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NITRIC OXIDE AS MEDIATOR OF BRADYKININ-INDUCED PANCREATIC CIRCULATORY AND METABOLIC RESPONSES

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Bradykinin (BK) is an endogenous nonapeptide with potent vasodilator properties of the visceral circulation. BK alters vascular tone *via* two BK receptor subtypes, B_1 and B_2. Current experimental evidence suggests that the dilator action of BK in some vessels is mediated primarily by B_2 receptor activation. In addition, there are reports that BK increases endothelial generation of vasodilator factors, such as nitric oxide (NO). The present study had two aims. First, to explore the role of BK-receptors in the pancreatic vasodilatatory and metabolic responses to BK. Second aim was to examine whether endogenous NO play a role in the mediation of BK-receptors induced pancreatic circulatory and metabolic activity. In anesthetized dogs, the superior pancreatico-duodenal artery blood flow (SPBF) was measured by ultrasonic blood flowmeter (Transonic System T-206), pancreatic microcirculatory blood flow (PBF) was determined by laser Doppler flowmetry (Periflux 4001 Master). Pancreatic oxygen consumption (PVO_2) was calculated as the product of the arteriovenous oxygen difference (AVO_2) across the pancreatic circulation and SPBF. Drugs were infused into the superior pancreatico-duodenal artery. BK (0.01—1.0 mg/kg/min) increased maximally SPBF by $180 \pm 15\%$, PBF by $208 \pm 22\%$ and PVO_2 by $145 \pm 11\%$, respectively. Pretreatment with B_2-subtype receptor antagonist, D-Arg, [Hyp^3, $Thi^{5,8}$, D-Phe^7] BK inhibited significantly BK-induced increase in SPBF, PBF and PVO_2 by $86 \pm 8\%$, $73 \pm 7\%$ and $85 \pm 6\%$, respectively. A nitric oxide synthesis inhibitor (L-NNA) administered i.v. at dose of 25 mg/kg 20 min before BK, inhibited significantly the pancreatic hyperemic and metabolic responses. The results presented emphasize an important role of B_2 receptors in the mediation of pancreatic circulatory and metabolic responses to bradykinin. Endogenous NO plays a mediatory role in the pancreatic vascular and metabolic responses due to stimulation of B_2-receptors.

Key words: *pancreatic circulation, oxygen uptake, bradykinin receptors, nitric oxide*

INTRODUCTION

Pancreatic blood flow is controlled by multiple extrinsic mechanisms such as, the autonomic nervous system, nonadrenergic, noncholinergic (NANC) vasodilator nerves, circulating hormones and various humoral factors (1—3). There is also sufficient reason to support the suspicion that intrinsic factors are

especially important in the control of blood flow through pancreatic vasculature. Two mechanisms are commonly invoked to explain intrinsic regulation of pancreatic blood flow and pancreatic tissue oxygenation, i.e. myogenic and metabolic mechanisms. Both mechanism predict decreases in pancreatic vascular resistance and increases in capillary exchange capacity in response to a fall in arterial pressure. Among other considerations, the pancreatic blood flow has been found to exhibit a variety of discrete autoregulatory responses in which the challenge of either a diminished blood flow or an increased oxygen demand is met by a localized hyperemia of the pancreas. Examples of such pancreatic autoregulatory phenomena include pressure-flow autoregulation, reactive hyperemia, venous pressure elevation and functional hyperemia. Intrinsic control of the pancreatic circulation is related to the exo- and endocrine function of the gland (2—4). Claude Bernard (5) was the first to observe flushing and congestion of the pancreas during digestion. The relationship between blood flow and secretion has been confirmed under various experimental conditions, using both the stimulants and inhibitors or pancreatic exocrine secretion. The results indicate that the increase in pancreatic secretion is preceded and accompanied by a prompt increase in the blood flow to the gland, while decreased secretion is accompanied by a reduction in the blood flow (1—3, 6).

Several factors have been implicated as mediators of functional hyperemia in the pancreas. Among these factors may by either a direct effect of decrease of tissue PO_2, release of vasodilator metabolites e.g. adenosine, K^+ or increased interstitial osmolarity (4, 7). Glandular kallikreins have been implicated in the regulation of pancreatic blood flow through the generation of plasma kinins e. g. bradykinin (BK) (6, 8—10).

BK is an endogenous nonapeptide with potent vasodilator properties in gastrointestinal tract and visceral organs including pancreas (11—14). The exact mechanism by which BK induces pancreatic vasodilatation is uncertain. The present study was undertaken to estimate the role of BK receptors in the pancreatic vasodilatatory and metabolic responses to BK and to examine the role of nitric oxide (NO) in the mediation of BK-induced pancreatic circulatory and metabolic responses.

MATERIAL AND METHODS

Experiments were performed on 15 mongrel dogs of either sex weighing 16—29 kg. The animals were fasted 24 hrs before the experiment with free access to water.

