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ROLE OF NITRIC OXIDE IN THE PANCREATIC VASCULAR AND METABOLIC RESPONSES ASSOCIATED WITH ACTIVATION OF CAPSAICIN-SENSITIVE NEURONS

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The effects of local periarterial placement of capsaicin upon pancreatic blod flow, oxygen extraction from pancreatic circulation and oxygen consumption by pancreatic tissue were measured in anesthetized dogs. These studies explored also the possible role of endogenous nitric oxide (NO) in the pancreatic vascular and metabolic responses to periarterial capsaicin. In anesthetized dogs, superior pancreatico-doudenal artery blood flow (SPBF) was measured with an ultrasonic blood flowmeter. Microcirculatory pancreatic blood flow (PBF) was measured with laser-Doppler flowmeter. Arteriovenous oxygen difference (AVO_2) across the pancreatic circulation was determined spectrophotometrically. Pancreatic oxygen uptake was calculated as the product of AVO_2 and SPBF. Capsaicin applied periarterialy induced initial increase in SPBF, PBF and oxygen uptake. The acute capsaicin-induced vascular dilation was followed by steady state response characterized by significant decrease in SPBF, PBF and oxygen uptake. Inhibition of NO synthase by N^{ω} — nitro-L-arginine (L-NNA) induced pancreatic ischemia and hypoxia. After pretreatment with L-NNA the acute capsaicin-induced pancreatic vascular dilation and the increase in pancreatic oxygen uptake were significantly reduced. Above circulatory and metabolic effects of L-NNA were significantly attenuated when administration of L-NNA was combined with L-arginine. The results of these studies indicate that sensory C-fibers at rest and when actived play a role in the control of pancreatic blood flow and tissue oxygenation. These findings support also the hypothesis that NO plays a role in the mediation of pancreatic vasodilatory action of neuropeptides released from sensory C-fibers.

Key words: *pancreatic circulation, oxygen, sensory neurons, nitric oxide.*

INTRODUCTION

Factors regulating pancreatic blood flow include general hemodynamic factors, the autonomic nervous system, circulating hormones, peptides, various humoral agents and certain local properties of pancreatic vasculature. Among these factors neural control of the pancreatic vasculature plays an important role in the modulation of vascular responses to tissue metabolic requirements

and adaptation of pancreatic vascular resistance to general hemodynamic. The pancreatic vasculature is innervated by the extrinsic nervous system which contains the sympathetic vasomotor fibers which mediate constrictor responses, the parasympathetic fibers which elicit vasodilation and nonadrenergic, noncholinergic (NANC) vasodilator nerves. Within the extrinsic pancreatic vascular nerves there are visceral afferent neurons which are largely unmyelinated. Approximately 70% of these neurons are afferent sensory C-fibers, which contain a variety of vasoactive peptide neurotransmitters, such as CCK, CGRP, substance P, VIP. C-fibers contain also nitric oxide synthase (1—4). It has been demonstrated that C-fibers and sensory peptides play a role in the modulation of pancreatic vascular responses at basal conditions and in acute experimental pancreatitis (5, 6). Available evidence suggests that afferent C-fibers may regulate resting tone of the pancreatic vasculature.

The present study was undertaken to estimate the role of sensory afferent neurons in the regulation of the resting pancreatic blood flow and oxygenation of pancreatic tissue. These studies explored also the possible role of NO in the pancreatic vascular and metabolic responses at basal conditions and hyperemia due to activation of sensory neurons in the pancreas.

MATERIAL NAD 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 penthobarbital (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 continuos monitoring of systemic arterial pressure (AP) and another for siphoning arterial blood into an arterial cuvette of spectrophotometric oxygen content difference analyzer (AVOX System, San Antonio). The femoral vein was also cannuled 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 suplied 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 System 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 AP, 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.

