, and kininases. Pharmacol. Rev. 44: 1-80, 1992.

Carter, R. D., W. L. Joyner, and E. M. Renkin. Effect of histamine and some other substances on molecular selectivity of the capillary wall to plasma proteins and dextran. Microvasc. Res. 7: 31-48, 1974.

Cerasi, E. An analogue computer model for the insulin response to glucose infusion. Acta Endocrinol. 55 : 163-183, 1967.

Cerasi, E., and R. Luft. Insulin response to glucose infusion in diabetic and non-diabetic monozygotic twin pairs. Genetic control of insulin response. Acta Endocrinol. 55: 330-345, 1967.

Collier, H. O., J. A. Holgate, M. Schachter, and P. G. Shorley. The bronchoconstrictor action of bradykinin in the Guinea pig. Br. J. Pharmacol. 15: 290-297, 1962.

Drouhault, R. T., N. Abrous, J. P. David, and B. Dufy. Bradykinin parallels thyrotrophin-releasing hormone actions on prolactin release from rat anterior pituitary cells. Neuroendocrinology 46: 360-364, 1987.

Figueroa, C. D., L. M. Henderson, R. W. Colman, R. A. De La Cadena, W. Müller-Esterl, and K. D. Bhoola. Immunoreactive L- and H-kininogen in human neutrophils. J. Physiol. (Lond.) 425: 65, 1990.

Goke, R., B. Wagner, H. C. Fehmann, and B. Goke. Glucose-dependency of the insulin stimulatory effect of glucagon-like peptide-1 (7-37) amide on the rat pancreas. Res. Exp. Med. Berl. 193: 97-103, 1993.

Goldstein, R. H., M. Wall, F. G. Graeff, A. P. Corrado, I. R. Pela, and R. Capek. Activation of protein formation and cell division by bradykinin and Des-Arg^9-bradykinin. J. Biol. Chem. 259: 9263-9268, 1984.

Grodsky, G. M., and L. L. Bennett. Insulin released from the isolated pancreas in the absence of insulinogenesis. Proc. Soc. Exp. Biol. Med. 114: 769-771, 1963.

Grodsky, G. M., D. Curry, H. Landahl, and L. L. Bennett. Further studies on the dynamic aspects of insulin release in vitro with evidence for a two-compartmental storage system. Acta. Diabetol. Lat. 6: 554-579, 1969.

Hale, C. N., and P. J. Randle. Immunoassay of insulin with insulin antibody precipitate. Biochem. J. 88:137-146, 1963.

Hargrove, D. M., N. A. Nardone, L. M. Persson, J. C. Parker, and R. W. Stevenson. Glucose-dependency action of glucagon-like peptide-1 (7-37) in vivo during short- or long-term administration. Metabolism 44: 1231-1237, 1995.

Jaffa, A. A., J. Pratt, A. Ashford, and G. S. Bailey. Studies of the effects of insulin , bradykinin, and captopril on blood glucose levels of alloxan-diabetic rats. Adv. Exp. Med. Biol. 198: 373-378, 1986.

Jauch, K. W., B. Gunther, W. Hartl, K. Rett, M. Wicklmayr, and G. Dietze. Improvement of impaired postoperative insulin action by bradykinin. Biol. Chem. Hoppe-Seyler. 367: 207-210, 1986.

Jauch, K. W., B, Gunther, K. Bett, W. Hartl, M. Wicklmayr, G. Dietze, E. Fink, M. Georgieff, and D. Neumeier. Dose-dependent effect of bradykinin infusion in post-operative patients. Adv. Exp. Med. Biol. 247A: 603-608, 1989.

Jones, T. H., B. L. Brown, and P. R. M. Dobson. Evidence that angiotensin II is a paracrine agent mediating gonadotrophin-releasing hormone, inositol phosphate production and prolactin secretion in the rat. J. Endocrinol. 116: 367-371, 1988.

Kellett, G. L., and E. D. Barker. The stimulation of glucose absorption and metabolism in rat jejunum by bradykinin: dependence on the composition of commercial diets. Biochem. Biophys. Acta. 21: 128-130, 1989.

Kimura, I., N. Nakashima, T. Komori, Y. Kameda, and M, Kimura. Dependence of cholecystokinin-8-stimulated insulin release on high glucose levels is evidenced by pseudo-alpha-D-glucose in rat pancreas and islets. Jpn J. Pharmacol. 64: 103-107, 1994.

Lacy, P.E. Beta cell secretion-From the standpoint of a pathobiologist. The Banting Memorial Lecture Diabetes 19: 895-905, 1970.

Lacy, P. E., M. M. Walker, and C. J. Fink. Perfusion of isolated rat islets in vitro: participation of the microtubular system in the biphasic release of insulin. Diabetes 21: 987-998, 1972.

Marceau, F., and B. Tremble. Mitogenic effect of bradykinin and Des-Arg^9-bradykinin on cultured fibroblast. Life Sci. 39: 2351-2358, 1986.

Needleman, P., S. L. Key, S. E. Denny, P. C. Isakson, and G. R. Marshall. The mechanism and modification of bradykinin-induced coronary vasodilation. Proc. Natl. Acad. Sci. USA 72: 2060-2063, 1975.

Nwator, I. A. A. and E. T. Whalley. Angiotensin converting enzyme inhibitors and expression of des-Arg^9-BK (kinin B$_1$) receptors in vivo. Eur. J. Pharmacol. 160: 125-13, 1989.

Pan, H. L., C. L. Stebbins, and J. C. Longhurst. Bradykinin contributes to the exercise pressor reflex: mechanism of action. J. Appl. Physiol. 75: 2061-2068, 1993.

Pinkus, G. S., M. Maier, D. C. Seldin,O. Ole-Moiyoi, K. F. Austen, and J. Spragg, Immunohistochemical localization of glandular kallikrein in the endocrine and exocrine pancreas. J. Histochem. Cytochem. 31: 1279-1288, 1983.

Pisano, J. J. and K. F. Austen. Chemistry and biology of the Kallikrein-kinin system in health and disease. National Institutes of Health, Bethesda, Maryland, Oct.20-23, DHEW publication No.(NIH) 76-79, 1974.

Reddigari, S., and A. P. Kaplan. Quantification of human high molecular weight kininogen by immunoblotting with a monoclonal anti-light chain antibody. J. Immunol. Methods 119: 19-25, 1989.

Roberts, R. A. Bradykinin receptors: Characterization, distribution and mechanisms of signal transduction. Prog. Growth Factor Res. 1:237-252, 1989.

Schmaier, A. H., D. Schutsky, A. Farber, L. D. Silver, H. N. Bradford, and R. W. Colman. Determination of bifunctional properties of high molecular weight kininogen by studies with monoclonal antibodies directed to each of its chains. J. Biol. Chem 262: 1405-1411, 1987.

Scicli, A. G., T. B. Ørstavik, S. F. Rabito, F. D. Murray, and O. A. Carretro. Blood kinins after sympathetic nerve stimulation of the rat submaxillary gland. Hypertension 5: 1101-1106, 1983.

Sharp, P. A., and E. S. Debnam. Rapid stimulatory effect of bradykinin on glucose transport across the brush-border and basolateral membranes of rat jejunal enterocytes. Exp. Physiol. 77: 913-916, 1992.

Staszewska-Barczak, J., and J. R. vane. The release of catecholamines from the adrenal medulla by peptides. Br. J. Pharmacol. 30: 655-667, 1967.

Steranka, L. R., D. C. Manning, C. J. Dehaas, J. W. Ferkany, S. A. Borosky, J. R. Connor, R. J. Vavrex, J. M. Sreward, and S. H. Snyder. Bradykinin as a pain mediators: receptors are localized to sensory neurons and antagonists have analgesic action. Proc. Natl. Acad. Sci. USA 85: 3245-3249, 1988.

Symington, R. P., A. Ashford, and G. Bailey. Effect of bradykinin and insulin on glucose oxidation in adipocytes from normal and streptozotocin diabetic rats. Biochem. Soc. Trans. 16: 1012-1013, 1988.

Wicklmayr, M., and G. Dietze. Effect of oral kallikrein and intrabrachial-arterial bradykinin on forearm-metabolism in maturity onset diabetes. In: Kininogenases, Kallikrein 4, edited by G. L. Haberland, J. W. Rohen, and T. Suzuki, Stuttgart, New York, Schattauer Verlag, 1977, p. 299-308.

Yang, C., and W. H. Hsu. Stimulatory effect of bradykinin on insulin release from the perfused rat pancreas. Am. J. Physiol. 268: E1027-E1030, 1995.

CHAPTER 4. THE EFFECT OF BRADYKININ ON THE SECRETION OF INSULIN, GLUCAGON AND SOMATOSTATIN FROM THE PERFUSED RAT PANCREAS

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Chi Yang and Walter H. Hsu

Abstract

In order to evaluate the effect of bradykinin (BK) on rat islet α-cells, β cells and δ-cells, rat pancreata were perfused in situ with BK (1 μM) for 30 min via a cannula placed in the celiac artery. We measured insulin, glucagon and somatostatin concentrations in the effluents to determine the effect of BK on the secretion of these hormones. The basal secretion of insulin, glucagon and somatostatin in the medium containing 6 mM glucose was maintained at 6.5 ± 0.5 ng/ml (n =12), 124 ± 8 pg/ml (n =8) and 511 ± 22 pg/ml (n = 12), respectively. BK (1 μM) induced a biphasic insulin secretion which was characterized by a transient peak which was 3.7-fold of the baseline concentration at ≤ 3 min and a sustained phase. BK also transiently increased glucagon secretion with a peak which was 1.7-fold of the baseline concentration at ≤ 3 min , without a sustained secretion phase. BK also caused a reduction in somatostatin secretion within 3 min to a level of 60-70 % of the baseline concentration. We concluded that BK stimulated a biphasic insulin secretion response, transiently increased glucagon secretion and decreased somatostatin secretion during the 30 min perfusion of the rat pancreas.

Introduction

Kinins, including bradykinin (BK), display an extraordinarily high degree of physiological activities. They influence a number of biological processes, including blood pressure and local blood flow (Pan et al., 1993), pain (Steranka et al., 1988) and inflammation (Figueroa,1990), increased vascular permeability and local edema (Carter et al., 1974), contraction of smooth muscle (Collier et al., 1962), decreased blood glucose concentration, (Wicklmayr and Dietze, 1977), promotion of cell proliferation (Goldstein et al., 1984), increased glucose uptake by intact intestine (Sharp and Debnam, 1992), and other cellular functions.

Kinins also stimulate the secretion of other hormones, such as renin from isolated rat glomeruli (Beierwaltes et al., 1985), vasopressin from rat posterior pituitary gland (Baertschi et al., 1981), prolactin and growth hormone from rat anterior pituitary gland (Drouhault et al., 1987) , catecholamines from vas deferens nerve terminals (Liona et al., 1991). At least two BK-receptor have been characterized, BK_1 and BK_2, but most of the effects of BK are mediated through BK_2-receptors (Roberts, 1989).

In our previous study, administration of BK for 5 min dose-dependently stimulated insulin secretion from the perfused rat pancreas and this was mediated by BK_2-receptors. The BK_2-receptors blocker, HOE 140, reduced the basal insulin secretion, abolished the BK-induced insulin release, and attenuated the glucose-stimulated insulin release. BK also increased glucose (10 mM)-induced insulin release (Yang and Hsu, 1995). Our findings suggest that, BK may be physiologically involved in maintaining the basal and glucose-induced insulin secretion. However, in addition to insulin-secreting cells (β cells), islets also have glucagon-secreting cells (α cells), somatostatin-secreting cells (δ cells), and other types of cells for controlling glucose homeostasis (Hazelwood, 1989). The effects of BK on these types of cells are still not understood. Thus, the present study was undertaken to investigate the effect of BK on insulin, glucagon and somatostatin secretion.

Materials and Methods

Male Sprague-Dawley rats (300-400 g) were anesthetized with pentobarbital sodium (60 mg/kg ip; Fort Dodge Labs, Fort Dodge, IA) and kept on a 37°C hot plate. The rat pancreatic perfusion was performed as previously described (Yang and Hsu, 1995). After celiac arteries were cannulated, the rat pancreata were immediately perfused in situ with oxygenated (95% O_2/5% CO_2) Krebs-Ringer bicarbonate (KRB) solution supplemented with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 6 mM glucose, 0.2% bovine serum albumin and pH 7.4 as basal medium. The perfusion rate was maintained at 1 ml/min and the effluent was collected from a cannula placed in the portal vein.

After the rat pancreatic preparations were established, the first 10-15 min of perfusion was considered as an equilibration period. Subsequently, the effluent fluid was collected each min for 10 min as baseline insulin secretion and the rat pancreata were continuously perfused with BK (1 μM) (Sigma Chemicals, St. Louis, MO) for 30 min followed by a 5 min perfusion with the basal

medium. In our previous study, BK (0.01 - 1 μM) dose-dependently increases insulin release and BK 1 μM induces a about 3.7-fold increase of the baseline level (Yang and Hsu, 1995). Therefore, we used 1 μM BK in these experiments. The rat pancreata of the control group were perfused with 6 mM glucose in KRB medium only.

The effluents were centrifuged (1000× g), then 0.5 ml of the effluents was mixed with 1 ml acid-ethanol (95% of 95% ethanol and 5% of 1 N HCl). The mixtures were centrifuged (1000× g) again and the supernatants were collected, stored at -20°C for measuring the concentrations of glucagon and somatostatin by using radioimmunoassay (RIA). An aliquot of the effluent was kept at 4°C and subsequently assayed within 12 h for insulin by using RIA as previously described (Hale and Randle, 1963). The glucagon RIA kit was purchased from Linco Research, INC. St. Charles, MO and the measurement of glucagon was determined according to the instructions of the manufacturer. Somatostatin was measured by an RIA as previously described (Patel and Reichlin, 1978).

Values are shown as means ± SE. Data were analyzed by using analysis of variance (ANOVA) to determine the significance of treatment and time. The treatment x time interaction was used as an error term to determine the effect of treatment. The significance of treatment was determined from the conservative F value. Tukey's highly significant different test was used to determine the differences between treatments for which the ANOVA indicated a significant (P ≤ 0.05) F ratio.

Results

The rat pancreata in the control group were only perfused with 6 mM glucose, and the baseline insulin concentrations were 6.7 ± 0.5 ng/ml throughout the perfusion period. BK (1 μM) induced a biphasic insulin release pattern (Fig. 1). The onset time of insulin release was less than 1 min and reached a peak which was 270 % over the baseline concentration at ≤ 3 min; then the insulin concentrations decreased gradually within 5 min to a sustained level which was still significantly higher (~50 %) than that of the control group throughout the perfusion period. The combination of BK and 6 mM glucose in insulin secretion was significantly higher than that of 6 mM glucose alone.