Anesthesia was induced by intravenous injection of pentobarbital (Vetbutal 0,5 ml/kg). The animals were ventilated with room air using a positive pressure respirator (Ugo Basile). Both femoral arteries were exposed. One femoral artery was cannulated and connected with a pressure transducer (Statham) for continuous monitoring of systemic arterial pressure (SAP) and another for

siphoning arterial blood into an arterial cuvette of spectrophotometric oxygen content difference analyzer (AVOX System, San Antonio) (15). The femoral vein was also cannulated for periodic injection of anesthetic as needed. After a midline laparotomy, the pancreas was exposed and all nonpancreatic branches of the superior pancreatico-duodenal artery and vein were ligated. A polyethylene catheter was introduced into one of the pancreatic veins draining the vascular area of the pancreas supplied by the superior pancreatico-duodenal artery. This catheter was used to obtain venous blood from the pancreas for perfusion through the arteriovenous oxygen content difference analyzer. The superior pancreatico-duodenal artery blood flow (SPBF) was determined by means of ultrasonic blood flow probe (1,5—2,0 mm) placed around the artery. The probe was connected to a blood flow amplifier (Transonic Systems Inc. T-206, Ithaca, USA).

Pancreatic oxygen uptake (PVO_2) was calculated as the product of simultaneously measured blood flow to the pancreas (SPBF) and the arteriovenous oxygen content difference (AVO_2), and was expressed in ml O_2/min. After administration of heparin the pancreatic venous catheter and the femoral arterial catheter were attached to a constant flow pump (Medipan, Poland) to permit pancreatic venous and femoral arterial blood flow through separate cuvettes of the oxygen-content difference analyzer with the rate of 7,0 ml/min. The blood flow from the analyzer returned to the animal *via* the femoral vein catheter. Continuous recordings of SAP, SPBF and AVO_2 were made on the polygraph (Sensor Medics Dynograph, model R 611).

Continuous tissue microcirculatory blood flow (PBF) was determined by laser Doppler flowmetry (Periflux 4001 Master). A fiberooptic probe was positioned against the surface of the corpus of pancreas and was secured outside the animal to prevent any movement of the tip of the probe. The change in PBF was calculated in terms of percentage of control.

When circulatory and metabolic parameters stabilized one of three experimental protocols was initiated. In each protocol, a group of five dogs was studied.

In group I, first we determined the pancreatic circulatory and metabolic responses to exogenous bradykinin. BK (Sigma) was infused into the superior pancreatico-duodenal artery at doses of 0.1, 0.25 and 0.5 μg/kg-min. In this group, BK at dose 0.1 μg/kg-min was also infused i.a. immediately after B_1-receptor antagonist and than after B_2-receptor antagonist. BK B_1-receptor antagonist des-Arg^9, [Leu^8]BK (B7276) Sigma or BK B_2-receptor antagonist D-Arg, [Hyp^3, Thi^{5,8}, D-Phe^7]BK (B5630) Sigma were infused i.a. at a rate of 100 μg/kg-min for 5 min.

In group II, the SPBF, PBF, PVO_2 and AP responses to BK were studied before and after NO synthase blockade using N^G-nitro-L-arginine (L-NNA) (Sigma). The drug was dissolved freshly in isotonic saline and given i.v. as slow injection in a dose of 15 mg/kg.

In group III, the pancreatic vascular and metabolic responses to BK were studied before and after combined pretreatment with L-Arginine + L-NNA. L-Arginine (Sigma) was injected i.v. in a dose of 100 mg/kg and 15 minutes later, L-NNA was administered as previously. Then 30 min after onset of L-NNA administration bradykinin was infused.

All data are presented as means ± SEM. The significance of changes in measured values from control was determined using the Student's test for either grouped or paired data with a confidence limit of less than 5%.

RESULTS

The control values in these three groups of experiments were: SAP 128 ± 6 mmHg and was significantly altered only after pretreatment with L-NNA, SPBF 37 ± 3.0 ml/min, PBF 380 ± 26 PU, AVO_2 3.7 ± 0.7 ml O_2 /100 ml of blood and PVO_2 1.8 ± 0.2 ml/min.

In groups I, II and III dogs control infusion of BK elicited significant increase in SPBF characterized by an early peak increase which was followed by a slightly lower plateau phase of increased SPBF (*Fig. 1*). Similar pattern of vasodilatatory response was observed in PBF. At the some period of time AVO_2 decreased below control level while PVO_2 was increased and SAP did not change (*Fig. 1*).