After the surgical preparation was completed, hemodynamic and metabolic parameters were allowed to stabilize for 30 min. Then three experimental protocols were initiated. In each protocol, a group of 5 dogs was studied. In group I, first we determined the pancreatic circulatory and metabolic responses to periarterial capsaicin. Capsaicin (CAP) (Fluka) was applied as a 1% solution in 10% ethanol, 10% Tween 80, and 80% 0,9% NaCl. A silastic-coated sponge cuff was placed around the trunk of the superior pancreaticoduodenal artery and 0.3 ml of capsaicin solution was injected into the cuff. In group II, the pancreatic hypermia and metabolic responses to the presence of capsaicin on the superior pancreatico-duodenal artery were studied after NO synthase blockade using L-NNA (Sigma, Chemical Co.). 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 SPBF, PBF, PVO_2 and AP response to capsaicin were studied 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 capsaicin was applicated.

All data are presented as means $\pm$ 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

Control values for 15 animals were: SPBF 41 ± 2.5 ml/min, AVO_2 4.6 ± 0.6 ml O_2/100 ml of blood, PVO_2 1.9 ± 0.1 ml/min, and PBF 3.4 ± 0.3 V. Mean systemic arterial pressure ranged from 120 to 135 mmHg and was significantly altered only after pretreatment with L-NNA.

In group I immediately after periarterial application of capsaicin, an increase in SPBF was observed. The maximal hyperemic response was observed about 8 min after onset of drug administration, when SPBF was increased by $25 \pm 3\%$ ($p < 0.05$) and PBF increased by $35 \pm 6\%$ ($p < 0.01$). At the same period of time AVO_2 decreased below control level by $4.2 \pm 1.0\%$ ($p < 0.05$) while PVO_2 was increased by $19 \pm 3\%$ ($p < 0.05$). Subsequently a slow recovery of SPBF and PBF towards the control value was observed. This vasodilatory response to periarterial capsaicin had a letency of up to 25 min and was followed by a long period of vasoconstriction. The new steady-state of pancreatic circulatory and metabolic parameters was obtained at 25 min after onset of capsaicin application. The analysis of the changes observed during vasoconstrictory response to capsaicin showed that at that time SPBF was decreased by $21 \pm 3\%$ ($p < 0.01$) and PBF by $38 \pm 4\%$ ($p < 0.01$). PVO_2 was decreased $24 \pm 4\%$ ($p < 0.05$) and AVO_2 increased by $3.1 \pm 1.9\%$ ($p < 0.05$). AP was not significantly altered in this experimental group. (*Figs. 1 and 2*).

