PRELIMINARY REPORT

The Effect of Bradykinin on Secretion of Insulin, Glucagon, and Somatostatin From the Perfused Rat Pancreas

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To evaluate the effect of bradykinin (BK) on rat islet α, β, and δ cells, the rat pancreas was perfused in situ with BK (1 μmol/L) for 30 minutes via a cannula placed in the celiac artery. Insulin, glucagon, and somatostatin concentrations in the effluent were measured to determine the effect of BK on the secretion of these hormones. The BK concentration of the rat pancreas was also measured. Basal secretion of insulin, glucagon, and somatostatin in medium containing 6 mmol/L glucose was maintained at 6.5 ± 0.5 ng/mL, 124 ± 8 pg/mL, and 511 ± 22 pg/mL (n = 12), respectively. BK (1 μmol/L) induced a transient peak that was 3.7-fold of the baseline concentration within 3 minutes, followed by a sustained level that was approximately 50% higher than baseline. BK also transiently increased glucagon secretion with a peak that was 1.7-fold of the baseline concentration within 3 minutes, without a sustained secretion phase. BK caused a reduction in somatostatin secretion within 3 minutes to a level of 60% to 70% of the baseline concentration. The BK concentration of the rat pancreas was 3.42 ± 1.45 μg/g protein (n = 5), which was approximately 3 μmol/L. We concluded that BK stimulated insulin secretion, transiently increased glucagon secretion, and decreased somatostatin secretion during the 30-minute perfusion of the rat pancreas.

KININS, including bradykinin (BK), display an extraordinarily broad range of physiological activities. They influence a number of biological processes including blood pressure, local blood flow, pain, and inflammation, causing increased vascular permeability and local edema, contraction of smooth muscle, decreased blood glucose concentration, promotion of cell proliferation, increased glucose uptake by intact intestine, and other cellular functions.[1]

Kinins also stimulate secretion of other hormones, such as renin from isolated rat glomeruli, vasopressin from rat posterior pituitary gland, catecholamines from vas deferens nerve terminals,[1] and prolactin and growth hormone from rat anterior pituitary gland.[2] At least two BK receptors have been characterized, BK_1 and BK_2, with most of the effects of BK being mediated through BK_2 receptors.[1]

In our previous study, administration of BK for 5 minutes stimulated insulin secretion from the perfused rat pancreas in a dose-dependent manner, and this effect was mediated by BK_2 receptors. The BK_2 receptor blocker, HOE 140, reduced basal insulin secretion, abolished BK-induced insulin release, and attenuated glucose-stimulated insulin release. BK also increased glucose-induced insulin release.[3] Our findings suggest that BK may be physiologically involved in maintaining basal and glucose-induced insulin secretion. However, in addition to insulin-secreting cells (β cells), islets also contain glucagon-secreting cells (α cells), somatostatin-secreting cells (δ cells), and other types of cells for controlling glucose homeostasis.[4] The effects of BK on these cell types are still not understood. Thus, the present study was undertaken to investigate the effect of BK (1 μmol/L) on insulin, glucagon, and somatostatin secretion. A second objective was to determine the BK concentration of the pancreas to evaluate whether the BK level used in the perfusion study (1 μmol/L) was within the physiological range.

MATERIALS AND METHODS

Male Sprague-Dawley rats (300 to 400 g) were anesthetized with pentobarbital sodium (60 mg/kg intraperitoneally; Fort Dodge Laboratories, Fort Dodge, IA) and kept on a 37°C hot plate. Pancreatic perfusion was performed as previously described.[3] 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 preparation had been established, the first 10 to 15 minutes of perfusion was considered an equilibration period. Subsequently, the effluent fluid was collected each minute for 10 minutes and considered as baseline insulin secretion. The rat pancreas was continuously perfused with BK (1 μmol/L; Sigma Chemicals, St Louis, MO) for 30 minutes, followed by a 10-minute perfusion with the basal medium. Thus, the effluent fluid also was collected for 40 minutes after the baseline had been established. In our previous study, 0.01 to 1 μmol/L BK increased insulin release in a dose-dependent manner, and 1 μmol/L BK induced a 3.7-fold increase in the baseline level.[3] Therefore, we used 1 μmol/L BK in these experiments. The pancreata of the control group were perfused with 6 mmol/L glucose in Krebs-Ringer bicarbonate buffer only.