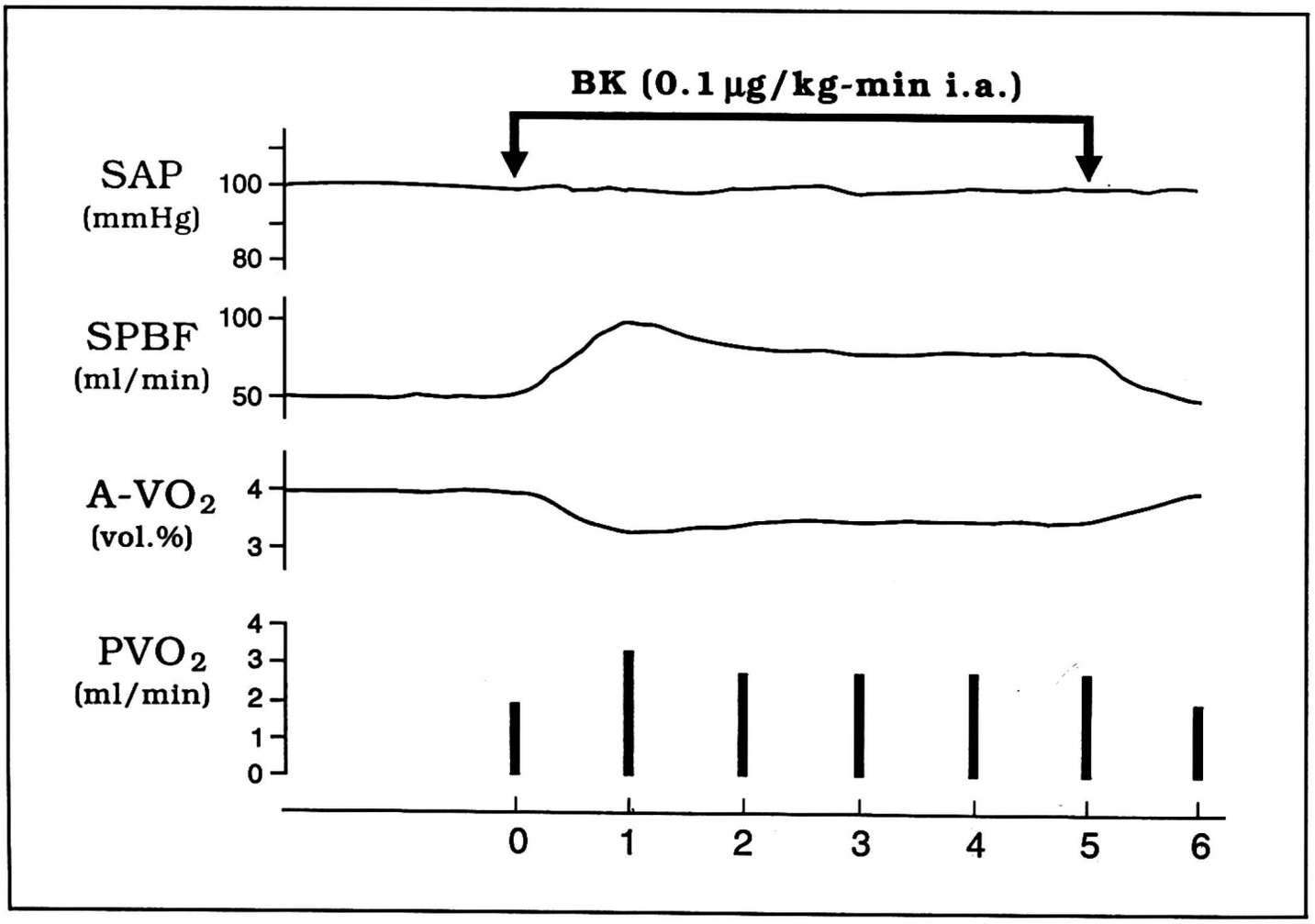

Fig. 1. The effect of intraarterial infusion of bradykinin (BK) on systemic arterial blood pressure (SAP), superior pancreatico-duodenal artery blood flow (SPBF), pancreatic arteriovenous oxygen difference (AVO_2) and pancreatic oxygen consumption (PVO_2).

The analysis of the changes observed during vasodilatatory response to BK showed dose-dependent type of circulatory and metabolic response. BK in dose of 0.1µg/kg-min i.a. increased PBF by $112 \pm 13\%$ ($p > 0.01$) and SPBF by $32 \pm 7\%$ ($p > 0.01$). AVO_2 was decreased $39 \pm 3.0\%$ ($p > 0.05$) and PVO_2 increased by $58 \pm 3.5\%$ ($p > 0.05$). Higher dose of BK (0.25 µg/kg-min i.a.) increased PBF by $139 \pm 10\%$ ($p > 0.001$), SPBF by $113 \pm 8\%$ ($p > 0.01$) while decreased AVO_2 by $42 \pm 4\%$ ($p > 0.05$) and increased PVO_2 by $67 \pm 5\%$ ($p > 0.05$). BK (0.5 mg/kg-min i.a.) increased PBF by $208 \pm 12\%$ ($p > 0.01$), SPBF by $180 \pm 11\%$ ($p > 0.01$) and PVO_2 by $145 \pm 11\%$ ($p > 0.01$), respectively, while decreased AVO_2 $59 \pm 9\%$ ($p > 0.05$) (*Fig. 2*).