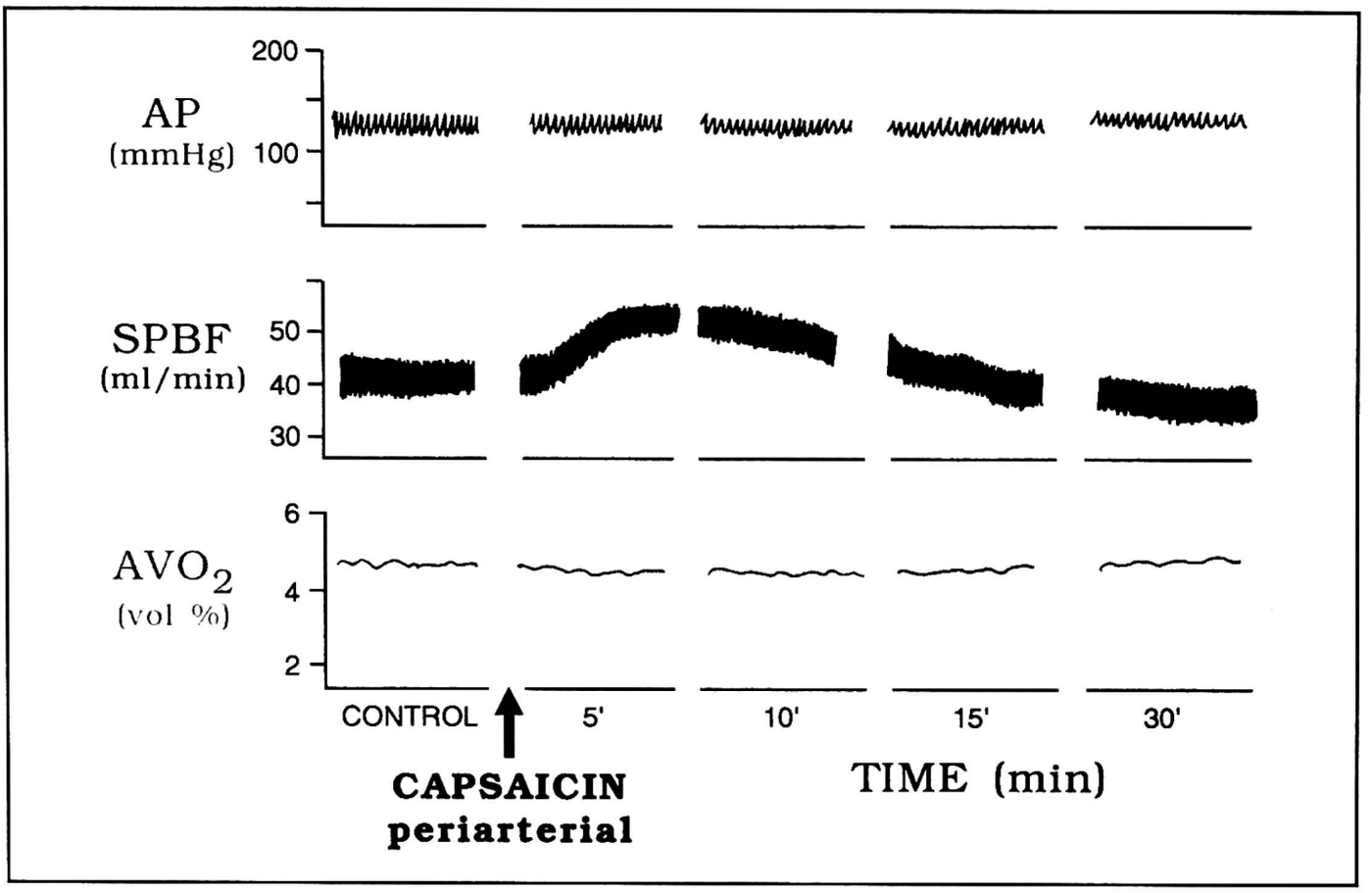

Fig. 1. The effect of periarterial administration of capsaicin on AP, PBF and AVO_2 values.