The concentrations of glucagon in the control group were maintained at a steady level which was ~ 128 ± 8 pg/ml throughout the perfusion period. Due to the increased variance, there
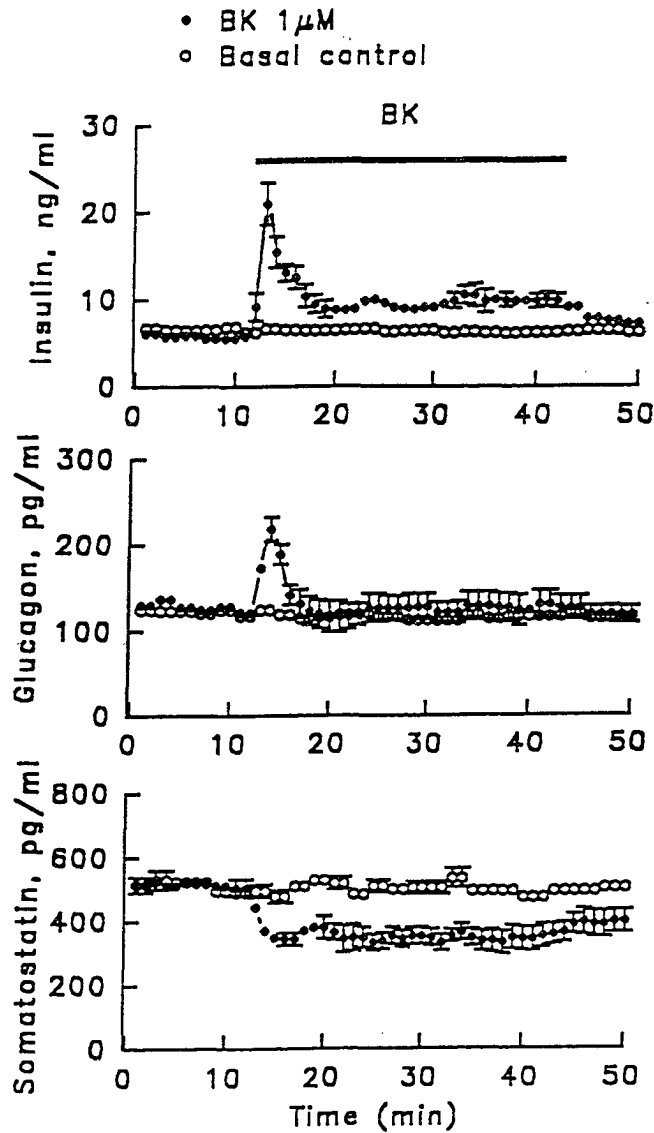
Fig. 1. Effect of 1 μM bradykinin (BK) in the medium containing 6 mM glucose on the secretion of insulin, glucagon and somatostatin from the perfused rat pancreas. BK was administrated for 30 min (bar). Values are shown as means ± SE. There was an equilibration period of 10 to 15 min in which basal medium containing 6 mM glucose was perfused before time 0. The baseline concentrations of insulin, glucagon and somatostatin in the effluents were 6.7 ± 0.5 ng/ml (n = 4 rats) and 6.6 ± 0.5 ng/ml (n = 8 rats), 128 ± 8 pg/ml (n = 4 rats) and 119 ± 7 pg/ml (n = 4 rats), 522 ± 25 pg/ml (n = 4 rats) and 505 ± 20 pg/ml (n = 8 rats) for control and bradykinin + glucose groups, respectively.

was no significance difference between the treatments in the 30 min perfusion period. However, BK (1 μM) transiently and significantly increased glucagon secretion which reached a peak within 3 min, and was ~70 % greater than the baseline concentration; the concentrations of glucagon of the effluent decreased to the baseline concentration within 3 min (Fig. 1).

The somatostatin concentrations of the effluent in the control group were maintained at 522 ± 25 pg/ml throughout the perfusion period. Administration of BK (1 μM) significantly decreased somatostatin concentrations in the effluents to ~ 60 % of the baseline concentration within 3 min and maintained steadily at this level to the end of the perfusion (Fig. 1).

Discussion

Although, the physiological concentration of BK in the pancreas is not known, the precursors, kininogens are abundant in plasma (55 μg/ml) (Bhoola et al., 1992; Reddigari and Kaplan, 1989) and the circulatory kininogens bind to the surface receptors of the endothelial cells as a substrate for BK synthesis (Schmaier et al., 1987). The catalytic enzymes, kallikreins are synthesized and rich in the pancreas (Bhoola et al., 1992; Pinkus et al., 1983). In addition, the concentrations of BK are detected in the range of ng/mg protein or μM in the rat submaxillary gland (Scicli et al., 1983) and kidney (Barabe et al., 1988).

Glucose, at higher concentrations of ≥ 10 mM, induces a biphasic insulin secretion which was characterized by a transient insulin release followed by a sustained insulin secretion phase in isolated perfused rat pancreas (Grodsky et al., 1969) and in humans (Cerasi and Luft, 1967). However, the present results showed that glucose of 6 mM, similar to normal physiological concentration, only maintained a steady insulin secretion at 6.5 ± 0.5 ng/ml (n =12).

BK (1 μM) induced a biphasic insulin secretion and transiently increased glucagon secretion, but decreased the secretion of somatostatin during the 30 min period of the pancreatic perfusion. The initial transient insulin release was similar to our previous study in which rat pancreata were perfused with BK only for 5 min (Yang and Hsu, 1995). BK also induced a second plateau phase of insulin secretion in this 30 min perfusion.

Intra-islet communication among α, β and δ cells may occur through paracrine (interstitial) and/or vascular routes. The microvascular circulating cellular order, from β cells to α cells and from α cells to δ cells, also influences the secretion of islet hormones (Stagner et al., 1988). Thus insulin regulates the secretion of glucagon, and both insulin and glucagon regulate the secretion of

somatostatin (Samols and Stagner, 1990). The intra-islet insulin promotes the transport of glucose entering α cells and inhibits the secretion of glucagon (Greenbaum et al., 1991). In addition, the amount of released insulin during the early period of stimulation determines the rapidity and magnitude of inhibition to the secretory function of α cells (Leclercq-meyer et al., 1983). In the present study, BK stimulated α cells and transiently increased glucagon release, and the BK-induced increase in insulin secretion inhibited glucagon secretion, thus the glucagon concentrations returned to the baseline levels.

In isolated perfused rat pancreas, glucose stimulates the secretion of insulin and somatostatin. The released somatostatin concentration is insufficient to inhibit glucose-induced insulin secretion (Sorenson et al., 1980). However, exogenous insulin suppressed glucose- and arginine-induced somatostatin secretion (Gerber et al., 1981). In our studies, the reduction in the somatostatin secretion may have been caused by a direct action of BK or BK-induced insulin release. At present, the mechanisms of action of BK on the secretion of glucagon and somatostatin are unknown. Glucagon- and somatostatin-secreting cell lines are needed to explore the direct action of BK on the secretion of these hormone secretion and to investigate the mechanisms of action of BK.

In the cellular order of microvascular circulation, δ cells are last and thus δ cells are not a paracrine regulator of islet hormone secretion, but may be important in the regulation of exocrine function (Samols and Stagner, 1990). The periacinal islet hormones are high (Bendayan, 1993) and islet hormone (insulin and somatostatin) receptors exist on the plasma membrane of pancreatic acinar cells (Bendayan, 1990). The released insulin directly regulates the release of zymogen, glucose transport, and protein synthesis (Williams and Goldfine, 1985) and increases secretin-induced pancreatic juice release, but not the release of protein, whereas both glucagon and somatostatin inhibit secretin-induced pancreatic juice and protein secretion (Hasegawa et al., 1993). Therefore, BK may play a physiological role in the control of the function of exocrine pancreas via its effects on endocrine pancreas.

Acknowledgments

References

Baertsci, A. J., H. H. Zingg, and J. J. Dreifuss. Enkephalins, substance P, bradykinin,and angiotensin II: differential sites of action on the hypothalmo-neurohypophysial system. Brain Res. 220: 107-119, 1981.

Barabe, J., D. Huberdean, and A. Bernoussi. Influences of sodium balance on urinary excretion of immunoreactive kinin in rats. Am. J. Physiol. 254: F484-F491, 1988.

Bendayan, M. Anatomic basis of islet-acinar interaction in the pancreas: A quarterly clinical review. Regul. pept. lett. 11: 1-7, 1990.

Bendayan, M. Pathway of insulin in pancreatic tissue on its release by the B-cell. Am. J. Physiol. 264: G187-194, 1993.

Beierwaltes, W. H., L. Prado, and O. A. Carretero. Kinin stimulation of renin release in isolated rat glomeruli. Am. J. Physiol. 248: F757-F761, 1985.

Bhoola, K. D., C. D. Figueroa, and K. Worthy. Bioregulation of kinin: kallikrein, kininogen, and kininases. Pharmacol. Rev. 44: 1-80, 1992.

Carter, R. D., W. L. Joyner, and E. M. Renkin. Effect of histamine and some other substances on molecular selectivity of the capillary wall to plasma proteins and dextran. Microvasc. Res. 7: 31-48, 1974.

Cerasi, E., and R. Luft. Insulin response to glucose infusion in diabetic and non-diabetic monozygotic twin pairs. Genetic control of insulin response. Acta Endocrinol. 55: 330-345, 1967.

Collier, H. O., J. A. Holgate, M. Schachter, and P. G. Shorley. The bronchoconstrictor action of bradykinin in the Guinea pig. Br. J. Pharmacol. 15: 290-297, 1962.

Drouhault, R. T., N. Abrous, J. P. David, and B. Dufy. Bradykinin parallels thyrotrophin-releasing hormone actions on prolactin release from rat anterior pituitary cells. Neuroendocrinology 46: 360-364, 1987.

Figueroa, C. D., L. M. Henderson, R. W. Colman, R. A, De La Cadena, W. Müller-Esterl, and K. D. Bhoola. Immunoreactive L- and H-kininogen in human neutrophils. J. Physiol. (Lond.) 425: 65, 1990.

Gerber, P. P., E. R. Trimble, C. B. Wollheim, and A. E. Renold. Effect of insulin on glucose- and arginine-stimulated somatostatin secretion from the isolated perfused rat pancreas. Endocrinology 109: 279-283, 1981.

Goldstein, R. H., M. Wall, F. G. Graeff, A. P. Corrado, I. R. Pela, and R. Capek. Activation of protein formation and cell division by bradykinin and Des-Arg^9-bradykinin. J. Biol. Chem. 259: 9263-9268, 1984.

Greenbaum, C. J., P. J. Havel, G. J. Jr. Taborsky, and L. J. Klaff. Intra-islet insulin permits
glucose to directly suppress pancreatic A cell function. J. Clin. Invest. 88: 767-773, 1991.

Grodsky, G. M., D. Curry, H. Landahl, and L. L. Bennett. Further studies on the dynamic
aspects of insulin release in vitro with evidence for a two-compartmental storage system.
Acta Diabetol. Lat. 6: 554-579, 1969.

Hale, C. N., and P. J. Randle. Immunoassay of insulin with insulin antibody precipitate.
Biochem. J. 88:137-146, 1963.

Hasegawa, H., Y. Okabayshi, M. Koide, Y. Kido, T. Okutani, K. Matauahita, M. Otsuki, and M.
Kasuga. Effect of islet hormones on secretion-stimulated exocrine secretion in isolated
perfused rat pancreas. Dig. Dis. Sci. 38: 1278-1283, 1993.

Hazelwood, R. L. The endocrine pancreas. Prentice Hall, Englewood Cliffs, New Jersey. pp. 9-
83, 1989.

Leclercq-Meyer, V., J. Marchand, W. J. Malaisse. Role of glucose and insulin in the dynamic
regulation of glucagon release by the perfused rat pancreas. Diabetologia 24: 191-195,
1983.

Liona, I., X. Galleguillos, and J. Belmar. Bradykinin modulates the release of noradrenaline from
vas deferens nerve terminals. Life Sci. 48: 2585-2592, 1991.

Pan, H. L., C. L. Stebbins, and J. C. Longhurst. Bradykinin contributes to the exercise
pressor reflex: mechanism of action. J. Appl. Physiol. 75: 2061-2068, 1993.

Patel, Y. C., and S. Reichlin. "Somatstatin," in Methods of Hormone Radioimmunoassay, 2nd ed.,
B. M. Jaffa and H. R. Behrman (eds.), Academic Press, New York, 1978, p. 77.

Pinkus, G. S., M. Maier, D. C. Seldin, O. Ole-Moiyoi, K. F. Austen, and J. Spragg.
Immunohistochemical localization of glandular kallikrein in the endocrine and exocrine
pancreas. J. Histochem. Cytochem. 31: 1279-1288, 1983..

Reddigari, S., and A. P. Kaplan. Quantification of human high molecular weight kininogen by
immunoblotting with a monoclonal anti-light chain antibody. J. Immunol. Methods 119:
19-25, 1989.

Roberts, R. A. Bradykinin receptors: Characterization, distribution and mechanisms of signal
transduction. Prog. Growth Factor Res. 1:237-252, 1989.

Samols, E., and J. I. Stagner. Islet somatostatin--microvacular, paracrine, and pulsatile regulation.
Metabolism. 39: 55-60, 1990.

Schmaier, A. H., D. Schutsky, A. Farber, L. D. Silver, H. N. Bradford, and R. W. Colman.
Determination of bifunctional properties of high molecular weight kininogen by studies
with monoclonal antibodies directed to each of its chains. J. Biol. Chem 262: 1405-1411,
1987.

Scicli, A. G., T. B. Ørstavik, S. F. Rabito, F. D. Murray, and O. A. Carretro. Blood kinins after sympathetic nerve stimulation of the rat submaxillary gland. Hypertension 5: I101-I106, 1983.

Sharp, P. A., and E. S. Debnam. Rapid stimulatory effect of bradykinin on glucose transport across the brush-border and basolateral membranes of rat jejunal enterocytes. Exp. Physiol. 77: 913-916, 1992.

Sorenson, R. L., D. V. Lindell, and R. P. Elde. Glucose stimulation of somatostatin and insulin release from the isolated, perfused rat pancreas. Diabetes 29: 747-751, 1980.

Steranka, L. R., D. C. Manning, C. J. Dehaas, J. W. Ferkany, S. A. Borosky, J. R. Connor, R. J. Vavrex, J. M. Sreward, and S. H. Snyder. Bradykinin as a pain mediators: receptors are localized to sensory neurons and antagonists have analgesic action. Proc. Natl. Acad. Sci. USA 85: 3245-3249, 1988.

Stagner, J. I., E. Samols, and W. S. Bonner. beta---alpha---delta pancreatic islet cellular perfusion in dogs. Diabetes 37: 1715-1721, 1988.

Wicklmayr, M., and G. Dietze. Effect of oral kallikrein and intrabrachial-arterial bradykinin on forearm-metabolism in maturity onset diabetes. In: Kininogenases, Kallikrein 4, edited by G. L. Haberland, J. W. Rohen, and T. Suzuki, Stuttgart, New York, Schattauer Verlag, 1977, p. 299-308.

Williams, J. A., and I. D. Goldfine. The insulin-pancreatic acinar axis. Diabetes 34: 1980-986. 1985.

Yang, C., and W. H. Hsu. Stimulatory effect of bradykinin on insulin release from the perfused rat pancreas. Am. J. Physiol. 268: E1027-E1030, 1995.