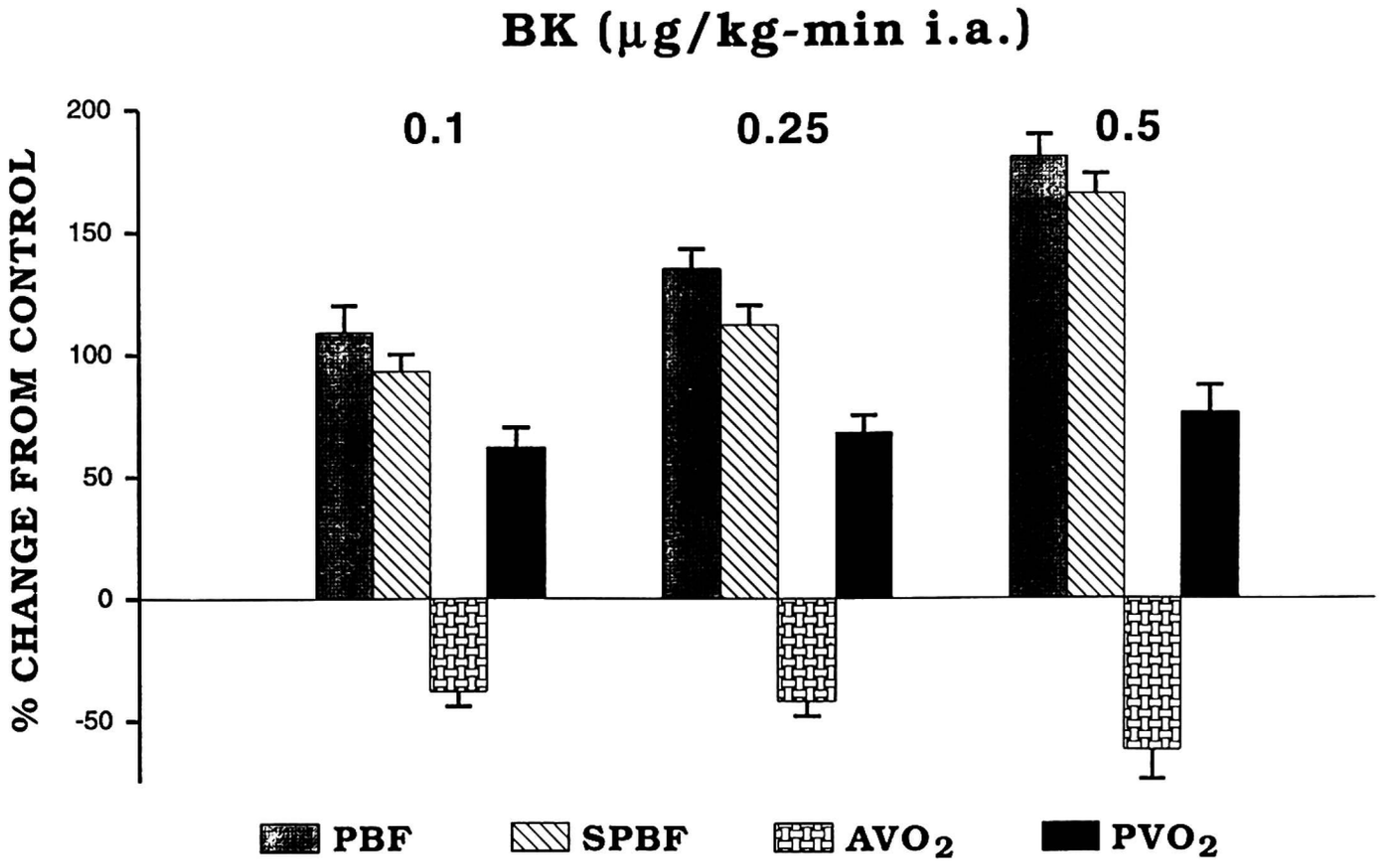

Fig. 2. Effects of three doses of BK on pancreatic microcirculatory blood flow (PBF), SPBF, AVO_2 and PVO_2.