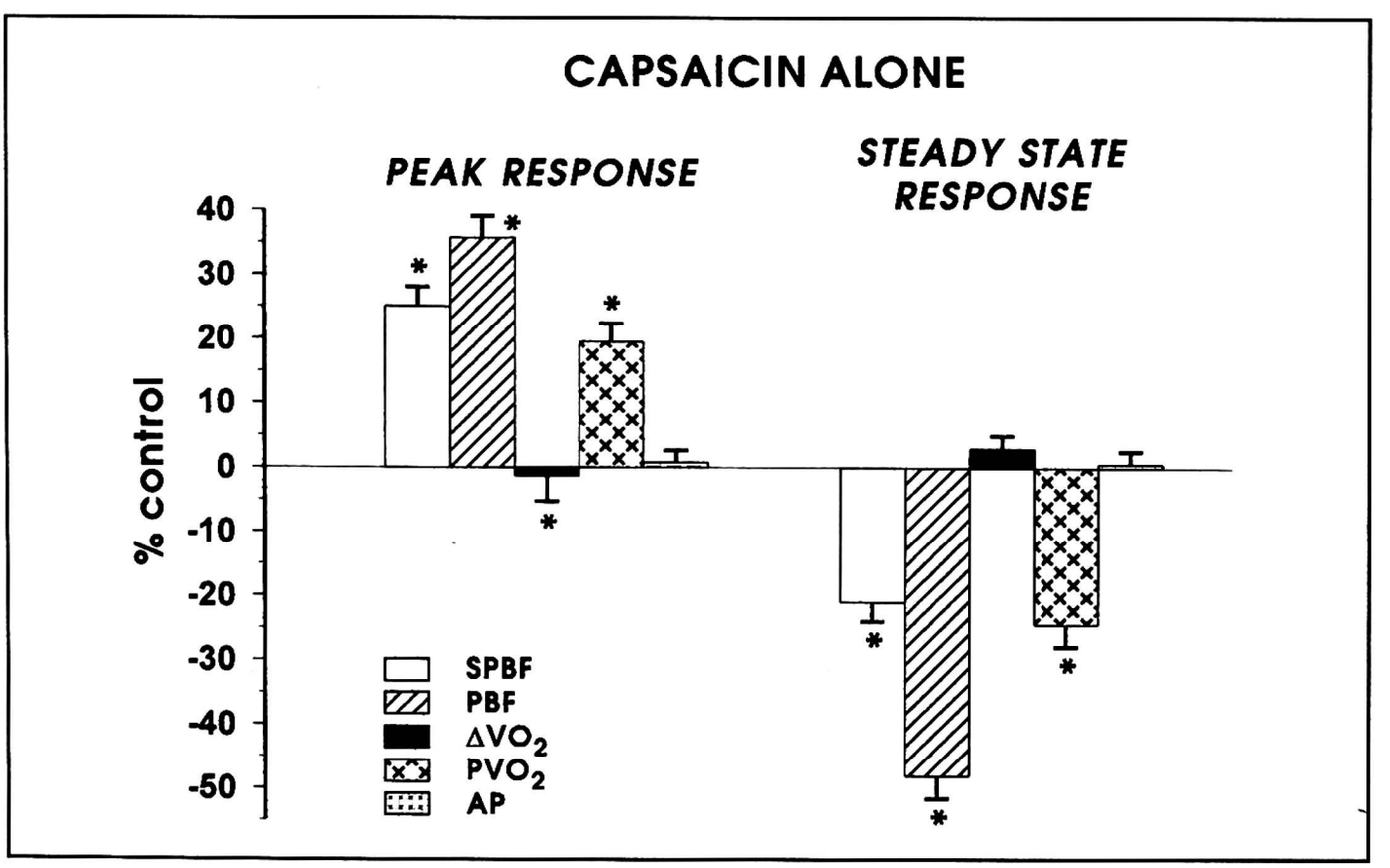

Fig. 2. Effect of periarterial placement of capsaicin on early (peak) and late (steady state) response of superior pancreatico-duodenal artery blood flow (SPBF), microcirculatory pancreatic blood flow (PBF), arteriovenous oxygen difference (AVO_2) across the pancreatic circulation, pancreatic oxygen uptake (PVO_2) and the mean arterial pressure (AP). Asterisk indicates significant difference from control.