CHAPTER 5. MECHANISMS OF BRADYKININ-INDUCED INSULIN SECRETION AND A RISE IN CYTOSOLIC Ca²⁺ IN A CLONAL β-CELL LINE RINm5F

Chi Yang, Bumsup Lee, Ter-Hsin Chen and Walter H. Hsu

Abstract

The present study investigated the mechanisms underlying the bradykinin (BK)-induced rise in $[Ca^{2+}]_i$ and insulin secretion using a clonal β-cell line RINm5F. BK concentration-dependently increased insulin secretion from 10 nM to 10 μM and $[Ca^{2+}]_i$ from 100 nM to 100 μM. In Ca^{2+}-containing medium, BK (1 μM) induced a biphasic $[Ca^{2+}]_i$ rise which was characterized by a Ca^{2+} spike and a sustained Ca^{2+} phase. In the Ca^{2+}-free medium, BK failed to increase insulin secretion and induced only a Ca^{2+} spike without the sustained Ca^{2+} phase. Thapsigargin (TG) (1 μM), an inhibitor of the Ca^{2+} pump in the endoplasmic reticulum (ER), abolished both the Ca^{2+} spike and the sustained phase. Nimodipine (1 μM), a voltage-dependent Ca^{2+} channel blocker, abolished the BK-induced sustained Ca^{2+} phase and inhibited BK-induced insulin release. The BK_1-receptor agonist desArg⁹-BK (1 μM) did not change $[Ca^{2+}]_i$ or insulin secretion. Both BK-induced insulin secretion and a rise in $[Ca^{2+}]_i$ were dose-dependently inhibited by a selective BK_2-receptor antagonist HOE140 (3.3 to 100 nM), but were not inhibited by a BK_1-receptor antagonist desArg⁹,leu⁸-BK (1 μM). Pertussis toxin (PTX) (0.1 μg/ml) did not block the BK-induced insulin secretion or an increase in $[Ca^{2+}]_i$. U-73122 (4, 6 and 8 μM), a phospholipase C (PLC) inhibitor, dose-dependently inhibited both BK-induced insulin secretion and an increase in $[Ca^{2+}]_i$ in a parallel manner. BK increased intracellular concentrations of IP_3, but did not increase cAMP formation. Ro 31-8220 (3 and 10 μM), a PKC inhibitor, dose-dependently increased BK-induced insulin secretion and an increase in $[Ca^{2+}]_i$, whereas, OAG, an analogue of diacylglycerol (DAG), dose-dependently inhibited the BK-induced insulin secretion and the increase in $[Ca^{2+}]_i$. Ro 31-8220 increased the BK-induced Ca^{2+} spike and the sustained Ca^{2+} phase. OAG (30 μM) inhibited the BK-induced sustained Ca^{2+} phase. In Ca^{2+}-free medium, Ro 31-8220 and OAG did not alter the BK-induced increase in $[Ca^{2+}]_i$. Nimodipine abolished BK-induced Ca^{2+} influx, but did not abolish the BK-induced Ca^{2+} influx in Ro 31-8220 pretreated cells. ACA (100

μM), a PLA_2 inhibitor, or L- NAME (100 μM), a nitric oxide synthase inhibitor, did not inhibit these effects of BK. Taken together, these findings suggested that in β-cells, BK activates the BK_2-receptor which, in turn, activates PTX-insensitive G protein, probably G_q. The G protein activates PLC which promotes the formation of inositol, 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases $[Ca^{2+}]_i$ from the intracellular Ca^{2+} stores, probably the ER, which triggers Ca^{2+} influx via voltage-dependent Ca^{2+} channels and thus increases insulin secretion. DAG activates protein kinase C (PKC) which, in turn, brings $[Ca^{2+}]_i$ back to normal by promoting Ca^{2+} efflux and inhibiting Ca^{2+} influx.

Introduction

The autacoid bradykinin (BK) is a potent vasoactive nonapeptide. Kinins including BK and kallidin, an analog of BK, influence a number of biological processes. They regulate blood pressure and local blood flow (Pan et al., 1993), produce pain (Steranka et al., 1988) and inflammation (Figueroa,1990), increase vascular permeability and local edema (Carter et al., 1974), contract smooth muscle (Collier et al., 1962), increase cell proliferation (Marceau and Tremble, 1986), increase glucose uptake (Sharp and Debnam, 1992) and decrease blood glucose concentration (Wicklmayr and Dietze, 1977), and affect other cellular functions. The effects of BK are mediated by at least two groups of BK receptors, BK_1- and BK_2. BK_1-receptors mediate the acute inflammatory response, whereas BK_2-receptors are responsible for most of the biological activities of kinins (Regoli et al., 1993).

In our previous report, we demonstrated that BK stimulated insulin secretion via BK_2- receptors in a dose-dependent manner from the perfused rat pancreas (Yang and Hsu, 1995). However, the mechanisms underlying BK-induced insulin secretion remain unknown. In general, kinin receptors are coupled to G proteins which may be pertussis toxin (PTX)-sensitive or - insensitive (Bhoola et al., 1992), and at least four possible signal transduction pathways may mediate the effects of BK in other cellular systems: 1) phospholipase C (PLC) which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to form inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG); IP_3 mobilizes $[Ca^{2+}]_i$ from the intracellular Ca^{2+} store of the endoplasmic reticulum (ER) (Takeuchi et al., 1988); 2) phospholipase A_2 (PLA_2) which increases the formation of arachidonic acid (Birch and Axelrod, 1987), arachidonic acid, in turn, mobilizes Ca^{2+} from the ER and opens voltage-dependent Ca^{2+} channels (Konrad et al., 1992; Fernandez and Balsinde,

1991); 3) adenylate cyclase which increases cAMP formation (Suidan et al., 1991); and 4) nitric oxide synthase (NOS) which increases the formation of nitric oxide (NO) (Mombouli and Vanhoutte, 1995). Therefore, in this study, we sought to determine which signal transduction pathways mediate the effects of BK on insulin secretion.

We proposed that in β-cells, the effects of BK-induced insulin secretion and an increase in $[Ca^{2+}]_i$ are mediated through the PLC-IP$_3$ signal transduction pathway. Therefore, we used a clonal insulin-secreting β-cell line RINm5F to explore the mechanisms underlying BK-induced insulin secretion.

Materials and Methods

Cell culture

A clonal insulin-secreting β-cell line, RINm5F, donated by Dr. S. B. Pek of the University of Michigan, Ann Arbor, was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and aerated with 5% CO_2/ 95% air, as described by Thomas et al. (1989). The cells were cultured for 5 days and the passages from 42 to 55 were used for these experiments.

Insulin secretion and measurement

RINm5F cells were plated into 24-well plastic plates (Corning Glass Works, Corning, NY) at 2×10^5 cells/130 mm well and grown for 5 days. During the experiments, the culture medium was removed and replaced with Krebs-Ringer Bicarbonate (KRB) solution containing (in mM): 136 NaCl, 4.8 KCl, 1.2 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 5 $NaHCO_3$, 10 HEPES, 4 glucose, 0.1% bovine serum albumin, pH 7.4. The cells were preincubated for 15 min at 37°C, then were incubated in KRB with the test agent as described by Hsu et al (1991). When needed, cells were pretreated with HOE140 (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]BK), desArg^9,Leu^8-BK, U-73122 and U-73343 (1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione), ACA (p-amylcinnamoyl) anthranilic acid), NAME (N^G-nitro-L-arginine methyl ester), or nimodipine for 5 min before BK administration. Cells were pretreated with Pertussis toxin (PTX) (0.1 μg/ml) for 16 h and Ro-31-8220 for 30 min. OAG (1-oleoyl-2-acetyl glycerol) was given 30 sec before BK administration. The supernatants were collected, kept at 4°C, and subsequently assayed within 12 hours for insulin by using radioimmunoassay (RIA) as described by Hsu et al. (1991).

Measurement of $[Ca^{2+}]_i$

    Cultured RINm5F cells of ~ 30 x 10^6 cells were loaded with 2 μM fura-2

acetoxymethylester (AM) in KRB for 30 min at 37°C. Then the loaded cells were centrifuged

(200 x g) and resuspended in KRB, and kept at room temperature for the $[Ca^{2+}]_i$ measurement.

The cells were resuspended at a concentration of 10^6/ml and 1.5-ml aliquots were used for $[Ca^{2+}]_i$

measurement at room temperature (24°C). In the absence of extracellular Ca^{2+}, cells were

centrifuged (200 x g) and were resuspended in Ca^{2+}-free/EGTA (10 μM) medium. The 340

nm/380 nm fluorescence ratios were monitored in a SLM-8000 fluorescence spectrophotometer

(SLM, Urbana, IL). $[Ca^{2+}]_i$ was calibrated after cell lysis as described by Hsu et al. (1991). When

needed, cells were pretreated with HOE140, desArg[9],Leu[8]-BK, U-73122, ACA, NAME,

nimodipine for 100-sec before BK administration. Cells were pretreated with PTX (0.1 μg/ml) for

2 hour, thapsigargin and Ro 31-8220 for 30 min before $[Ca^{2+}]_i$ measurement. OAG was given 30

sec before BK administration.

Measurement of IP_3 (inositol 1,4,5-trisphosphate)

    Intracellular IP_3 concentration was measured using a radioreceptor-binding assay kit

purchased from DuPont Co., Boston, MA. 1.5 x 10^6 cells in 1 ml KRB were placed in

polypropylene tubes and the treatment with BK was terminated in 10 sec by adding 20% (w/v) of

ice-cold trichloroacetic acid. The concentration of IP_3 was determined according to instructions of

the manufacturer.

Cyclic AMP measurement

Cyclic AMP concentrations in cultured cell monolayers were studied under conditions similar to

the insulin secretion experiments and were determined by using RIA as previously described

(Richards et al., 1979).

Data expression and statistical analysis.

    Data are expressed as means ± SE . Results were analyzed using the Student's t test for

paired and unpaired values. The significant level was set at $P \leq 0.05$.

Materials

All chemicals were from Sigma Chemical Co. (St. Louis, MO), except that HOE140 was donated by Hoechst-Roussel Pharmaceuticals (Somerville, NJ); U-73122, U-73343 and ACA were from Biomol Research Laboratory (Plymouth Meeting, PA); nimodipine was from Research Biochemicals International (Natick, MA); Ro-31-8220 was donated by Roche product (Herfordshire, UK); fura-2 AM was from Molecular Probes (Eugene, OR).

Results

Effects of BK on Insulin Secretion and $[Ca^{2+}]_i$ Increase

BK (10 nM to 10 μM) dose-dependently increased insulin secretion (Fig. 1A). BK (1 μM) significantly increased insulin secretion which was 3.8 times of the basal control level. The insulin concentrations of basal control group were 0.35 ± 0.02 ng/well/ml (n = 12). BK (1 μM) failed to induce insulin secretion in Ca^{2+}-free medium (control = 0.34 ± 0.02 ng/well/min; BK = 0.35 ± 0.01 ng/well/min, n = 4, $P > 0.05$).

BK (100 nM to 100 μM) increased $[Ca^{2+}]_i$ in a dose-dependent manner (Fig. 1B). BK (1 μM) significantly increased $[Ca^{2+}]_i$ which was 98 % (at the peak) over the baseline level. The $[Ca^{2+}]_i$ of baseline was 120 ± 5 nM (n = 4) (Fig. 1B). In Ca^{2+}-containing medium, BK (1 μM) induced a transient Ca^{2+} spike which reached a peak (238 ± 6 nM, n = 8) at ~ 20 sec and this followed by a sustained Ca^{2+} plateau phase which gradually declined to the baseline level over 4 min (Fig. 2). In the Ca^{2+}-free medium, BK (1 μM) only induced a transient Ca^{2+} spike (168 ± 5 nM, n = 8) without the sustained Ca^{2+} phase (Fig. 2). The baseline $[Ca^{2+}]_i$ in Ca^{2+}-free/EGTA medium was 88 ± 4 nM (n = 8) (Fig. 2).

Effects of BK-receptor Agonists and Antagonists on Insulin Secretion and $[Ca^{2+}]_i$ Increase