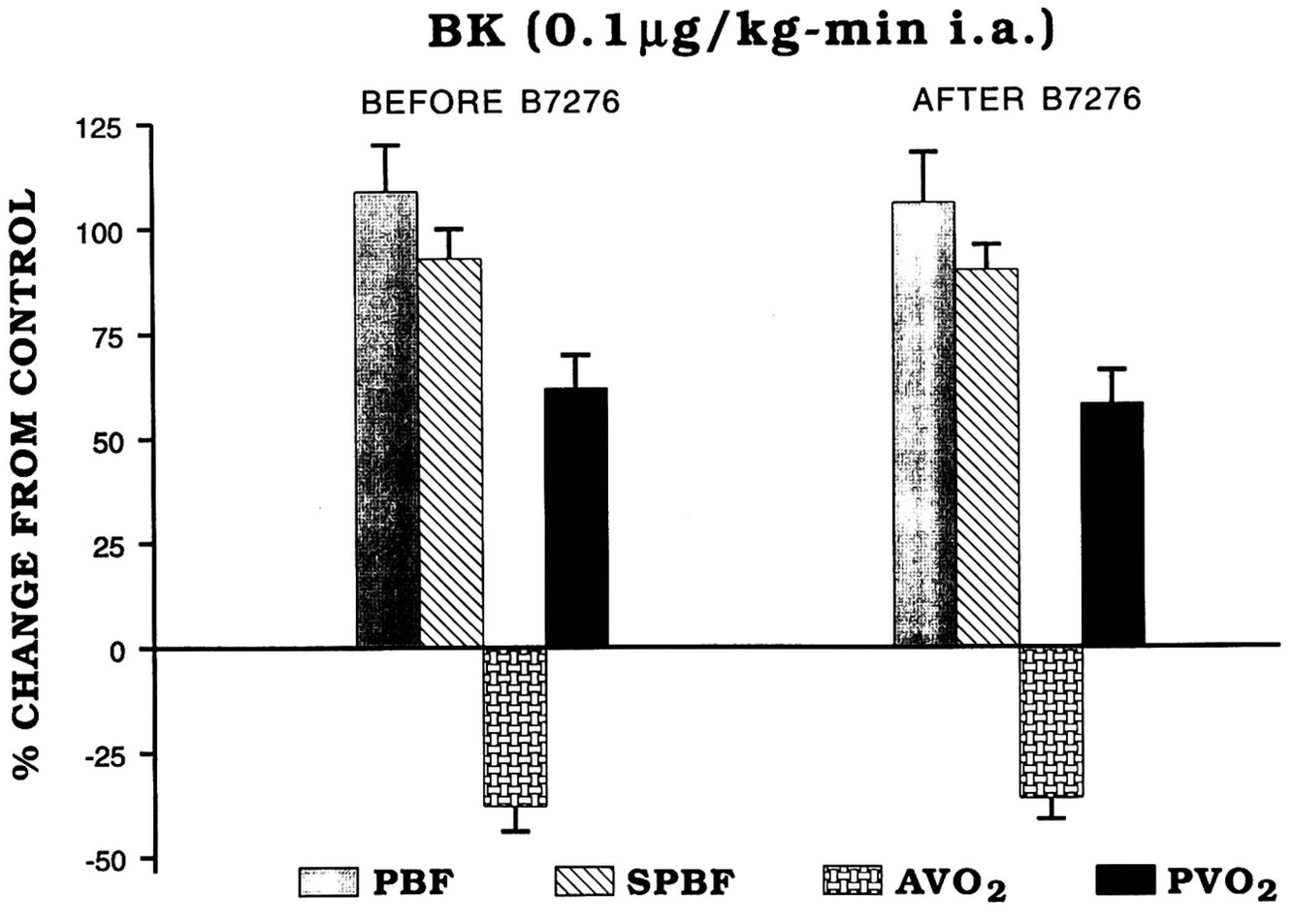

Fig. 3. BK-induced pancreatic hyperemic and metabolic responses before and after pretreatment with B7276.