The effluents were centrifuged (1,000 × *g*), and then 0.5 mL effluent was mixed with 1 mL acid-ethanol (95% ethanol and 5% 1N HCl). The mixtures were centrifuged (1,000 × *g*) again, and the supernatants were collected and stored at −20°C for determination of glucagon and somatostatin by radioimmunoassay (RIA). The glucagon RIA kit was purchased from Linco Research (St. Charles, MO), and the glucagon concentration was measured according to the instructions of the manufacturer. Insulin and somatostatin levels were measured by RIA as previously described.[5,6]

For determination of BK concentration in the pancreas, pancreata were expanded with 1% trifluoroacetic acid (TFA) through the bile duct of anesthetized rats. The pancreatic tissues were mixed with an equal volume of 30% TFA, homogenized, and centrifuged at 2,000 × *g* for 10 minutes. The supernatant was applied to Sep-Pak C18 cartridges (Waters, Millford, MA), eluted with 3 mL 50% acetonitrile in 1% TFA, and lyophilized. BK was determined by RIA as previously described.[7]

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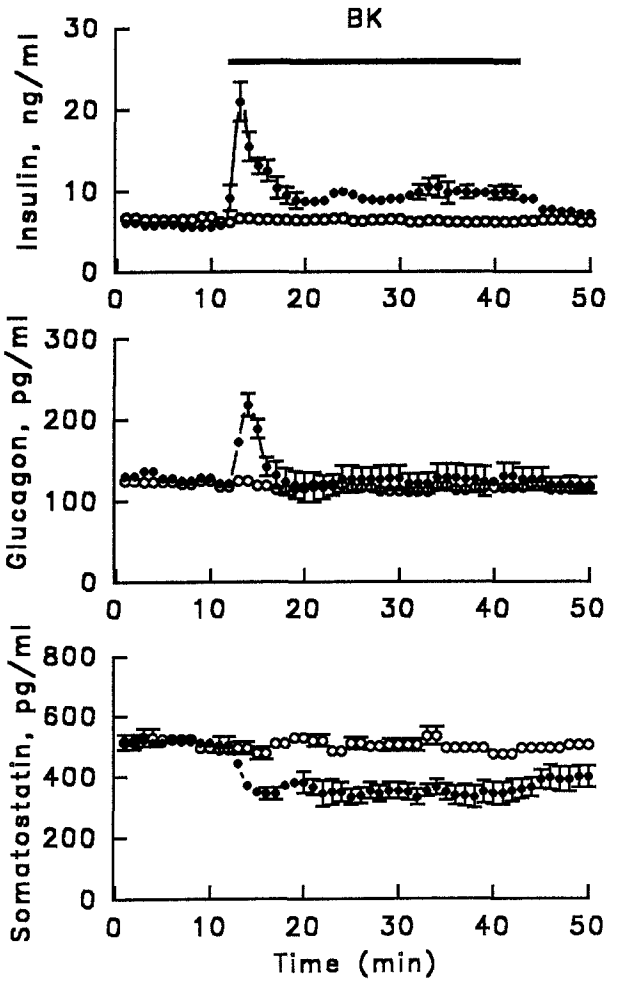

Fig 1. Effect of 1 μmol/L BK in medium containing 6 mmol/L glucose on the secretion of insulin, glucagon, and somatostatin from perfused rat pancreas. BK was administered for 30 minutes (bar). Values are the mean ± SE; n = 4 for control group (○) and n = 8 for BK-treated group (●). There was an equilibration period of 10 to 15 minutes, in which the basal medium containing 6 mmol/L glucose was perfused before time 0.

Values are shown as the mean ± SE. Data were subjected to ANOVA using a split-plot design[8] to determine the significance of treatment and time (first 40 minutes). The treatment by × 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.

RESULTS

Rat pancreata in the control group were perfused with 6 mmol/L glucose alone, and baseline insulin concentrations were 6.7 ± 0.5 ng/mL throughout the perfusion period. BK (1 μmol/L) induced a peak increase in insulin release, which was followed by a sustained level (Fig 1). The onset time of insulin release was less than 1 minute and reached a peak that was 270% of the baseline concentration within 3 minutes of BK administration. Subsequently, insulin decreased gradually in 5 minutes to a sustained level that was still significantly ($P < .05$) higher (~50%) than that of the control group throughout the perfusion period.