BK (1 μM) significantly induced insulin secretion (Fig. 3) and an increase in $[Ca^{2+}]_i$ (at the peak) (Fig.4), but desArg[9]-BK, a BK_1-receptor agonist, did not induce insulin secretion (Fig. 3) or an increase in $[Ca^{2+}]_i$ (Fig.4). HOE140 (1 μM), a BK_2-receptor antagonist, inhibited both the BK-induced insulin secretion (Figs. 3 and 5A) and an increase in $[Ca^{2+}]_i$ (Figs. 4 and 5B). In contrast, desArg[9], leu[8]-BK (1 μM), a BK_1-receptor antagonist, failed to alter this effect of BK (Fig. 3 and 4). HOE140 (3 - 100 nM) dose-dependently inhibited both the BK-induced insulin secretion (Fig. 5A) and $[Ca^{2+}]_i$ increases (Fig. 5B). HOE140 at 100 nM abolished the BK-induced insulin
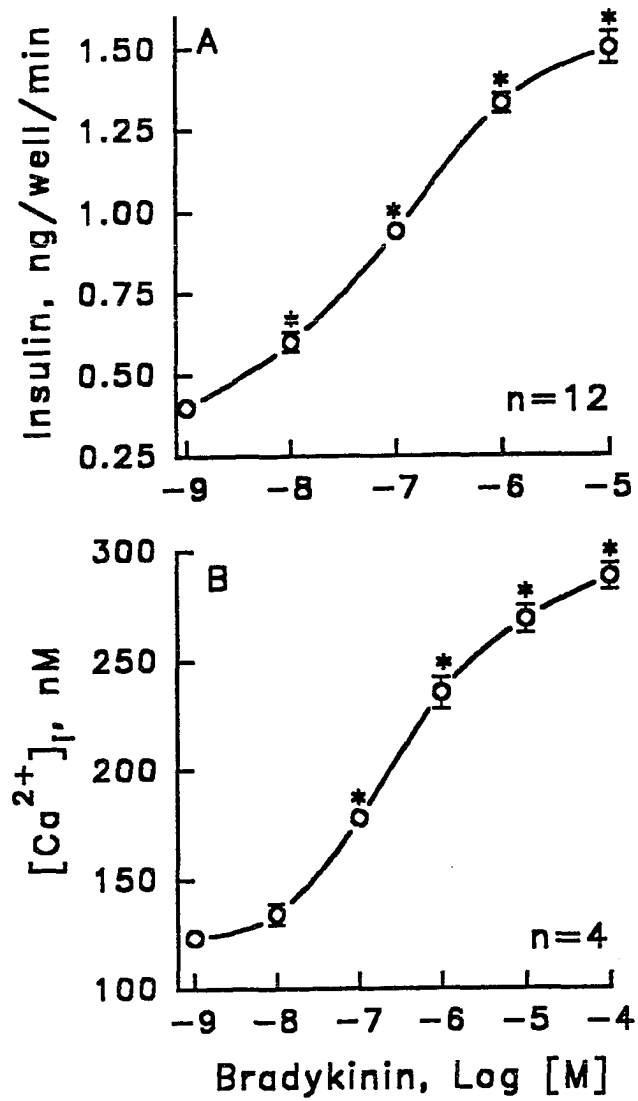
Fig. 1. Effects of bradykinin (BK) on insulin secretion (A) and peak $[Ca^{2+}]_i$ increase (B) in RINm5F cells. In this and the following figures, static incubation for 15 min was performed to measure insulin secretion. *p < 0.05, compared with the insulin concentration of basal control group which was 0.35 ± 0.02 ng/well/min (n = 12, A) or with the baseline level of $[Ca^{2+}]_i$ which was 120 ± 5 nM (n = 24, B).
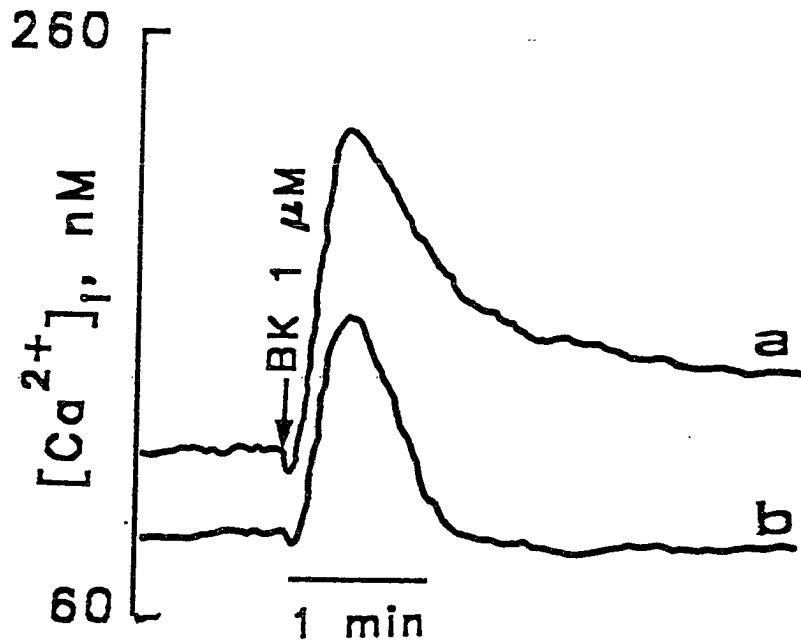
Fig. 2. Effects of bradykinin (BK) on the increase of $[Ca^{2+}]_i$ in the Ca^{2+}-containing (a) or Ca^{2+}-free (b) medium. BK (1 μM) was administered at 1 min. Data shown are representative of eight experiments. Fura-2-loaded RINm5F cells were suspended in 1.5 mM of Ca^{2+} or Ca^{2+}-free medium.
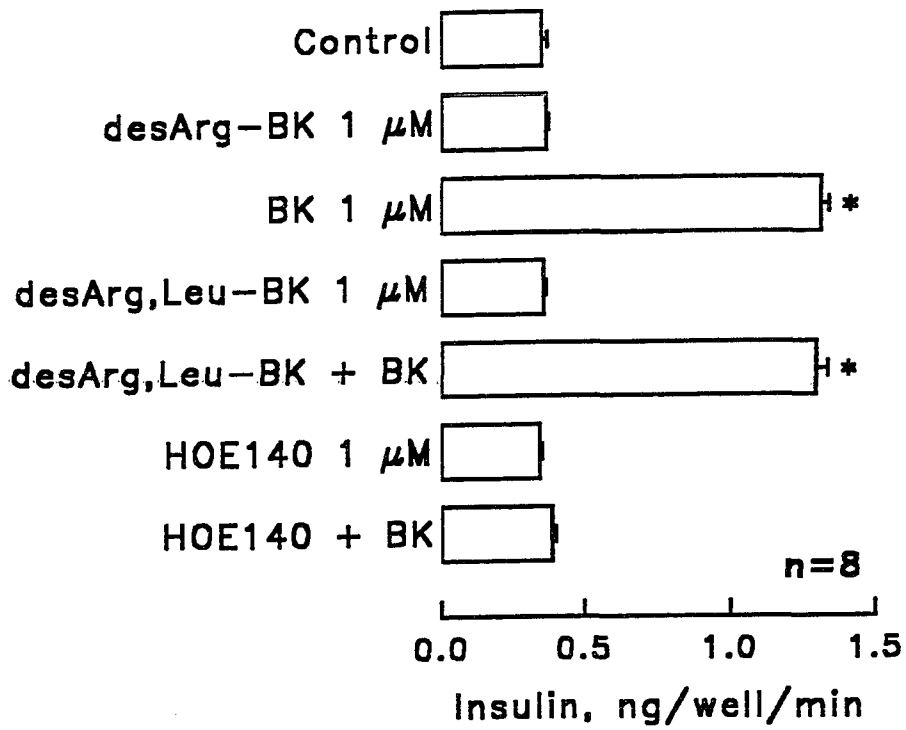
Fig. 3. Effects of bradykinin (BK) and desArg-BK on insulin secretion, and desArg,Leu-BK and HOE140 on BK-induced insulin secretion in RINm5F cells. desArg,Leu-BK or HOE140 was given 5 min before BK administration. *p < 0.05, compared with the insulin concentration of the basal control group.
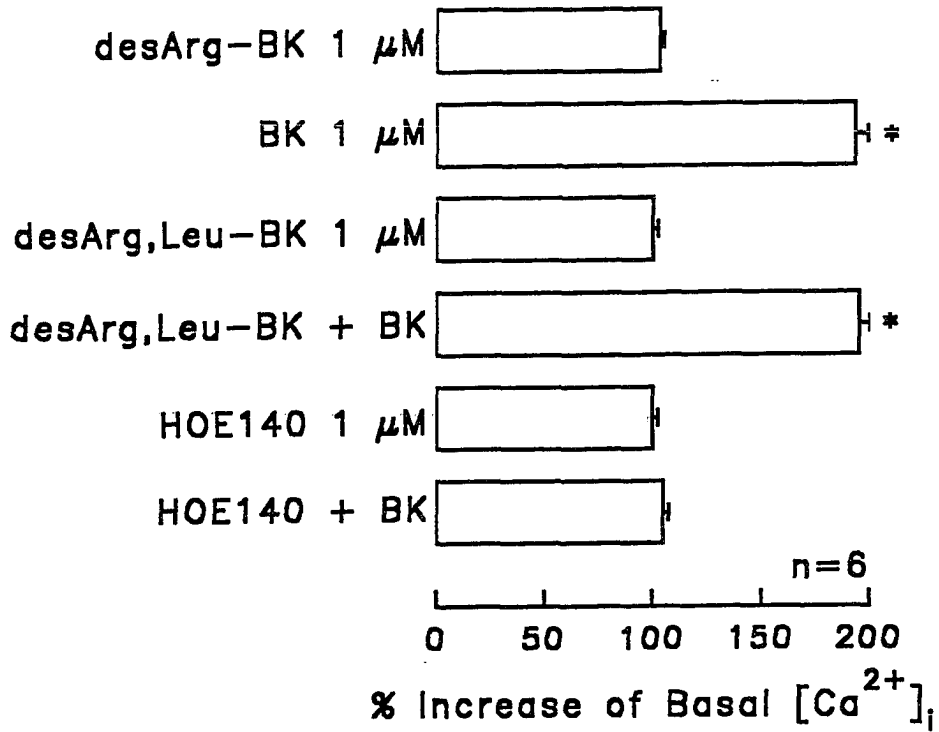
Fig. 4. Effects of bradykinin (BK) and desArg-BK on peak $[Ca^{2+}]_i$ increase, and desArg,Leu-BK and HOE140 on BK-induced peak $[Ca^{2+}]_i$ increase in RINm5F cells. desArg,Leu-BK or HOE140 was given 100 sec before BK administration. $*p < 0.05$, compared with the control group.
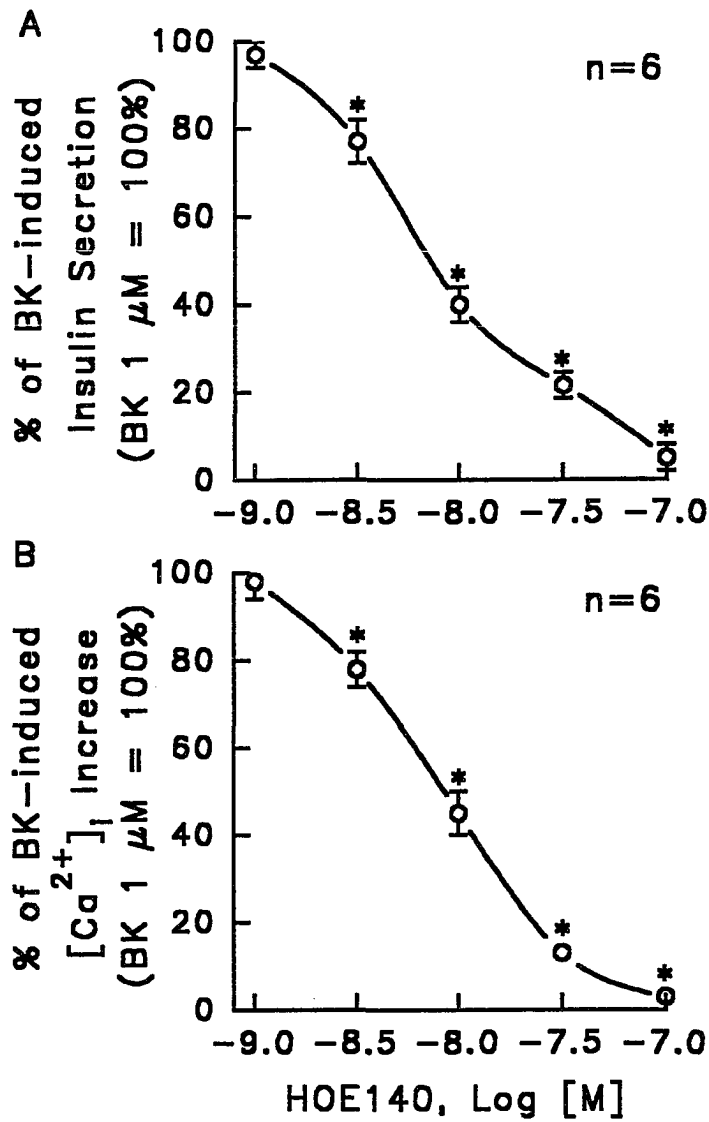
Fig. 5. Effects of HOE140 on bradykinin (BK)-induced insulin secretion (A) and peak $[Ca^{2+}]_i$ increase (B) in RINm5F cells. HOE140 was given 5 min and 100 sec before BK administration in insulin secretion and $[Ca^{2+}]_i$ measurement, respectively. *$p < 0.05$, compared with BK (1 µM) control group. The concentrations of BK-induced insulin secretion were 1.35 ± 0.03 ng/well/ml (n = 6, A) and BK-induced peak $[Ca^{2+}]_i$ were 235 ± 7 nM (n = 6, B).

secretion (Fig. 5A) and $[Ca^{2+}]_i$ increases (Fig. 5B).

Effects of PTX and U-73122 on BK-Induced Insulin Secretion and $[Ca^{2+}]_i$ Increase

Pretreatment with PTX (0.1 µg/ml) for 16 and 2 h, failed to inhibit either BK (1 µM)-induced insulin secretion (BK = 1.31 ± 0.04 ng/well/min; PTX + BK = 1.26 ± 0.05 ng/well/min, n = 8; p> 0.05) or an increase in $[Ca^{2+}]_i$ (BK = 212 ± 5 nM; PTX + BK = 210 ± 4 nM, n = 6; P>0.05), respectively. PTX (0.1 µg/ml) alone did not induce insulin secretion (control = 0.35 ± 0.03 ng/well/min; PTX = 0.36 ± 0.02 ng/well/min, n = 8; P > 0.05) or an increase in $[Ca^{2+}]_i$ (control = 110 ± 5 nM; PTX = 112 ± 3 nM, n = 6; P > 0.05). U-73122 (4, 6 and 8 µM), a PLC inhibitor, dose-dependently inhibited both BK-induced insulin secretion (Fig. 6A) and $[Ca^{2+}]_i$ increases (Fig. 6B). U-73122 at 8 µM prevented the BK-induced insulin secretion and $[Ca^{2+}]_i$ increase by ~ 80%, but the analog, U-73343 (8 µM), failed to alter the BK-induced $[Ca^{2+}]_i$ increases (data not shown). U-73122 alone significantly increased $[Ca^{2+}]_i$ which were 8.6 ± 1 and 11.4 ± 2 over the baseline after administered with U-73122 at the dosages of 6 and 8 µM, respectively (n = 8 for each dosage level). After 100 sec, $[Ca^{2+}]_i$ were decreased close to baseline level. The baseline $[Ca^{2+}]_i$ was 123 ± 1.8 nM (n = 32).

Effect of BK on Intracellular IP_3 and Cyclic AMP Concentrations

The basal level of IP_3 was 54 ± 2.5 pmol/1.5 million cells. BK significantly increased intracellular IP_3 concentrations by ~ 50 % over the control level within 10 s of administration (Fig. 7). BK failed to increase intracellular cAMP concentration (data not shown).

Effects of Thapsigargin (TG) and Nimodipine on BK-Induced Insulin Secretion and $[Ca^{2+}]_i$ increase