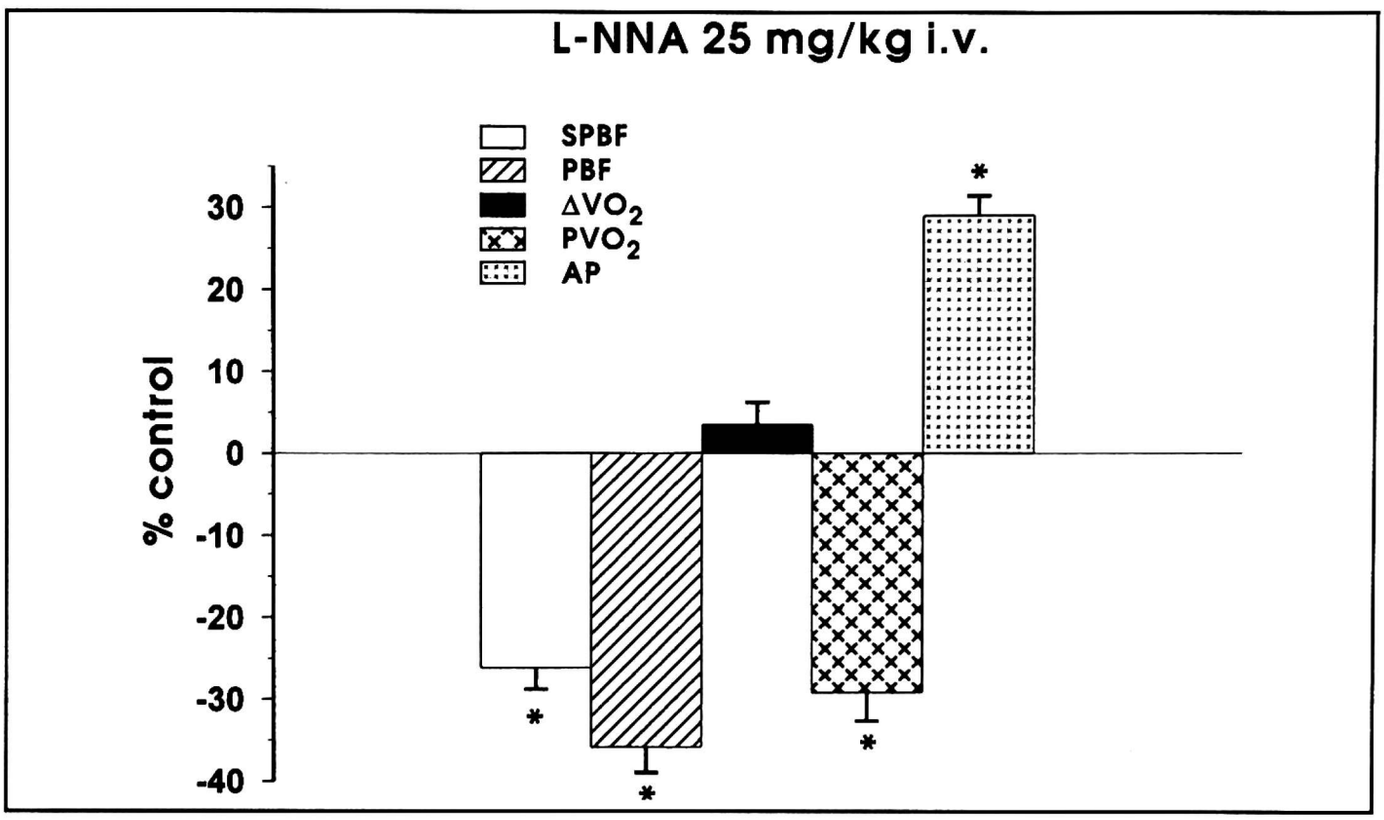

Fig. 3. Effects of L-NNA on the superior pancreatico-duodenal artery blood flow (SPBF), microcirculatory pancreatic blood flow (PBF), arteriovenous oxygen difference (AVO_2) accros the pancreatic circulation, pancreatic oxygen uptake (PVO_2) and the mean systemic arterial pressure (AP). Each column represents mean ± SE in % control of 5 dogs. Asterisk indicates significant difference from control.