Effluent concentrations of glucagon in the control group were maintained at a steady level of 128 ± 8 pg/mL throughout the perfusion period. There was no significant difference ($P > .05$) between BK and control groups during the 40-minute perfusion period. However, when data were analyzed for the first 20-minute period, 1 μmol/L BK significantly ($P < .05$) and transiently increased glucagon secretion, reaching a peak within 3 minutes that was approximately 70% greater than the baseline concentration; glucagon in the effluent decreased to the baseline concentration 2 minutes after reaching the peak (Fig 1).

Effluent somatostatin concentrations of the control group were maintained at 522 ± 25 pg/mL throughout the perfusion period. Administration of BK (1 μmol/L) significantly ($P < .05$) decreased effluent somatostatin to about 60% of the baseline concentration within 3 minutes, and it was maintained steadily at this level to the end of the perfusion (Fig 1).

BK concentration of the rat pancreas was 3.42 ± 1.45 μg/g protein (n = 5). BK concentrations of rat pancreata were in the micromolar range, and were similar to levels found in the rat submaxillary gland[9] and kidney.[10]

DISCUSSION

We found that BK (1 μmol/L) induced an increase in insulin secretion and transiently increased glucagon secretion but decreased somatostatin secretion during a 30-minute period of pancreatic perfusion. The initial transient insulin release was similar to that found in our previous study, in which rat pancreata were perfused with BK only for 5 minutes.[3] BK-induced insulin secretion maintained a sustained level in the 30-minute period of perfusion.

Intraislet communication among α, β, and δ cells may occur through paracrine (interstitial) and vascular routes. The microvascular circulating cellular order, from β cells to α cells and from α cells to δ cells, also influences secretion of islet hormones.[11] Thus, insulin regulates secretion of glucagon, and both insulin and glucagon regulate secretion of somatostatin.[12] Intraislet insulin promotes the transport of glucose entering α cells and inhibits the secretion of glucagon.[13] In addition, the amount of insulin released during the early phase of stimulation determines the rapidity and magnitude of inhibition of the secretory function of α cells.[14] In the present study, BK only transiently increased glucagon release from the perfused pancreas, unlike BK-induced insulin secretion, which lasted at least 30 minutes. It is probable that the BK-induced increase in insulin secretion inhibited glucagon secretion, and thus glucagon returned to the baseline level 2 minutes after reaching the peak. In our preliminary perfusion experiments with a clonal α-cell line, In-R1-G9, BK increased glucagon secretion for longer than 10 minutes (Yang and Hsu, unpublished results, 1996).

The BK-induced decrease in somatostatin secretion may have been caused by a direct action of BK or BK-induced insulin release, since a high insulin concentration in the pancreatic perfusate suppresses somatostatin secretion.[15,16] Further studies using streptozotocin-pretreated rats (to destroy β cells) are needed to examine the effect of BK on somatostatin and glucagon secretion. The BK-induced reduction in somatostatin secretion should not be attributed to an increase in glucagon secretion, since glucagon stimulates, rather than inhibiting, somatostatin secretion.[17]

Tissue kallikrein in the pancreas is found in both acini and β cells, and most of it is located in the acini.[18] Thus, it is probable

that acinar kinins can alter endocrine function of the islet. To date, there is no direct evidence that acinar tissues affect islet functions, but clinical observations support this premise. Human patients with chronic pancreatitis or acinar atrophy develop glucose intolerance as a result of insufficient insulin release.[19] This reduction in insulin secretion may be due to a lack of an acinus-islet interaction, because the reduction of β cells in these human patients appears too small to explain the impaired insulin secretion in response to various stimuli.[20] However, one cannot exclude the possibility that the islets and individual islet cells are structurally deranged in chronic pancreatitis; altered cells may not respond normally even if the total population of cells remain unchanged. In addition, in rats with pancreatic acinar atrophy induced with a Cu^{2+}-deficient diet, both baseline and glucose-stimulated plasma insulin concentrations were lower than in controls.[21] Further studies using the acinar atrophy model may provide insight into the physiological role of kinins in the regulation of endocrine function in pancreatic islets.

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