Pretreatment of RINm5F cells with TG (1 µM), a selective inhibitor of Ca^{2+} pump in the ER, for 30 min abolished the BK-induced transient Ca^{2+} release phase and the sustained Ca^{2+} influx phase (Fig. 8A). TG (1 µM) alone significantly increased $[Ca^{2+}]_i$ with an onset of 5 sec and reached a peak which was 118 ± 9 nM (n = 6) over the baseline. The baseline $[Ca^{2+}]_i$ was 122 ± 3 nM (n = 6). Pretreatment of RINm5F cells with nimodipine (1 µM) for 5 min and 100 sec also dose-dependently inhibited BK-induced insulin release (Fig. 9) and abolished Ca^{2+} influx (Fig. 8B), respectively. Nimodipine (1 µM) alone did not change insulin secretion (control = 0.35 ± 0.02 ng/well/min; nimodipine = 0.39 ± 0.04 ng/well/min, n = 8; P > 0.05) or an increase in $[Ca^{2+}]_i$
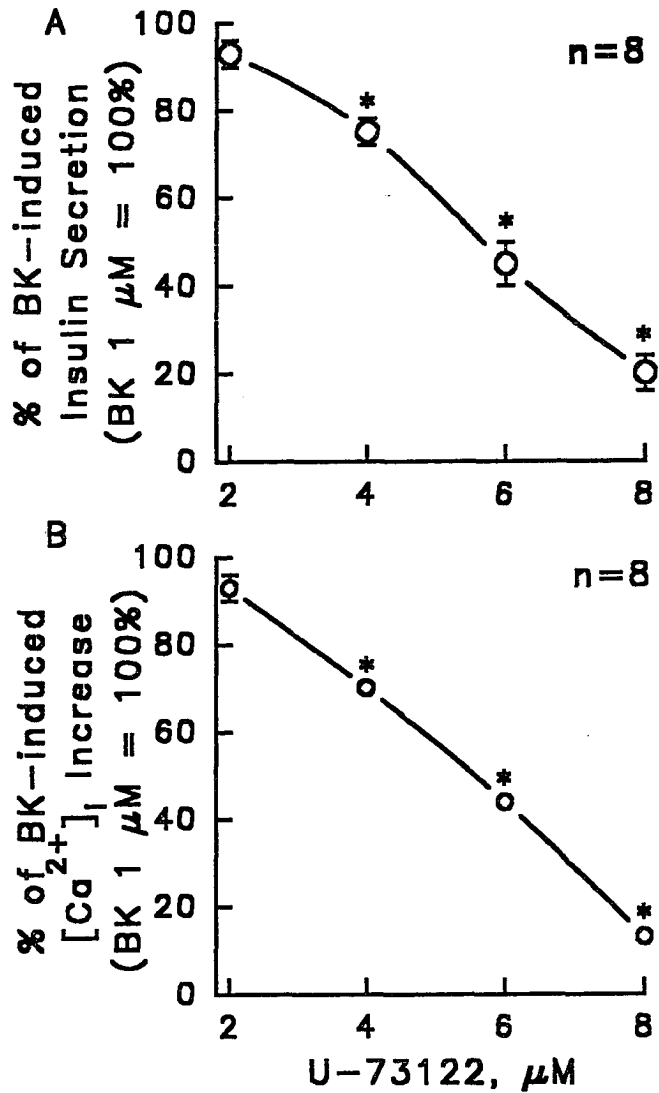
Fig. 6. Effect of U-73122 on bradykinin (BK)-induced insulin secretion (A) and peak $[Ca^{2+}]_i$ increase (B) in RINm5F cells. U-73122 was given 5 min and 100 sec before BK administration in insulin secretion and $[Ca^{2+}]_i$ measurement, respectively. *$p < 0.05$, compared with the BK (1 μM) control group. The concentrations of BK -induced insulin secretion were 1.40 ± 0.05 ng/well/ml (n=8, A) and BK-induced peak $[Ca^{2+}]_i$ were 95 ± 3 nM (n=8, B).
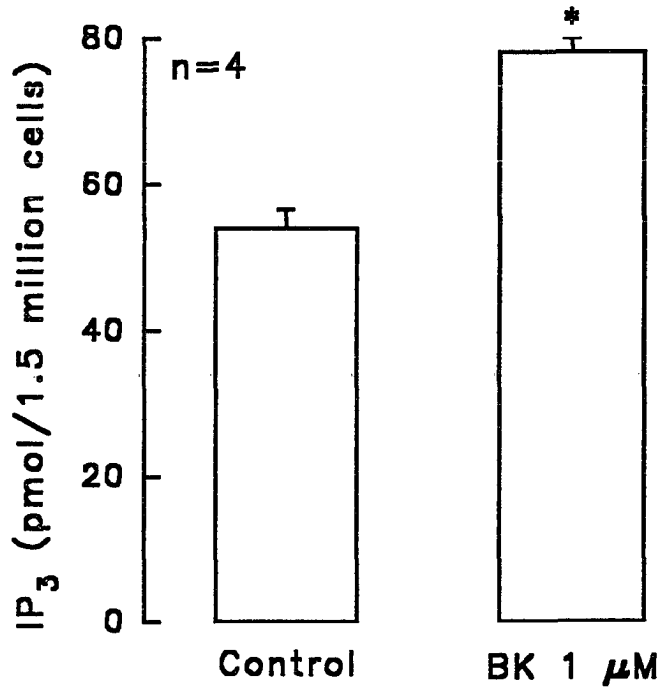
Fig. 7. Effect of bradykinin (BK) on intracellular IP_3 concentration in RINm5F cells. *$p < 0.05$, compared with the control group which was 54 ± 2.5 pmol/1.5 million cells (n=4).
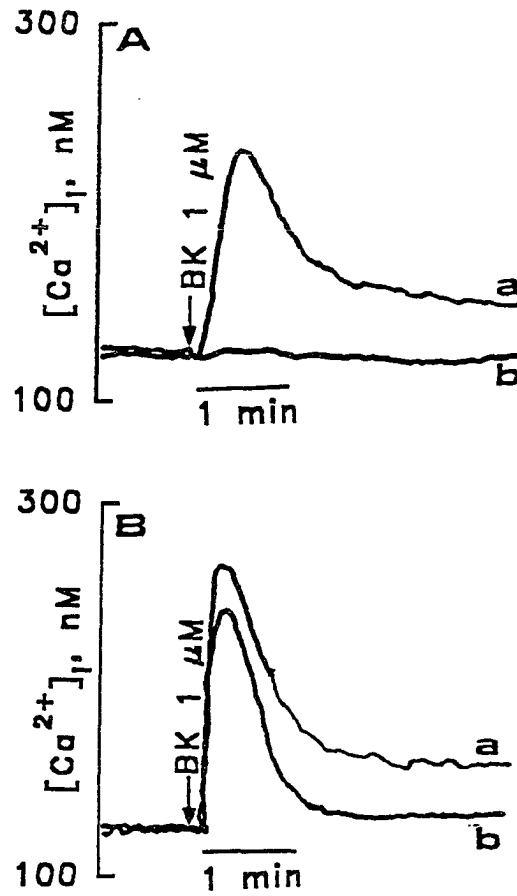
Fig. 8. Effects of thapsigargin (TG) (A) and nimodipine (B) on BK-induced $[Ca^{2+}]_i$ rise in RINm5F cells. A: curve a shows data of BK (1 μM) alone as control; curve b shows the effects of pretreatment with TG for 30 min before BK administration. B: curve a shows data of BK (1 μM) alone as control; curve b shows the effects of pretreatment with nimodipine for 100 sec before BK administration. Data shown are representative of six experiments

Fig. 9. Effects of nimodipine (NIM) on bradykinin (BK)-induced insulin secretion in RINm5F cells. Nimodipine was given 5 min before BK administration. *$p < 0.05$, compared with BK (1 μM) alone group.

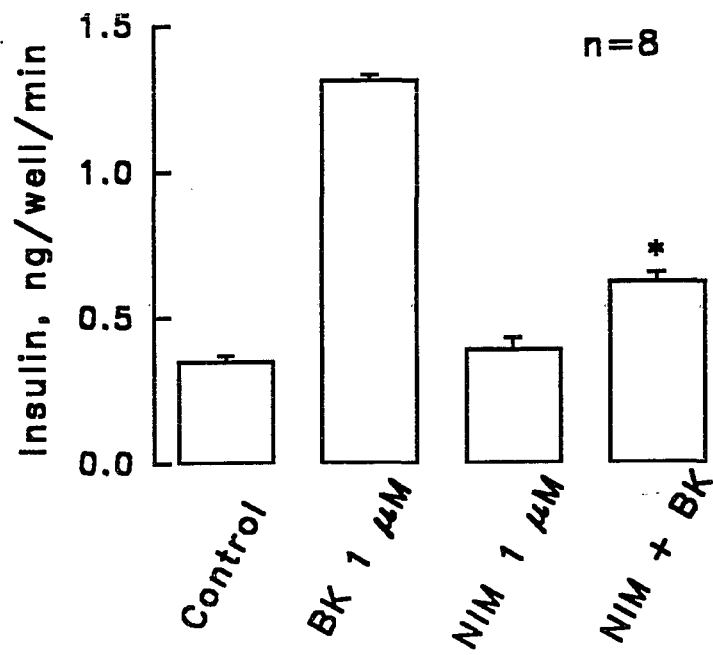(control = 112 ± 4 nM; nimodipine = 114 ± 2 nM, n = 6; $P > 0.05$).

Effects of the Activator and Inhibitor of PKC on BK-Induced Insulin Secretion and $[Ca^{2+}]_i$ Increase

Pretreatment of RINm5F cells with a PKC activator OAG (3 - 30 μM) for 30 sec dose-dependently inhibited BK-induced insulin secretion (Fig. 10). OAG (30 μM) also abolished the BK-induced sustained Ca^{2+} phase (Fig. 11A). OAG (30 μM) alone did not increase $[Ca^{2+}]_i$ (control = 120 ± 3 nM; OAG = 119 ± 2 nM, n = 4; $P > 0.05$). The PKC inhibitor Ro 31-8220 (3-10 μM) increased BK-induced insulin secretion in a dose-dependent manner (Fig. 12). Ro 31-8220 (10 μM) alone significantly increased insulin secretion which was 0.5 % over the basal control group (control = 0.36 ± 0.03 ng/well/min; Ro 31-8220 = 0.54 ± 0.02 ng/well/min, n = 8, P < 0.05). Ro 31-8220 at 10 μM further increased the BK-induced rise in $[Ca^{2+}]_i$ (Fig. 11B). Both the BK-induced Ca^{2+} spike and sustained Ca^{2+} phase were enhanced by the pretreatment with Ro 31-8220 (Fig. 11B). In the Ca^{2+}-free medium, Ro 31-8220 (10 μM) did not alter the BK-induced increase in $[Ca^{2+}]_i$ (BK = 75 ± 4 nM; Ro 31-8220 + BK = 78.4 ± 6 nM (n = 6). Nimodipine (1 μM) reduced , but failed to abolish, the Ro 31-8220-enhanced BK-induced $[Ca^{2+}]_i$ increase (Fig. 11B).

Effect of ACA and L-NAME on BK-Induced Insulin Secretion

Neither the PLA_2 inhibitor ACA (100 μM), nor the NOS inhibitor L-NAME (100 μM) affected BK (1 μM)-induced insulin secretion (ACA + BK = 1.24 ± 0.04 ng/well/min; L-NAME + BK = 1.20 ± 0.04 ng/well/min; BK = 1.27 ± 0.03 ng/well/min, n = 8; P > 0.05, compared with each other).

Discussion

The results of the present study suggested that BK increases $[Ca^{2+}]_i$ and insulin secretion in RINm5F cells by activating BK_2-receptors, because BK, the nonselective BK-receptor agonist (Farmer 1992), induced insulin secretion and $[Ca^{2+}]_i$ increase, but a BK_1-receptor agonist, desArg^9-BK (Farmer, 1992), did not. In addition, the BK-induced insulin secretion and $[Ca^{2+}]_i$ increase were inhibited by a BK_2-receptors antagonist HOE140 (Cuthbert et al., 1992), but were not altered by a BK_1-receptor antagonist desArg^9,leu^8-BK (Goldstein and Wall, 1984). This finding is
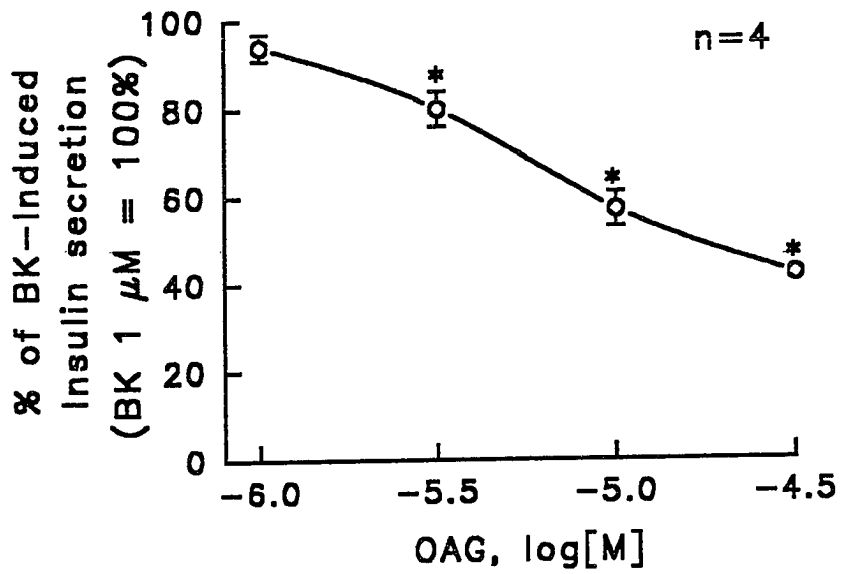
Fig. 10. Effect of 1-oleoyl-2-acetyl glycerol (OAG) on bradykinin (BK)-induced insulin secretion in RINm5F cells. OAG was given 30 sec before BK administration. $*p < 0.05$, compared with the insulin concentration of BK 1 μM control group which was 1.45 ± 0.05 ng/well/ml (n = 4).
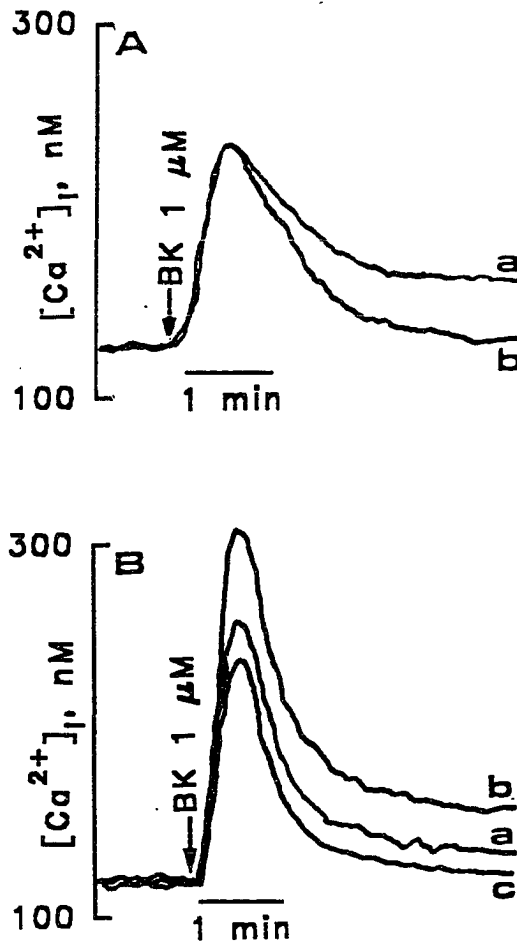
Fig. 11. Effect of an activator and inhibitor of PKC on BK-induced $[Ca^{2+}]_i$ increase in RINm5F cells. A: effect of 1-oleoyl-2-acetyl glycerol (OAG). Data shown are representative of four experiments. Curve a shows data of BK (1 μM) alone as control, and curve b shows those of the pretreatment with OAG (30 μM) for 30 sec before BK. B: effect of Ro 31-822. Data shown are representative of six experiments. Curve a shows data of BK alone as control, curve b shows those of the pretreatment with Ro 31-8220 (10 μM) for 30 min before BK and curve c shows those of the pretreatment with Ro 31-8220 (10 μM) for 30 min and with nimodipine (1 μM) for 100 sec before BK.

Fig. 12. Effect of Ro 31-8220 on bradykinin (BK)-induced insulin secretion in RINm5F cells. Ro 31-8220 was given 30 min before BK administration. *p < 0.05, compared with the BK (1 μM) control group.

consistent with that of our previous study in the perfused rat pancreas (Yang and Hsu, 1995). Further studies are needed to explore the BK_2-receptors of β cells using radioligand binding techniques.
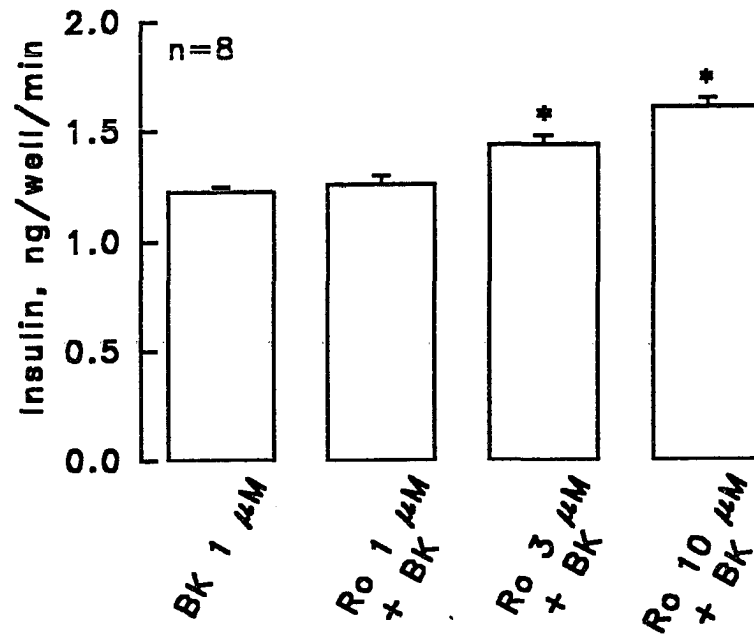
The BK_2-receptor protein has seven-transmembrane spanning domains, and are coupled to G-proteins (Hess et al., 1994). There are PTX-sensitive G-proteins, such as G_i and G_o (Hess et al., 1994; Schmidt et al., 1991) and PTX-insensitive G-proteins, such as the G_q family (Phaneuf et al., 1993). In bovine aortic endothelial cells, BK activates both G_i- and G_q-proteins (Liao and Homcy, 1993). We found that PTX at 0.1 μg/ml failed to inhibit the stimulatory effects of BK in the present study, but it abolished the inhibitory effect of $α_2$-adrenoceptor agonists on insulin secretion from RINm5F cells (Chen and Hsu, 1994) and $[Ca^{2+}]_i$ in HIT cells (Hsu et al., 1991) in previous studies. These results suggested that in RINm5F cells, BK_2-receptors are coupled to a PTX-insensitive G-protein, probably G_q, which mediates $[Ca^{2+}]_i$ and insulin secretion.