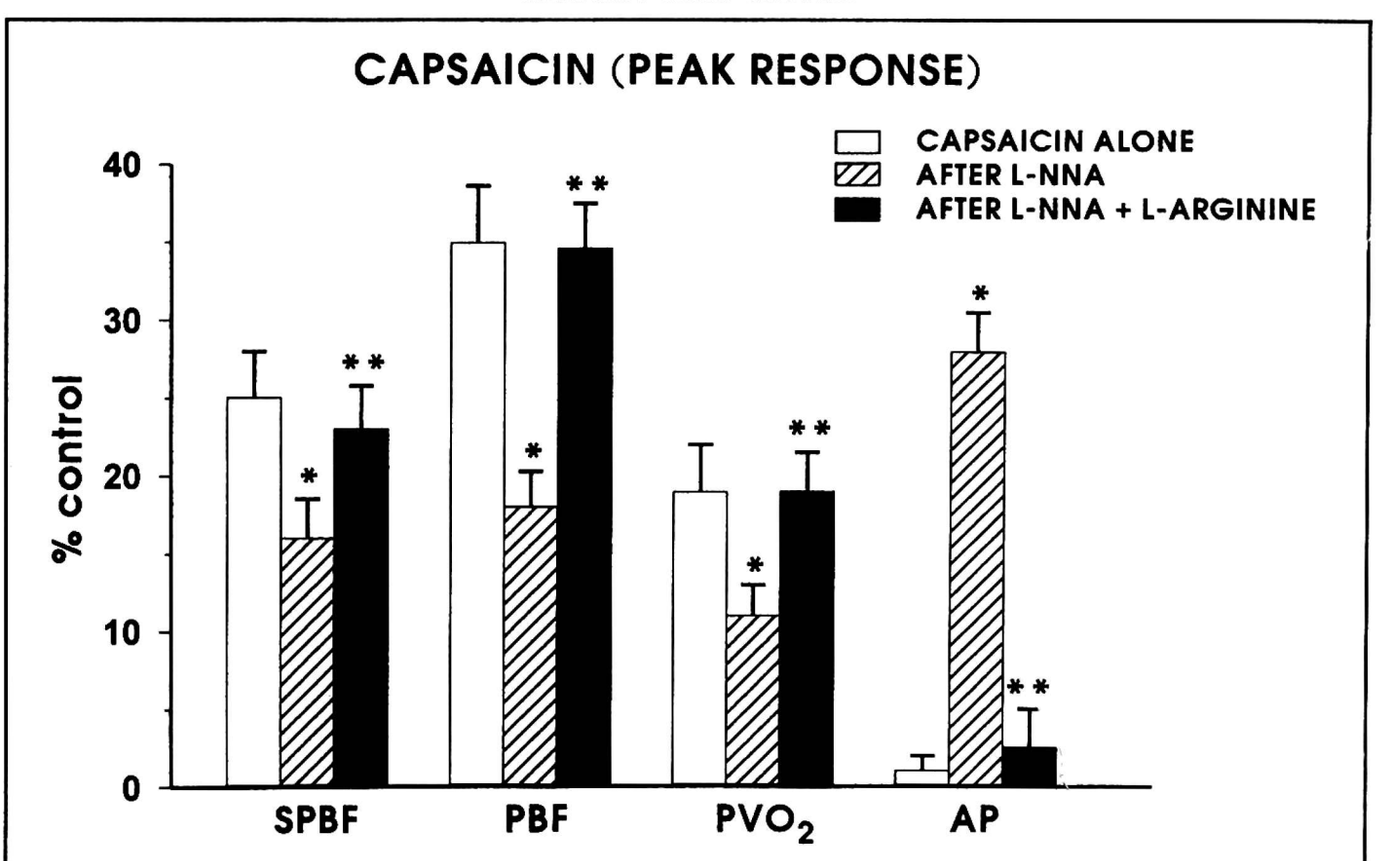

Fig. 4. Changes in capsaicin-induced peak hyperemic response of SPBF, PBF, PVO_2 and AP before and after pretreament with L-NNA, and after L-NNA + L-Arginine. Single asterisk indicates significant change from capsaicine alone. Double asterisk indicates significant change from capsaicin after L-NNA.

In group II intravenous injection of L-NNA decreased basal SPBF by $26 \pm 2.3\%$ ($p < 0.01$), PBF by $36 \pm 3.5\%$ ($p < 0.01$), PVO_2 by $29 \pm 3.1\%$ ($p < 0.01$), AVO_2 did not change significantly, and AP increased by $28 \pm 2\%$ ($p < 0.05$) (*Fig. 3*). After i.v. pretreatment with L-NNA, periarterial capsaicin was administered. The subsequent hyperemic response was similar in shape to the control and appeared 6 min. after onset of capsaicin application. Capsaicin-induced peak hyperemic response was significantly diminished in comparison with response observed in group I. Thus the increase in SPBF was $16 \pm 2.5\%$ ($p < 0.05$) in PBF $18 \pm 2.6\%$ ($p < 0.05$) and in PVO_2 $11 \pm 2.3\%$ ($p < 0.05$), likewise the steady state vasoconstrictory response was pronounced. SPBF, PBF and PVO_2 were decreased by 30 ± 2.8, 45 ± 5.2 and $29 \pm 2.4\%$ respectively. AVO_2 did not change (*Figs. 4 and 5*).