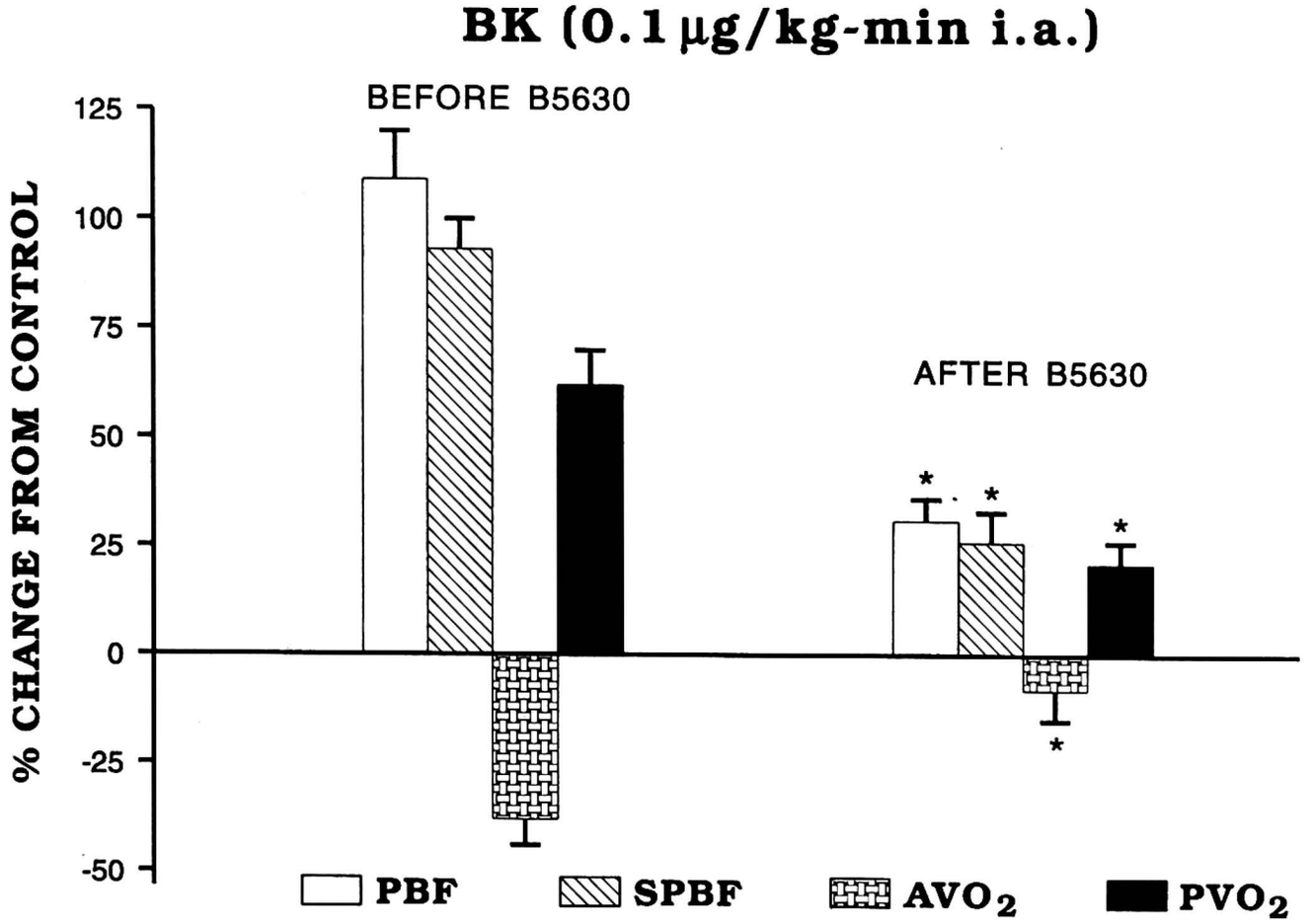

Fig. 4. Changes in BK-induced hyperemic and metabolic responses before and after pretreatment with B5630. Asterisk indicates significant difference from BK alone.

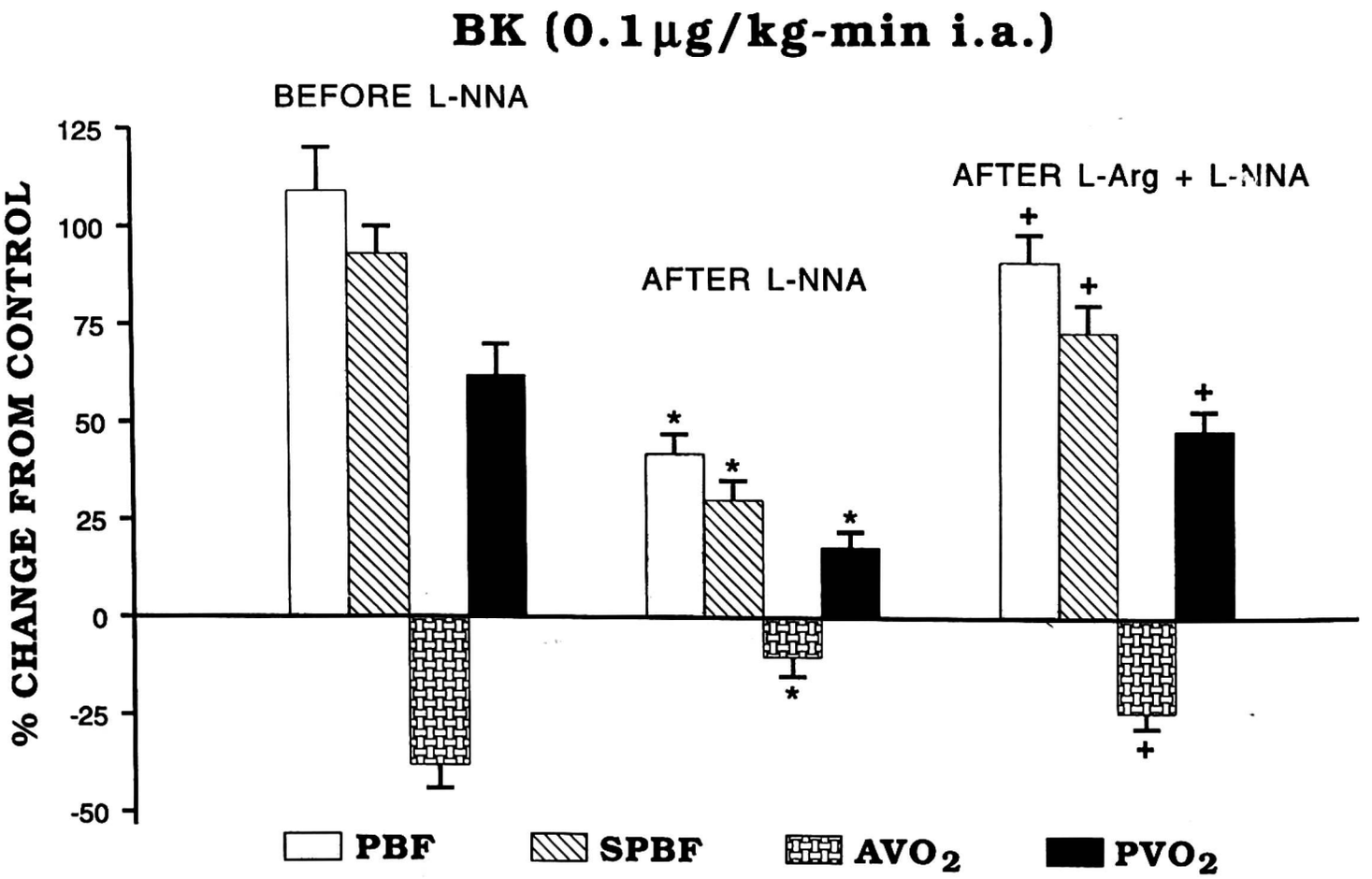

Fig. 5. Changes in BK-induced response of PBF, SPBF, AVO_2 and PVO_2 before and affter pretreatment with L-NNA and after L-NNA + L-Arg. Single asterisk indicates significant change from BK alone. Double asterisk indicates significant change from BK after L-NNA.