The G_q-proteins are usually coupled to PLC (Hepler and Gilman, 1992). The specific PLC inhibitor, U-73122, inhibits PLC in a variety of cells such as human neutrophils and platelets (Bleasdale et al., 1990), rat hepatocytes (Galan et al., 1991), pancreatic acinar cells (Yule and Williams, 1992) and β-cells (Chen and Hsu, 1994). In the present study, BK-induced insulin secretion and $[Ca^{2+}]_i$ increase were inhibited by U-73122 in a dose-dependent manner, but were not altered by the analog U-73343 which does not inhibit PLC activity (Smith et al., 1990). Thus, these results suggested that BK activates PLC which increases the formation of IP_3. IP_3, in turn, increases Ca^{2+} release from the ER (Berridge, 1993).

Intracellular Ca^{2+} is a major signal in insulin secretion. In many cell types, the stimulus-response usually couples to the elevation of $[Ca^{2+}]_i$ through Ca^{2+} release from intracellular stores and Ca^{2+} influx. Secretagogues, including hormones and neurotransmitters increase intracellular Ca^{2+} leading to insulin secretion (Li et al., 1992; Komatsu et al., 1988). In RINm5F cells, BK induced a transient Ca^{2+} peak which was immediately followed by a sustained Ca^{2+} phase which was attributed to Ca^{2+} influx. The transient Ca^{2+} peak is partly attributed to the release of Ca^{2+} from intracellular stores, probably the ER, because in Ca^{2+}-free medium, BK only induced a Ca^{2+} peak without the sustained Ca^{2+} phase. TG depletes the intracellular Ca^{2+} stores by inhibiting the Ca^{2+} uptake of Ca^{2+} pump (Thastrup et al., 1990). Our findings showed that TG abolished both the BK-induced transient Ca^{2+} spike and sustained Ca^{2+} phase, suggesting that the BK-induced Ca^{2+} influx depends on BK-induced Ca^{2+} release. The BK-induced $[Ca^{2+}]_i$ peak in the Ca^{2+}-containing medium was higher than that in the Ca^{2+}-free medium. Thus, the BK-induced Ca^{2+}

peak in Ca^{2+}-containing medium partly came from Ca^{2+} release and partly came from Ca^{2+} influx. The Ca^{2+} influx may be predominantly attributed to the opening of receptor-operated Ca^{2+} channels (ROCs), and/or voltage-dependent Ca^{2+} channels (VDCCs) (Fasolato et al., 1994; Leis et al., 1995). Our findings suggested that the BK-induced Ca^{2+} influx is predominantly mediated through the VDCCs, because the sustained Ca^{2+} influx was abolished by a VDCC blocker, nimodipine.

The results in the present study suggested that BK increases insulin secretion by activating the PLC-IP_3 signal transduction system. In addition to IP_3 formation, activation of PLC also promotes the formation of DAG (Takeuchi et al., 1988). In HIT cells, carbachol and bombesin increase $[Ca^{2+}]_i$ and the formation of DAG which activates PKC in insulin-secreting cells (Regazzi et al., 1990), and thus increases insulin secretion. However, the role of PKC activation in mediating insulin secretion is still a matter of considerable debate. In many cell systems, PKC has inhibitory and stimulatory effects (Nishizuka, 1988). TPA (12-O-tetradecanoylphorbol 13-acetate) activates PKC and induces insulin secretion in HIT cells (Swope and Schonbrunn, 1988). The overnight pretreatment of HIT cells with TPA which depletes PKC activity decreases acetylcholine and glucose-stimulated insulin secretion (Hughes et al., 1990). The activation of PKC by DAG maintains glucose-induced sustained insulin secretion in rat islet (Ganesan et al., 1990) and in mouse islet induced by interleukin-1 (Eizirik et al., 1995). In addition, a PKC inhibitor staurosporine decreases insulin secretion (Zawalich et al., 1991). These observations suggest that PKC enhances insulin secretion in pancreatic islets and insulin-secreting cell lines. On the other hand, RINm5F cells treated with TPA, which depletes intracellular PKC, displays an enhanced insulin secretion in response to vasopressin and carbachol by activating PLC (Li et al., 1990). OAG which depolarizes membrane and increases $[Ca^{2+}]_i$ also increases insulin secretion in PKC-deficient cells (Li et al., 1990). In addition, activation of PKC also inhibits the coupling of receptors to PLC and interferes with insulin secretion (Li et al., 1990). Activation of PKC in RINm5F cells lowers $[Ca^{2+}]_i$ by promoting an active extrusion of $[Ca^{2+}]_i$ (Berggren et al., 1989), thereby reducing insulin secretion. Our findings suggested that activation of PKC reduces BK-induced insulin secretion in RINm5F cells, because a PKC activator OAG inhibited BK-induced insulin secretion, whereas, a selective PKC inhibitor Ro 31-8220 (10 μM) (Dieter and Fitzke, 1991) increased BK-induced insulin secretion.

Activation of PKC may stimulate the Ca^{2+} pump or inhibit the Ca^{2+} channel of the plasma membrane, thereby decreasing $[Ca^{2+}]_i$ (Ziche et al., 1993; Arkhammar et al., 1994). Berggren and coworkers (1989) reported that PKC promotes the recovery of RINm5F cells from elevated $[Ca^{2+}]_i$

by increasing an active extrusion of Ca^{2+} to the extracellular space. Our findings suggested that PKC decreases BK-induced Ca^{2+} influx or increases BK-induced Ca^{2+} efflux, because in the Ca^{2+}-containing medium, OAG inhibited the BK-induced sustained Ca^{2+} phase, whereas Ro 31-8220 (10 μM) (Dieter and Fitzke, 1991) increased BK-induced sustained Ca^{2+} phase. In the Ca^{2+}-free/EGTA medium, Ro 31-8220 and OAG did not alter the BK-induced Ca^{2+} rise. Nimodipine (1 μM) inhibited BK-induced Ca^{2+} influx, but did not abolish BK-induced Ca^{2+} influx in RINm5F cells pretreated with Ro 31-8220. These results suggested that PKC may promote Ca^{2+} efflux and inhibit Ca^{2+} influx, thereby returning $[Ca^{2+}]_i$ to normal range. Further studies are required which use $^{45}Ca^{2+}$ to determine the effect of PKC on Ca^{2+} flux.

BK stimulates the formation of NO in vascular endothelial cells by activating PTX-insensitive G_q protein (Liao and Homcy, 1993). NO may increase insulin secretion by activating guanylate cyclase and generating cGMP (Laychock et al., 1991; Pueyo et al., 1994). Our present results suggested that the NO signal transduction pathway is not involved in BK-induced insulin secretion in RINm5F cells, because a NOS inhibitor, L-NAME (100 μM) failed to alter BK-induced insulin secretion and $[Ca^{2+}]_i$ increase. In Swiss 3T3 fibroblasts, BK also activates the PLA_2 signal transduction pathway via PTX-insensitive G-proteins (Burch and Axolrod, 1987). The activated PLA_2 increases endogenous arachidonic acid which mobilizes Ca^{2+} from the ER and regulates VDCCs (Konrad et al., 1992) and thus induces insulin secretion (Band et al., 1992). However, our findings indicated that PLA_2 is not involved in the BK-induced insulin secretion because the PLA_2 inhibitor ACA (100 μM) failed to alter the effects of BK. In another study, ACA inhibited glucose-induced activation of PLC in β-cells (Konrad et al., 1992). In cultured tracheal smooth muscle cells, BK activates adenylate cyclase and increases cAMP (Stevens et al., 1994). However, in the present study, BK (1 μM) did not alter the intracellular concentration of cAMP in RINm5F cells. Our results further suggested that an increase in cAMP concentration is not involved in BK-induced insulin secretion.

In summary, our results suggested that in β-cells, bradykinin activates BK_2-receptors which, in turn, activates a receptor-coupled PTX-insensitive G_q protein. The G_q protein activates PLC which increases the formation of IP_3 and DAG. IP_3 releases Ca^{2+} from the ER and triggers the Ca^{2+} influx, leading to insulin secretion. DAG activates PKC which may promote Ca^{2+} efflux and inhibit Ca^{2+} influx to return $[Ca^{2+}]_i$ to normal range.

References

Arkhammar, P., L. Juntti-Berggren, O. Larsson, M. Welsh, E. Nanberg, A. Sojoholm, M. Kohler, and P. O. Berggren. Protein kinase C modulates the insulin secretory process by maintaining a proper function of the beta-cell voltage-activated Ca^{2+} channels. J. Biol. Chem. 269: 2743-2749, 1994.

Band, A. M., P. M. Jones, and S. L. Howell. Arachidonic acid-induced insulin secretion from rat islets of Langerhans. J. Mol. Endocrinol. 8: 95-101, 1992.

Berrgren, P.O., P. Arkhammar, and T. Nilsson. Activation of protein kinase C assists insulin producing cell in recovery from raised cytoplasmic Ca^{2+} by stimulating Ca^{2+} efflux. Biochem. Biophys. Res. Commun. 165: 416-421, 1989.

Berridge, M. J. Inositol phosphate and calcium signalling. Nature 361: 315-325, 1993.

Bhoola, K. D., C. D. Figueroa, and K. Worthy. Bioregulation of kinin: kallikrein, kininogen, and kininases. Pharmacol. Rev. 44: 1-80, 1992.

Biden, T. J. B. Peter-Riesch, W. Schlegel, and C. B. Wollheim. Ca^{2+}-mediated generation of inositol 1,4 ,5-triphosphate in pancreatic islets. Studies with K^+, glucose, and carbamylcholine. J. Biol. Chem. 262: 3567-3571, 1987.

Bleasdale, J. E. N. R. Thakur, R. S. Gremban, G. L. Bundy, F. A. Fitzpatrick, R. J. Smith, and S. Bunting. Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. J. Pharmacol. Exp. Ther. 255: 756-768, 1990.

Burch, R.M., and J. Axelrod. Dissociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 fibroblasts. Evidence for a G protein regulation of phospholipase A_2. Proc. Natl. Acad. Sci. USA 84 :6347-6378, 1987.

Carter, R. D., W. L. Joyner, and E. M. Renkin. Effect of histamine and some other substances on molecular selectivity of the capillary wall to plasma proteins and dextran. Microvasc. Res. 7: 31-48, 1974.

Chen, T. H., and W. H. Hsu. Inhibition of insulin release by a formamidine pesticide amitraz and its metabolites in a rat β-cell line: An action mediated by α_2-adrenoceptors, a GTB-binding protein and a decrease in cyclic AMP. J. Pharmacol. Exp. Ther. 271: 1240-1245, 1994.

Chen, T. H., and W. H. Hsu. U-73122 inhibits carbachol-induced increases in $[Ca^{2+}]_i$, IP_3, and insulin release in beta-TC3 cells. Life Sci. 56: PL103-108, 1994.

Collier, H. O., J. A. Holgate, M. Schachter, and P. G. Shorley. The bronchoconstrictor action of bradykinin in the Guinea pig. Br. J. Pharmacol. 15: 290-297, 1962.

Cuthbert, A. W., L. J. MacVinish, and R. J. Pickles. Antagonism of kinin effects on epithelial by HOE 140: Apparently competitive and non-competitive interactions. Br. J. Pharmacol. 107: 797-802, 1992.

Dieter, P., and E. Fitzke. Ro 31-8220 and Ro 31-7549 show improved selectivity for protein kinase C over staurosporine in macrophages. Biochem. Biophys. Res. Commun. 181: 396-401, 1991.

Eizirik, D. L., S. Sandler, N. Welsh, L. Juntti-Berggren, and P. O. Berggren. Interleukin-1 beta-induced stimulation of insulin release in mouse pancreatic islets is related to diacylglycerol production and protein kinase C activation. Mol. Cell Endocrinol. 111: 159-165, 1995.

Farmer, S. G. Biochemical and molecular pharmacology of kinin receptors. Annu. Rev. Pharmacol. Toxicol. 32: 511-536, 1992.

Fasolato, C., B. Innocenti, and T. Pozzan. Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels. Trends Pharmacol. Sci. 15: 77-83, 1994.

Fernandez, B., and J. Balsinde. Receptor-mediated activation of arachidonic acid release in mouse peritoneal macrophages is linked to extracellular calcium influx . Biochem. Biophys. Res. Commun. 180: 1036-1040, 1991.

Figueroa, C. D., L. M. Henderson, R. W. Colman, R. A. De La Cadena, W. Müller-Esterl, and K. D. Bhoola. Immunoreactive L- and H-kininogen in human neutrophils. J. Physiol. (Lond.) 425: 65, 1990.

Galan, J., M. Trankina, R. Noel, E. Sprague, and W. Ward. Neomycin affects insulin internalization in rat hepatocytes. FASEB J. 5: A757, 1991.

Ganesan, S., R Calle, K. C. Zawalich, H. Smallwood, W. S. Zawalich, and H. Rasmussan. Glucose-induced translocation of protein kinase C in rat islets. Proc. Natl. Acad. Sci. USA 97: 9893-9897, 1990.

Goldstein, R. H. and M. Wall. Activation of protein formation and cell division by bradykinin and desArg^9-bradykinin. J. Biol. Chem. 259: 9263-9268, 1984.

Helper, J. R., and A. G. Gilman. G proteins. Trends Biochem. Sci. 17: 383-387, 1992.

Hess, J. F., J. A. Borkowski, G. Y. Stonesifer, T. Macneil, C. D. Strader, and R. W. Ransom. Cloning and pharmacological characterization of bradykinin receptors. Braz. J. Med. Biol. Res. 27: 1725-1731, 1994.

Hsu, W. H., H. Xiang, A. S. Rajan, D. L. Kunze, and A. E. Boyd III. Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta cell. J. Biol. Chem. 266: 837-843, 1991.

Hughes, S. J., J. G. Chalk, and S. J. Ashcroft. The role of cytosolic free Ca^{2+} and protein kinase C in acetylcholine-induced insulin release in the clonal beta-cell line, HIT-T15. Biochem. J. 267: 227-232, 1990.

Konrad, R. J., Y. C. Jolly, C. Major, and B. A. Wolf. Inhibition of phospholipase A_2 and insulin secretion in pancreatic islets. Biochim. Biophys. Acta. 1135: 215-220, 1992.

Komatsum M., T. Aizawa, N. Takasu, and T. Yamada. Glucose raises cytosolic free calcium in the rat pancreatic islets. Horm. Metabol. Res. 21: 405-409, 1989.

Laychock, S. G., M. E. Modica, and C. T. Cavanaugh. L-arginine stimulates cyclic guanosine 3', 5'-monophosphate formation in rat islets of Langerhans and RINm5F insulinoma cells: evidence for L-arginine:nitric oxide synthase. Endocrinology 129: 3043-3052, 1991.

Leis, H. J., D. Zach, E. Huber, L. Ziermann, H. Gleispach, and W. Windischhofer. On the inhibition of prostaniod formation by SK&F 96365, a blocker of receptor-operated calcium entry. Br. J. Pharmacol. 114: 598-601, 1995.