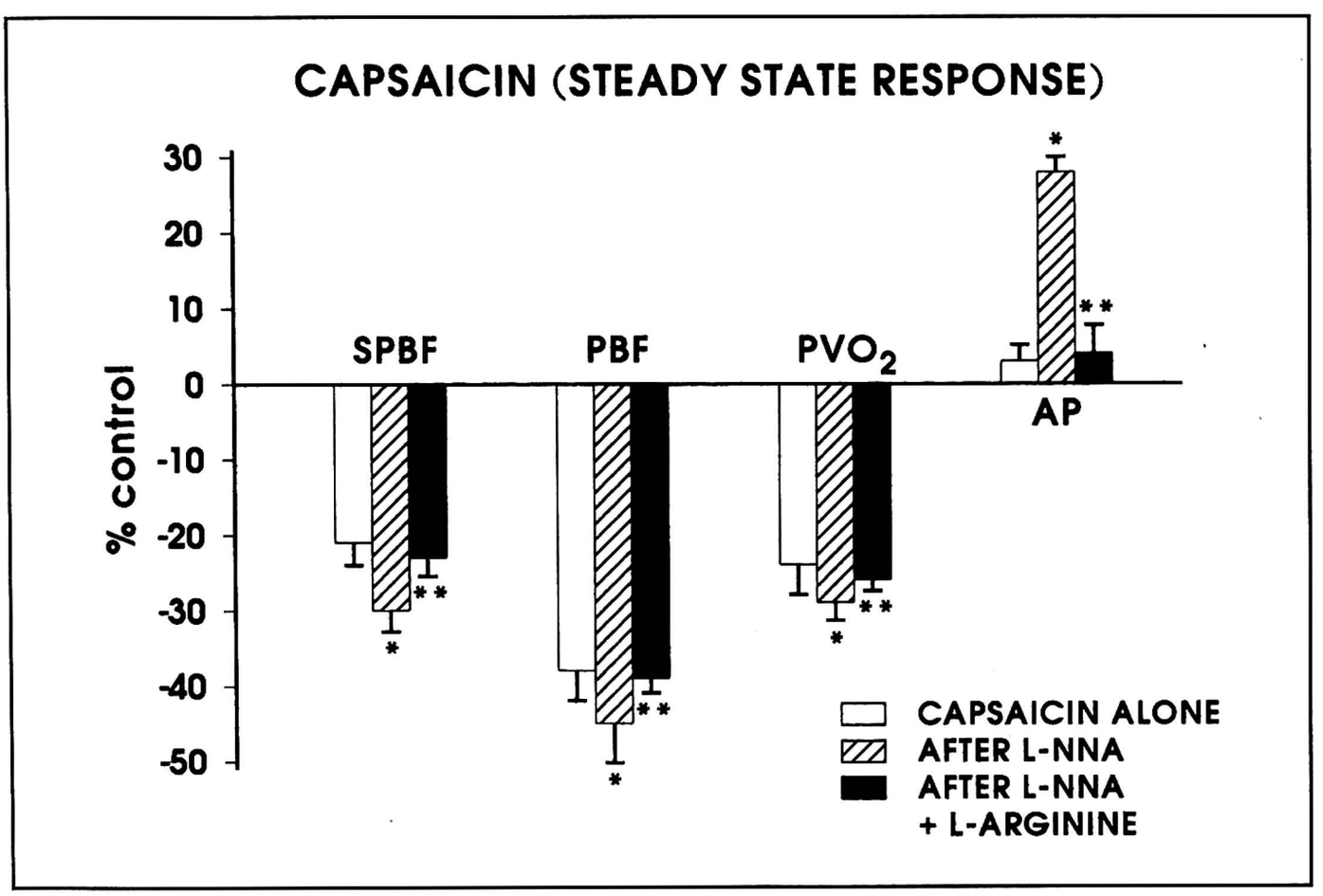

Fig. 5. Changes in capsaicin-induced steady state response of SPBF, PBF, PVO_2, and AP before and after pretreatment with L-NNA and after L-NNA + L-Arginine. Single asterisk indicates significant change from capsaicine alone. Double asterisk indicates significant change from capsaicin after L-NNA.