In group I dogs, pretreatment of animals with BK B_1-receptor antagonist was without any effect on BK-induced circulatory and metabolic responses (*Fig. 3*). Pretreatment with B_2-subtype receptor antagonist, inhibited significantly BK-induced increase in PBF, SPBF and PVO_2 by $86 \pm 8\%$, $73 \pm 7\%$ and $85 \pm 6\%$, respectively and reduced decrease in AVO_2 by $76 \pm 8\%$ (*Fig. 4*).

In group II i.v. injection of L-NNA decreased basal PBF by $34 \pm 2\%$ ($p > 0.05$), SPBF by $24 \pm 3\%$ ($p > 0.05$), PVO_2 by $27 \pm 3\%$ ($p > 0.05$), AVO_2 did not change significantly, and SAP increased by $26 \pm 3\%$ ($p > 0.05$). The subsequent vasodilatatory BK-induced response was significantly diminished in comparison with control BK infusion (*Fig. 5*). Thus the increase in PBF was $41 \pm 5\%$ ($p > 0.05$) in SPBF $35 \pm 4\%$ ($p > 0.05$) and in PVO_2 $20 \pm 3\%$ ($p > 0.05$) and the decrease in AVO_2 was $9 \pm 3\%$.

In group III pretreatment with L-Arginine significantly reversed effect of L-NNA on BK-induced pancreatic hyperemic and metabolic responses (*Fig. 5*).

DISCUSSION

Bradykinin is an endogenous nonapeptide which is formed by the action of the tissue and the circulating enzyme kallikrein on precursor kininogens and is metabolized by various peptidases and kininases (16—18). The kinin functions as a mediator of local vasodilatation in many vascular beds (12, 14, 18).

Experimental evidence accumulated supports the notion that BK is a physiologically important arterial vasodilator in the pancreas (6, 10). A considerable amount of evidence has also been presented that argues for a role for BK in acute experimental pancreatitis (19). Current experimental evidence suggests that the dilator action of BK in some vessels is mediated primarily by B_2-receptor activation. In addition there are reports that BK acts *via* an endothelial cell-receptor-mediated Ca^{2+}-dependent release of nitric oxide (20—23).

In the present study we have assessed the effect of exogenous BK on resting total pancreatic blood flow, microcirculatory blood flow through the gland and oxygenation of pancreatic tissue. The characteristics of the changes in the pancreatic and systemic circulation, which appeared after i. a. infusion of BK are consistent with previous reports (6, 11, 12). We found that BK elicited vasodilatory response from pancreatico-duodenal artery and the glandular microcirculation that appears to have an early peak increase in blood flow followed by a lower stable plateau phase of increased blood flow (*Fig. 1*). We also found that BK evoked significant increase in pancreatic oxygen consumption. The microcirculatory structures at which vasoactive factors act

in the pancreatic circulation are the arterioles which regulate resistance to the total blood flow through the pancreas and the precapillary sphincters which regulate the blood flow through the nutrient circulation. Since oxygen significantly exchanges only across the capillary endothelium, an increase uptake of oxygen ensues when capillary blood flow is increased. An accepted measurement of the nutrient circulation can be obtained using the local laser Doppler flowmetry. In the present studies BK increased pancreatic macro- and microcirculatory blood flow. The observed increase in pancreatic oxygen consumption could be due to either a direct metabolic effect of BK or simply to opering of the underperfused capillaries in the pancreatic microcirculation at basal conditions. During hyperemic response BK reduced pancreatic oxygen extraction while increasing pancreatic blood flow more proportionally than the increase in pancreatic oxygen uptake. The last findings agree with previous reports from our laboratory showing that numerous endo- and exogenous vasodilators evoke the same pattern of responses in the pancreatic circulation (24). Our study was also undertaken to explore the role of BK receptors and NO in the pancreatic vasodilatatory and metabolic responses mediated by exogenous BK. The observed in the present study pancreatic circulatory and metabolic responses evoked by BK were significantly inhibited in the animals pretreated with the B_2-receptor antagonist. In contrast pretreatment of the animals with B_1-receptor antagonist was without any effect on BK-induced local hemodynamic and metabolic responses. The above findings suggest that exogenous BK activates one receptor which relaxes pancreatic vasculature simultaneously increasing PBF, SPBF and PVO_2. The receptor appears to be a B_2-receptor.

Previous studies have presented evidence to support a physiological role for NO as a modulator of resting pancreatic blood flow and mediator of pancreatic functional hyperemia (15, 25). In order to determine the role of NO in the BK-induced pancreatic circulatory responses the NO production was impaired using L-NNA. The characteristic of the general and local pancreatic circulatory responses which we observed after inhibition of NO generation was consisted with earlier reports (15, 25). In the present study we found that endogenous NO participates in the mediation of BK-induced pancreatic vascular and metabolic responses since inhibition of NO synthase significantly diminished these pancreatic responses. Additional support for this suggestion is that inhibitory effects of L-NNA on above pancreatic responses were reversed by pretreatment with exogenous L-Arg.

In summary the findings of the present investigation support the concept that BK is a vasorelaxing factor in the pancreatic macro and microcirculation and regulator of the pancreatic tissues oxygenation during various physiological and pathological conditions. The BK-induced vasodilator response is mediated by B_2 receptors and nitric oxide.