Li, G., R. Regazzi, S. Ullrich, W F. Pralong, and C. B. Wollheim. Potentiation of stimulus-induced insulin secretion in protein kinase C-deficient RINm5F cells. Biochem. J. 272: 637-645, 1990.

Li, G., W. F. Pralong, D. Pittet, G. W. Mayr, W. Schlege, and C. B. Wollheim. Inositol tetrakisphosphate isomers and elevation of cytosolic Ca^{2+} in vasopressin-stimulated insulin-secreting RINm5F cells. J. Biol. Chem. 267: 4349-4356, 1992.

Liao, J. K. and C. J. Homcy. The G proteins of the G alpha i and G alpha q family couple the bradykinin receptor to the release of endothelium-derived relaxing factor. J. Clin. Invest. 92(5): 2168-2172, 1993.

Marceau, F., and B. Tremble. Mitogenic effect of bradykinin and Des-Arg[9]-bradykinin on cultured fibroblast. Life Sci. 39: 2351-2358, 1986.

Mombouli, J. V., and P. M. Vanhoutte. Kinins and endothelial control of vascular smooth muscle. Annu. Rev. Pharmacol. Toxicol. 35: 679-705, 1995.

Nishizuka, Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature 308: 693-698, 1984.

Nishizuka, Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334: 661-665, 1988.

Pan, H. L., C. L. Stebbins, and J. C. Longhurst. Bradykinin contributes to the exercise pressor reflex: mechanism of action. J. Appl. Physiol. 75: 2061-2068, 1993.

Phaneuf, S., G. N. Europe-Finner, M. Varney, I. Z. MacKenzie, S. P. Watson, A. Lôpez Bernal. Oxytocin-stimulated phosphoinositide hydrolysis in human myometrial cells: Involvement of pertussis toxin-sensitive and -insensitive G-proteins. J. Endocrinol. 136: 497-509, 1993.

Pueyo, M. E., W. Gonzalez, E. Pussard, and J. F. Arnal. Insulin secretion in rats with chronic nitric oxide synthase blockade. Diabetologia 37: 879-884, 1994.

Rasmussen, H., K. C. Zawalich, S. Ganesan, R. Calle, and W. S. Zawalich. Physiology and pathophysiology of insulin secretion. Diabetes Care 13: 655-666, 1990.

Regazzi, R., G. D. Li, J. Deshusses, and C. B. Wollheim. Stimulus-response coupling in insulin-secreting HIT cells. Effects of secretagogues on cytosolic Ca^{2+}, diacylglycerol, and protein kinase C activity. J. Biol. Chem. 265: 15003-15009, 1990.

Regoli, D. D. Jukic, F. Gobeil, and N. E. Rhaleb. Receptors for bradykinin and related kinin: a critical analysis. Can. J. Physiol. Pharmacol. 71(8): 556-67, 1993.

Richards, J. S. J. A. Jonassen, A. I. Rolfers, K. Jersey, and L. E. Jr. Reichert. Adenosine 3',5'-monophosphate, luteinizing hormone receptor, and progesterone during granulosa cell differentiation: Effect of estradiol and follicle-stimulating hormone. Endocrinology 104: 765-773, 1979.

Schmidt, A., J. Hescheler, S. Offermanns, K. Spicher, K. Hinsch, F. Klinz, J. Codina, L. Birnbaumer, H. Gausepohl, R. Frank, G. Schultz, and W. Rosenthal. Involvement of pertussis toxin-sensitive G-proteins in the hormonal inhibition of dihydropyridine-sensitive Ca^{2+} currents in an insulin-secreting cell line (RINm5F). J. Biol. Chem. 266: 18025-18033, 1991.

Sharp, P. A., and E. S. Debnam. Rapid stimulatory effect of bradykinin on glucose transport across the brush-border and basolateral membranes of rat jejunal enterocytes. Exp. Physiol. 77: 913-916, 1992.

Smith, R. J., L. M. Sam, J. M. Justen, G. L. Bundy, G. A. Bala, and J. E. Bleasdale. Receptor-coupled signal transduction in human polymorphonuclear neutrophils: effects of a novel inhibitor of phospholipase C-dependent processes on cell responsiveness. J. Pharmacol. Exp. Ther. 253: 688-697, 1990.

Steranka, L. R., D. C. Manning, C. J. Dehaas, J. W. Ferkany, S. A. Borosky, J. R. Connor, R. J. Vavrex, J. M. Steward, and S. H. Snyder. Bradykinin as a pain mediators: receptors are localized to sensory neurons and antagonists have analgesic action. Proc. Natl. Acad. Sci. USA 85: 3245-3249, 1988.

Stevens, P. A., S. Pyne, M. Grady, and N, J, Pyne. Bradykinin-dependent activation of adenylate cyclase activity and cyclic AMP accumulation in tracheal smooth muscle occurs via protein kinase C-dependent and -independent pathways. Biochem. J. 1994.

Suidan, H. S., R. D. Murrell, and A. M. Tolkovsky. Carbachol and bradykinin elevated cyclic AMP and rapidly deplete ATP in cultured rat sympathetic neurons. Cell Regul. 2: 13-25, 1991.

Swope, S. L., and A. Schonbrunn. The biphasic stimulation of insulin secretion by bombesin involves both cytosolic calcium and protein kinase C. Biochem. J. 253: 193-202, 1988.

Takeuchi, K., K. Abe, K. Maeyama, M. Sato, M. Yasujimam, K. Omata, Y. Kassai, T. Watanabe, and K. Yoshinaga. Relationship between bradykinin induced rise in cytosolic free calcium and prostacyclin synthesis in cultured rat vascular smooth muscle cells. pp. 117-122, 1988. in O. Iimora and H. S. Margolius eds. Renal function, hypertension and kallikrein-kinin system. University of Tokyo Press. Tokyo, Japan.

Thastrup, O., P. J. Cullen, B. K. DrØbak, M. R. Hanley, and A. P. Dawson. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} store by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. Proc. Natl. Acad. Sci. USA 87: 2466-2470, 1990.

Thomas, T. P., T. R. Ellis, and S. B. Pex. Insulin release in RINm5F cells and glyceraldehyde activation of protein kinase C. Diabetes 38: 1371-1376, 1989.

Wicklmayr, M., and G. Dietze. Effect of oral kallikrein and intrabrachial-arterial bradykinin on forearm-metabolism in maturity onset diabetes. In: Kininogenases, Kallikrein 4, edited by G. L. Haberland, J. W. Rohen, and T. Suzuki, Stuttgart, New York, Schattauer Verlag, 1977, p. 299-308.

Yang, C., and W. H. Hsu. Stimulatory effect of bradykinin on insulin release from the perfused rat pancreas. Am. J. Physiol. 268: E1027-E1030, 1995.

Yule, D. I., and J. A. Williams. U-73122 inhibits Ca^{2+} oscillations in response to cholecystokinin and carbachol but not to JMV-180 in rat pancreatic acinar cells. J. Biol. Chem. 267: 13830-13835, 1992.

Zawalich, W. S., K. C. Zawalich, S. Ganesan, R. Colle, and H. Rasmussen. Influence of staurosporine on glucose-mediated and glucose-conditioned insulin secretion. Biochem. J. 279: 807-813, 1991.

Ziche, M. D. Zawieja, R. K. Hester, and H. Granger. Calcium entry, mobilization, and extrusion in postcapillary venular endothelium exposed to bradykinin. Am. J. Physiol. 265: H569-H580, 1993.

CHAPTER 6. GENERAL CONCLUSIONS

General Discussion

BK influences a number of biological activities. However, the physiological function of kallikrein-kinin system in the pancreas is unclear, except that tissue kallikrein may be involved in the formation of insulin from proinsulin (Ole-Moiyoi et al., 1979) and it is suppressed in diabetic patients (Uehara et al., 1988). A hypoglycemic effect caused by kallikrein-kinin system was first reported by Frey in 1932 (Bhoola et al., 1992). Kallikreins decrease the blood glucose concentration in diabetic patients with NIDDM or diabetic dogs (Bhoola et al., 1992) by releasing kinins from kininogens. Kinins produce a hypoglycemic effect by increasing the uptake of blood glucose (Jauch et al., 1986, 1988), and reducing glucose production (Jauch et al., 1988). In alloxan-induced diabetic rats, captopril, an inhibitor of angiotensin-converting enzyme and kininase, enhances BK-induced hypoglycemia (Jaffa et al., 1986). However, kinins do not have direct actions to increase glucose uptake or utilization, since they increase glucose oxidation only in the presence of insulin in rat adipocytes (Symington et al., 1988). The role of the kallikrein-kinin system on insulin secretion was first reported by Vance and Bragg in 1969. Kallikrein increased insulin secretion, but BK alone failed to show a consistent increase in insulin secretion from the perfused canine pancreas (Vance and Bragg, 1969) or in isolated rat pancreatic islets (Schack et al., 1980). However, the stimulatory effect of BK from our preliminary data in the canine pancreatic perfusion and $[Ca^{2+}]_i$ measurement suggested that studies are needed to investigate the physiological role of kinins in the pancreas.

The present studies were undertaken by using an in situ perfusion technique to investigate the stimulatory effect of BK-induced insulin secretion in the rat pancreas and by using a β-cell line RINm5F to explore the mechanisms underlying the BK-induced insulin secretion. We perfused rat pancreata with BK, Lys-BK (kallidin) and T-kinin and they all induced a similar potency in insulin secretion. Because BK is the physiological kinin in rats (Bhoola et al., 1992), it was used to further explore the stimulatory effect of kinins on insulin secretion in the perfused rat pancreas and RINm5F cells. BK dose-dependently increased insulin secretion (Yang and Hsu, 1995), and enhanced glucose-induced insulin secretion in a glucose-dose-dependent manner from the perfused rat pancreas (Yang and Hsu, 1995). These effects of BK may be physiologically significant, since our preliminary data showed that the concentrations of BK in the pancreata were

in the range of 0.5 - 1.5 ng/mg or μM (assayed by Dr. Julie Chao, Medical University of South Carolina), which was similar to that in the rat submaxillary gland (Scicli et al., 1983) and kidney (Barabe et al., 1988).

At least two types of kinin receptors, BK_1- and BK_2-receptors, have been characterized (Regoli et al., 1992; Bhoola et al., 1992). Most of the kinin action is mediated through BK_2-receptors (Bhoola et al., 1992), whereas BK_1-receptors seem to be absent and expressed only in the pathological state, e.g., inflammation (Bhoola et al., 1992). The stimulatory effect of BK on insulin secretion was mediated through BK_2-receptors, because a selective BK_2-receptor antagonist HOE140 (Cuthbert et al., 1992) abolished BK-induced insulin secretion in perfused rat pancreas. This effect was further confirmed by using RINm5F cells, in which the BK-induced insulin secretion and an increase in $[Ca^{2+}]_i$ were abolished by HOE140, but were not altered by a BK_1-receptor antagonist, desArg^9,Leu^8-BK. In addition, a BK_1-receptor agonist, desArg^9-BK (Farmer, 1992) failed to induce insulin secretion or an increase in $[Ca^{2+}]_i$. Further studies are needed to quantify the BK_2-receptors of β cells using the radioligand binding technique.

HOE140 also reduced glucose-induced and basal insulin secretion from the perfused rat pancreas (Yang and Hsu, 1995). This probably was due to a specific effect on β-cells which blocks the stimulatory effect of endogenous BK, since HOE140 did not inhibit basal insulin secretion in RINm5F cells. Thus endogenous BK and their receptors may play an important role in maintaining the basal and glucose-induced insulin secretion. BK also potentiated glucose-induced insulin secretion in a glucose-dose-dependent manner. In high glucose concentrations (10 and 20 mM), BK induced insulin secretion with a higher sustained phase than the transient peak. Thus BK stimulated insulin synthesis, in addition to insulin release, because the transient insulin release peak is due to the rapid release of pre-existing insulin from the secretory granules and the sustained insulin secretion phase is due to the increased synthesis and release of stored insulin in the perfused rat pancreas (Lacy, 1970; Lacy et al., 1972). These effects of BK may maintain glucose homeostasis by a rapid return of blood glucose levels to the normal range after a large amount of intestinal absorption of glucose. Further studies are needed to determine the mechanisms by which BK potentiates glucose-induced insulin secretion.

The acinus-islet interaction on insulin secretion is not well-understood. Because the acini contain most of the tissue kallikrein found in the pancreas (Ørstavik et al., 1981; Pinkus et al., 1983), a reduction in acini tissue kallikrein content may decrease the formation of kinin in the pancreas, thereby decreasing insulin secretion. To date, we do not have direct evidence that acinar

tissue affects islet function, but clinical observations support this premise. Human patients with chronic pancreatitis or acinar atrophy develop glucose intolerance as a result of insufficient insulin secretion (Bendayan, 1990). This reduction in insulin secretion may be due to a lack of the islet-acinar interaction, because the reduction in β-cell numbers appears too small to explain the impaired insulin secretion. In rats with pancreatic acinar atrophy induced by a long-term Cu^{2+}-deficient diet (Fölsch et al., 1988), both the baseline and glucose-induced insulin secretion are lower than the controls, but the insulin content of the islets is not significantly different between normal rats and rats with acinar atrophy (Fölsch et al., 1988). In an unpublished study, we perfused the pancreas with acinar atrophy that had been induced with a Cu^{2+}-deficient diet. The results from that study was inconclusive, partly because the rats receiving Cu^{2+}-deficient diet for 3 months had lost body weight by 50% and were remarkably anemic. Thus, for further study, one needs to develop a method for inducing pancreatic acinar atrophy without causing severe health problem seen with the Cu^{2+}-deficient diet.

The results in the present study suggested that the effects of BK in β-cells are mediated through the PLC-IP$_3$ signal transduction pathway. Because the BK-induced insulin secretion and $[Ca^{2+}]_i$ increase were not altered by PTX pretreatment, the BK$_2$-receptor was coupled to a PTX-insensitive G-protein (G_q) which is coupled to PLC (Hess et al., 1994). In addition, the effect of BK was inhibited by U-73122, a specific PLC inhibitor (Bleasdale et al., 1990) and BK increased the formation of IP$_3$. On the other hand, our findings suggested that neither NO, cAMP, nor PLA$_2$ signal transduction pathway is involved in BK-induced insulin secretion in β-cells, because neither the NOS inhibitor L-NAME nor the PLA$_2$ inhibitor ACA altered the effects of BK. BK did not change the intracellular concentration of cAMP in RINm5F cells.

Intracellular Ca^{2+} is a major signal in insulin secretion. The stimulus-response usually couples to the elevation of $[Ca^{2+}]_i$ through Ca^{2+} release from the intracellular store and Ca^{2+} influx, leading to insulin secretion (Li et al., 1992; Komatsu et al., 1988). Our results showed that Ca^{2+} influx was responsible for BK-induced insulin secretion, but Ca^2 release alone did not induce insulin secretion, because in the Ca^{2+}-free medium, BK only induced a transient Ca^{2+} release without the sustained Ca^{2+} influx and did not induce insulin secretion. In Ca^{2+}-containing medium, BK induced a transient Ca^{2+} release and a sustained Ca^{2+} influx, and induced insulin secretion. However, the Ca^{2+}influx depends on the Ca^{2+} release, because TG, a Ca^{2+} pump inhibitor (Thastrup et al., 1990), abolished BK-induced transient Ca^{2+} release and sustained Ca^{2+} influx. Thus, in RINm5F cells, Ca^{2+} release was as important as Ca^{2+} influx in BK-induced insulin secretion.