In group III pretreatment with L-Arginine significantly reversed effect of L-NNA on capsaicin-induced peak hyperemic and metabolic responses, and steady state response compared with group I (*Fig. 4 and 5*).

DISCUSSION

Previous studies have presented evidence to support a physiological role for capsaicin-sensitive sensory nerves as modulators of resting pancreatic blood flow. It has been suggested that primary sensory neurons which are present in the pancreas, contain a variety of well recognized peptides, such as CCK, CGRP, substance P and VIP, could participate in the mediation of local vascular responses during experimental acute pancreatitis (5, 7, 8).

In the present study we have assessed the role of afferent C-fibres in the 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 acute periarterial application of capsaicin are consistent with previous reports. We found that capsaicin elicited a vasomotor response from the canine pancreatico-duodenal artery and pancreatic microcirculation that appears to have two components: an initial transient vasodilation that fades and a sustained constriction of greater amplitude than observed vasodilation. The capsaicin-induced increase in the pancreatic blood flow observed in the current study corresponds with earlier reports showing that sensory C-fibers when activated induce local vasodilation which is followed by long lasting vasoconstriction (7, 9). We aslo found that capsaicin evoked significant biphasic changes in the pancreatic oxygen uptake. The microcirculatory structures at which vasoactive factors act in the pancreatic circulation are the aterioles 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 in 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 capsaicin increased pancreatic macro- and microcirculatory blood flow. The observed increase in pancreatic oxygen consumption could be due to either a direct metabolic effect of acute activation of sensory neurons or simply to opening of the underperfused capillaries in the pancreatic microcirculation at basal conditions (10—12). During hypermic response capsaicin reduced pancreatic oxygen extraction while increasing pancreatic blood flow more proportionally than the increase in pancreatic oxygen uptake. These findings agree with previous reports from our laboratory showing that numerous vasodilatatory drugs including sensory peptides evoke the same pattern of responses in the pancreatic circulation (13, 14).

Recently, nitric oxide has been established as the potent endogenous vasodilator in the gastrointestinal circulation (15—19). However, the physiological importance of NO in local regulation of pancreatic blood flow remains to be clarified. It was, therefore of interest to determine the possible

contribution of NO in the pancreatic vasodilatatory responses mediated by activation of peptide-containing sensory nerves. In order to determine the role of NO in the capsaicin-induced pancreatic circulatory responses the NO generation was impaired using the selective inhibitor of NO synthase L-NNA. The characteristics of the pancreatic basal vascular response and AP changes, which we observed after acute L-NNA in fasted dogs are cosisted with previous reports that acute administration of L-NNA prompted a marked gastrointestinal and pancreatic ischemia and hypoxia (11, 15, 16). Our results support the finding of others that NO is the major mediator of the tonic vascular dilatation in the pancreatic circulation.

We have also confirmed the participation of endogenous NO in the mediation of capsaicin-induced peak hyperemic response of the pancreatic circulation. Inhibition of NO synthase significantly diminished the pancreatic vascular response due to acute application of capsaicin on the main trunk of the superior pancreatico-duodenal artery. Our present study with L-NNA suggests that pancreatic hyperemia due to activtion of sensory nevers appears to be mediated in part by NO. Additional support for this suggestion is that inhibitory effects of L-NNA on this pancreatic hyperemic and metabolic responses are reversed by exogenous L-Arginine. It has been demonstrated that acute periarterial capsaicin induces a vasodilation secondary to a local release of vasodilator neuropeptides (13, 14, 20). This finding also suggests a role for afferent peptidergic nerves in the pancreatic circulation which may account for relaxation of the pancreatic vasculature at basal conditions and during various physiological and pathological states.

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