REFERENCES

1. Konturek SJ, Holst JJ, Lamers CB, Singer MU, Pawlik WW. Advances in neuro-hormonal control of pancreatic secretion. *Int J Pancreatol* 1988; 3: Supp. 2: 163—171.
2. Pawlik WW. Pancreatic blood flow-control mechanism. Polish Physiological Society XIX Congress. Torun, Proc Sept 1993: 80—85.
3. Sendur R, Pawlik WW. Pancreatic blood flow and its regulation. *Przeg Lek* 1994; 51/5: 41—45.
4. Kvietys PR, McLendon JM, Bulkley GB, Perry MA, Granger DN. Pancreatic circulation: intrinsic regulation. *Am J Physiol* 1982; 242: G596—G602.
5. Bernard C. Memoires sur le pancreas. CR Seances Acad Sci 1856; Ser II Sci Vie Suppl 1: 379—563.
6. Hilton SM, Jones M. The role of plasma kinin in functional vasodilatation in the pancreas. *J Physiol (Lond)* 1968; 195: 521—533.
7. Broadie TA, Devedas J, Rysavy A *et al.* The effect of hypoxia and hypercapnia on canine pancreatic blood flow. *J Surg Res* 1979; 27: 114.
8. Berg T, Ostravic TB, Carretero OA, Scicli G. Kallikrein-kinin system in the regulation of submandibular gland blood flow. *Am J Physiol* 1982; 242: H1010—H1014.
9. Hilton SM, Jones M. Plasma kinin and functional vasodilatation in the pancreas. *J Physiol (Lond)* 1963; 165: 35—36P.
10. Pawlik WW, Gustaw P, Pawlik T, Sendur R, Czarnobilski K. Nitric oxide as mediator of bradykinin induced pancreatic vasodilatation. *Digestion* 1994; 55: 321—322.
11. Berguer R, Hottenstein OD, Palen TE, Steward JM, Jacobson ED. Bradykinin-induced mesenteric vasodilation is mediated by B_2-subtype receptors and nitric oxide. *Am J Physiol* 1993; 264: G492—G496.
12. Blumberg AL, Denny SE, Marshall GR, Needleman P. Blood vessel-hormone interactions: angiotensin, bradykinin, and prostaglandins. *Am J Physiol* 1977; 232: H305—H310.
13. Ferreira SH, Smaje LH. Bradykinin and functional vasodilatation in the salivary gland. *Br J Pharmacol* 1976; 58: 201—209.
14. Jacobson ED, Berguer R, Pawlik WW, Hottenstein OD. Mesenteric Purinergic and peptidergic vasodilators. Physiology of the Gastrointestinal Tract, L.R. Johnson (ed), Third Edition, Raven Press, New York 1994; 47: pp. 1647—1667.
15. Pawlik WW, Czarnobilski K, Sendur R, Konturek SJ. Role of nitric in the pancreatic vascular and metabolic responses associate with activation of capsaicin-sensitive neurons. *J Physiol Pharmacol* 1996; 47, 4: 601—609.
16. Bhoola KD, Lemon M, Mathews R. Kaelikrein in exocrine glands. In Handbook of Experimental Pharmacology. Bradykinin, Kallidin and Kallikrein, EG Erdos (ed), Berlin Springer-Verlag 1979; 25: pp. 489—523.
17. Carretero OA, Scicli AG. Kinins paracrine hormone. *Kidney Int* 1988; 34: S52—S59.
18. Regoli D. Neurohumoral regulation of precapillary vessels: the kallikrein-kinin system. *J Cardiovasc Pharmacol* 1984; 6: S401—S412.
19. Yotsumoto F, Manabe T, Ohshio G. Bradykinin involvement in the aggravation of acute pancreatitis in rabbits. *Digestion* 1993; 54: 224—230.
20. Regoli D, Rhaleb NE, Dion S, Drapeau G. New selective bradykinin receptor antagonist and bradykinin B_2 receptor characterization. *Trends Pharmacol Sci* 1990; 11: 156—161.
21. Farmer SG, Burch RM. The pharmacology of bradykinin receptors. Bradykinin antagonist: basic and clinical research. Marcel Dekker, New York 1991, pp. 1—31.
22. Burch RM, Kyle DJ. Recent developments in the understanding of bradykinin receptors. *Life Sci* 1992; 50: 829—838.

23. Schini VB, Boulanger C, Regoli D, Vanhoutte PM. Bradykinin stimulates the production of cyclic GMP *via* activation of B_2 kinin receptors in cultured porcine aortic endothelial cells. J *Pharmacol Exp Ther* 1990; 252: 581—585.
24. Pawlik WW, Konturek SJ, Gustaw P *et al.* Role of tachykinins in the control of pancreatic secretion and circulation. *J Physiol Pharmacol* 1992; 43(1): 43—57.
25. Konturek SJ, Bilski J, Konturek PK, Cieszkowski M, Pawlik W. Role of endogenous nitric oxide in the control of canine pancreatic secretion and blood flow. *Gastroenterology* 1993; 104: 896—902.

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