The activation of PLC also promotes the formation of DAG. DAG activates PKC (Regazzi et al., 1990; Berridge., 1987). The physiological function of PKC in insulin secretion is still unclear. In rat islets, α-PKC at least partially mediates glucose-induced insulin secretion (Ganesan et al., 1990). PMA, an PKC activator, induces the translocation of PKC to a membrane-bound state and induces insulin secretion in pancreatic islets (Easom et al., 1989), HIT cells (Swope and Schonbrunn, 1988) and RINm5F cells (Yada et al., 1989). HIT cells pretreated with PMA for 22-24 h which decreases PKC activity by 73% decreases acetylcholine- and glucose-induced insulin secretion in HIT cells (Hughes et al., 1990). Thus, the activation of PKC stimulates insulin secretion (Swope and Schonbrunn, 1988; Tuch et al., 1988; Arkhammar et al., 1986). However, glucose primarily depolarizes β-cells and promotes Ca^{2+} influx (Wollheim and Pozza, 1984). In addition, several studies have failed to demonstrate the involvement of PKC in glucose-induced insulin secretion (Easom et al., 1989, 1990; Regazzi et al., 1990). Although PMA and staurosporine have been used to study the positive effect of PKC on insulin secretion, PMA depolarizes plasma membrane and increases $[Ca^{2+}]_i$, thereby promoting insulin secretion (Yada et al., 1989), and at a nonstimulatory concentration of glucose (3 mM), PMA induces a slightly and slowly insulin secretion (Bozem et al., 1987). In the PKC-deficient rat islets, staurosporine still inhibits glucose-induced insulin secretion (Persaud et al., 1993). In other cell types, PMA activates protein kinase A (Plet et al., 1988) and PLA_2 (Perkins et al., 1991), and staurosporine inhibits not only PLC but also a variety of other protein kinases (Rüegg and Burgess, 1989). Thus PMA and staurosporine are not a selective activator and inhibitor of PKC, respectively.

However, PKC may exert an inhibitory effect on insulin secretion, according to others. In RINm5F cells, PKC inhibits the coupling of receptors to PLC (Li et al., 1990) and promotes an active extrusion of $[Ca^{2+}]_i$ (Berggren et al., 1989), thereby reducing insulin secretion. PKC may stimulate the Ca^{2+} pump or inhibit the Ca^{2+} channel of the plasma membrane, thereby decreasing $[Ca^{2+}]_i$ (Ziche et al., 1993; Arkhammar et al., 1994; Di-Virgilio et al., 1986). In addition, PKC-deficient RINm5F cells display an enhanced insulin secretion in response to vasopressin and carbachol (Li et al., 1990). Our findings in the present study showed that PKC inhibited the effect of BK on insulin secretion, because OAG, an analog of DAG, decreased BK-induced insulin secretion and sustained Ca^{2+} influx, whereas, a selective PKC inhibitor Ro 31-8220 (Dieter and Fitzke, 1991) increased BK-induced insulin secretion and sustained Ca^{2+} influx. Therefore, our findings confirmed those of others and suggested that PKC has an inhibitory effect on insulin secretion in RINm5F cells. A similar inhibitory effect of PKC was found in other cell type,

parathyroid cells, in which PKC serves as a negative-feedback signal to damp signaling through the receptor-PLC pathway by phosphorylating the extracelluar Ca^{2+}-receptor and decreasing the ability of this receptor to increase IP_3 and $[Ca^{2+}]_i$, thereby decreasing parathyroid hormone secretion (Nemeth, 1995). Further studies using more specific activators and inhibitors such as OAG and Ro 31-8220 are needed to determine the physiological significance of the inhibitory effect of PKC in insulin secretion.

In summary, our results suggested that in β-cells, bradykinin activates BK_2-receptors which, in turn, activates a receptor-coupled PTX-insensitive G-protein (G_q). The G_q protein activates PLC which increases the formation of IP_3 and DAG. IP_3 releases Ca^{2+} from the ER and triggers the Ca^{2+} influx, leading to insulin secretion. DAG activates PKC which may promote $[Ca^{2+}]_i$ efflux and inhibit Ca^{2+} influx to return $[Ca^{2+}]_i$ to the normal range.

References

Arkhammar, P., T. Nilsson, and P. O. Berggren. Stimulation of insulin release by the phorbol ester 12-0-tetradecanoylphorbol 13-actate in the clonal cell line RINm5F despite a lowering of the free cytoplasmic Ca^{2+} concentration. Biochim. Biophys. Acta. 887: 236-241, 1986.

Arkhammar, P., T. Nilsson, M. Welsh, N. Welsh, and P. O. Berggren. Effect of protein kinase C activation on the regulation of the stimulus-secretion coupling in pancreatic beta-cells. Biocehm. J. 264: 207-215, 1989.

Arkhammar, P., L. Juntti-Berggren, O. Larsson, M. Welsh, E. Nanberg, A. Sojoholm, M. Kohler, and P. O. Berggren. Protein kinase C modulates the insulin secretory process by maintaining a proper function of the beta-cell voltage-activated Ca^{2+} channels. J. Biol. Chem. 269: 2743-2749, 1994.

Barabe, J., D. Huberdean, and A. Bernoussi. Influences of sodium balance on urinary excretion of immunoreactive kinin in rats. Am. J. Physiol. 254: F484-F491, 1988.

Bendayan, M. Anatomic basis of islet-acinar interaction in the pancreas. Regul. Pept. Lett. 2: 8-11, 1990.

Berrgren, P.O., P. Arkhammar, and T. Nilsson. Activation of protein kinase C assists insulin producing cell in recovery from raised cytoplasmic Ca^{2+} by stimulating Ca^{2+} afflux. Biochem. Biophys. Res. Commun. 165: 416-421, 1989.

Berridge, M. J. Inositol triphosphate and diacylglycerol: two interacting second messengers. Annu. Rev. Biochem. 56: 159-193, 1987.

Bhoola, K. D., C. D. Figueroa, and K. Worthy. Bioregulation of kinin: kallikrein, kininogen, and kininases. Pharmacol. Rev. 44: 1-80, 1992.

Bleasdale, J. E., N. R. Thakur, R. S. Gremban, G. L. Bundy, F. A. Fitzpatrick, R. J. Smith, and S. Bunting. Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. J. Pharmacol. Exp. Ther. 255: 756-768, 1990.

Bozem, M., M. Nenquin, J. C. Henquin. The ionic, electrical, and secretory effects of protein kinase C activation in mouse pancreatic B-cells: studies with a phorbol ester. Endocrinology 121: 1025-1033, 1987.

Cuthbert, A. W., L. J. MacVinish, and R. J. Pickles. Antagonism of kinin effects on epithelial by HOE 140: Apparently competitive and non-competitive interactions. Br. J. Pharmacol. 107: 797-802, 1992.

Dieter, P., and E. Fitzke. Ro 31-8220 and Ro 31-7549 show improved selectivity for protein kinase C over staurosporine in macrophages. Biochem. Biophys. Res. Commun. 181: 396-401, 1991.

Di-Virgilio, F., T. Pozzan, C. B. Wollheim, L. M. Vicentini, and J. Meldolesi. Tumor promotor phorbol myristate acetate inhibits Ca^{2+} influx through voltage-gated Ca^{2+} Channels in two secretory cell line, PC12 and RINm5F. J. Biol. Chem. 261: 32-35, 1986.

Easom, R. A., J. H. Hughes, M. Landt, B. A. Wolf, J. Turk, and M. L. McDaniel. Comparison of effects of phorbol esters and glucose on protein kinase C activation and insulin secretion in pancreatic islets. Biochem. J. 264: 27-33, 1989.

Farmer, S. G. Biochemical and molecular pharmacology of kinin receptors. Annu. Rev. Pharmacol. Toxicol. 32: 511-536, 1992.

Fölsch, U. R., M. Fussek, R. Ebert, and W. Creutzfeldt. Endocrine pancreatic function during atrophy of the exocrine gland. Pancreas 3. : 536-542, 1988.

Ganesan, S., R Calle, K. C. Zawalich, H. Smallwood, W. S. Zawalich, and H. Rasmussan. Glucose-induced translocation of protein kinase C in rat islets. Proc. Natl. Acad. Sci. USA 97: 9893-9897, 1990.

Hess, J. F., J. A. Borkowski, G. Y. Stonesifer, T. Macneil, C. D. Strader, and R. W. Ransom. Cloning and pharmacological characterization of bradykinin receptors. Braz. J. Med. Biol. Res. 27: 1725-1731, 1994.

Hughes, S. J., J. G. Chalk, and S. J. Ashcroft. The role of cytosolic free Ca^{2+} and protein kinase C in acetylcholine-induced insulin release in the clonal beta-cell line, HIT-T15. Biochem. J. 267: 227-232, 1990.

Jaffa, A. A., J. Pratt, A. Ashford, and G. S. Bailey. Studies of the effects of insulin, bradykinin, and captopril on blood glucose levels of alloxan-diabetic rats. Adv. Exp.Med. Biol. 198: 373-378, 1986.

Jauch, K. W., B. Gunther, W. Hartl, K. Rett, M. Wicklmayr, and G. Dietze. Improvement of impaired postoperative insulin action by bradykinin. Biol. Chem. Hoppe-Seyler. 367: 207-210, 1986.

Jauch, K. W., W. Hartl, M. Georgieff, R. R. Wolfe, G. J. Dietze, and B. Gunther. Low-dose bradykinin infusion reduces endogenous glucose production in surgical patients. Metabolism 38: 878-882, 1988.

Komatsum M., T. Aizawa, N. Takasu, and T. Yamada. Glucose raises cytosolic free calcium in the rat pancreatic islets. Horm. Metabol. Res. 21: 405-409, 1989.

Lacy, P.E. Beta cell secretion-From the standpoint of a pathobiologist. The Banting Memorial Lecture Diabetes 19: 895-905, 1970.

Lacy, P. E., M. M. Walker, and C. J. Fink. Perfusion of isolated rat islets in vitro: participation of the microtubular system in the biphasic release of insulin. Diabetes 21: 987-998, 1972.

Li, G., W. F. Pralong, D. Pittet, G. W. Mayr, W. Schlege, and C. B. Wollheim. Inositol tetrakisphosphate isomers and elevation of cytosolic Ca^{2+} in vasopressin-stimulated insulin-secreting RINm5F cells. J. Biol. Chem. 267: 4349-4356, 1992.

Nemeth, E. F. Ca^{2+} receptor-dependent regulation of cellular functions. News Phsiol. Sci. 10: 1-5, 1995

Ole-Moiyoi, O., D. C. Seldin, J. Spragg, G. Pinkus, and K. F. Austein. Sequential cleavage of proinsulin by human pancreatic kallikrein and a human pancreatic kinase. Proc. Natl. Acad. Sci. USA 76: 3612-3616, 1979.

Ørstavik, T. B., I. B. Brekke, J. Alumets, and O. A. Carretero. Kallikrein in rat pancreatic tissue after beta cell destruction or acinar cell atrophy. J. Histochem. Cytochem. 29: 1431-1436, 1981.

Perkins, P. S., L. H. Bahrami, L. W. Lenhard, and S. J. Pandol. Intracelllular mechanisms involved in short-term regulation of net protein synthesis in pancreatic acini. Biochim. Biophys. Acta. 1092: 145-152, 1991.

Persaud,S. J., P. M. Jones, D. Sugden, and S. L. Howell. Translocation of protein kinase C in rat islets of Langerhans. Effect of a phobor ester, carbachol and glucose. FEBS. Lett. 245: 80-84, 1989.

Persaud,S. J., P. M. Jones, and S. L. Howell. Staurosporine inhibits protein kinase activated by Ca^{2+} and cyclic AMP inaddition to inhibiting protein kinase C in rats islets of Langerhans. Mol. Cell Endocrinol. 94: 55-60, 1993.

Pinkus, G. S., M. Maier, D. C. Seldin, O. Ole-Moiyoi, K. F. Austen, and J. Spragg. Immunohistochemical localization of glandular kallikrein in the endocrine and exocrine pancreas. J. Histochem. Cytochem. 31: 1279-1288, 1983.

Plet, A., P. Gerbaud, W. B. Anderson, and D. Evain-Brion. Effects of phorbol ester on cyclic adenosine 3':5'-monophosphate-dependent protein kinases in PYS teratocarcinoma cells and counteraction withretinoic acid. Cancer Res. 48: 3993-3997, 1988.

Regazzi, R., G. D. Li, J. Deshusses, and C. B. Wollheim. Stimulus-response coupling in insulin-secreting HIT cells. Effects of secretagogues on cytosolic Ca^{2+}, diacylglycerol, and protein kinase C activity. J. Biol. Chem. 265: 15003-15009, 1990.

Regoli, D., D. Jukic, C. Tousignant, and N. E. Rhaleb. Kinin receptor classification. Agents actions Suppl. 38: 475-486, 1992.

Rüegg, U. T., and G. M. Burgess. Staurosporine, K-252 and UCN-01: potent but nonspecific inhibitors of protein kinases. Trends Pharmacol. Sci. 10:218-220, 1989.

Schack, L., Rohen, J. W., and Haberland, G. L. 1980. Experimental studies on the influence of the kallikrein-kinin system on glucose utilization. Agents Actions 10: 344-348.

Scicli, A. G., T. B. Ørstavik, S. F. Rabito, F. D. Murray, and O. A. Carretro. Blood kinins after sympathetic nerve stimulation of the rat submaxillary gland. Hypertension 5: 1101-1106, 1983.

Swope, S. L., and A. Schonbrunn. The biphasic stimulation of insulin secretion by bombesin involves both cytosolic calcium and protein kinase C. Biochem. J. 253: 193-202, 1988.

Symington, R. P., A. Ashford, and G. Bailey. Effect of bradykinin and insulin on glucose oxidation in adipocytes from normal and streptozotocin diabetic rats. Biochem. Soc. Trans. 16: 1012-1013, 1988.

Thastrup, O., P. J. Cullen, B. K. DrØbak, M. R. Hanley, and A. P. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} store by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. Proc. Natl. Acad. Sci. USA 87: 2466-2470, 1990.

Tuch, B. E., Z. Palavidis, and J. R. Turtle. Activators of protein kinase C stimulate insulin secretion from the human fetal pancreas. Pancreas 3: 675-680, 1988.

Uehara, S., S. Isogawa, W. Sakamoto, and A. Hirayama. Kallikrein-kinin system in diabetic patients. Arzneimittelforschung 38: 721-723, 1988.

Vance, J. E., and Bragg, S. C. 1969. Insulin release in response to microvasoactive substances. Diabetes 18: 326-327.

Wollheim, C. B., and T. Pozza. Correlation between cytosolic free Ca^{2+} and insulin release in an insulin-secreting cell line. J. Biol. Chem. 259: 2262-2267, 1984.

Yada, T., L. L. Russo, and G. W. Sharp. Phorbol ester-stimulated insulin secretion by RINm5F insulinoma cells is linked with membrane depolarization and an increase in cytosolic free Ca^{2+}concentration. J. Biol. Chem. 264: 2455-2462, 1989.

Yang, C., and W. H. Hsu. Stimulatory effect of bradykinin on insulin release from the perfused rat pancreas. Am. J. Physiol. 268: E1027-E1030, 1995.

Ziche, M. D. Zawieja, R. K. Hester, and H. Granger. Calcium entry, mobilization, and extrusion in postcapillary venular endothelium exposed to bradykinin. Am. J. Physiol. 265: H569-H580, 1993.

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