ELSEVIER

European Journal of Pharmacology 312 (1996) 75–81

ejp

Influence of the neurotoxin capsaicin on rat pancreatic islets in culture, and on the pancreatic islet blood flow of rats

Per-Ola Carlsson *, Stellan Sandler, Leif Jansson

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

Received 11 June 1996; accepted 21 June 1996

Abstract

The importance of peptidergic nerve fibres for the regulation of whole pancreatic and islet blood flow was studied by administration of the neurotoxin capsaicin. Administration of capsaicin induces an acute release and depletion of mainly substance P and calcitonin gene-related peptide from sensory nerve fibres. When given repeatedly to adult rats for several days, the neuropeptides are irreversibly depleted from the nerve endings. Depletion of substance P was confirmed by immunohistochemical stainings in the present study. A bolus dose of capsaicin (4 μg/kg body weight) reduced both whole pancreatic and islet blood flow in anesthetized rats, whereas repeated treatment with capsaicin led to an increase in both pancreatic and islet blood flow. In vitro experiments on isolated islets exposed to capsaicin (0.25 and 2.5 μM) for 4 days showed no effect on β-cell function. We conclude that peptidergic nerves have an important role for the maintenance of basal vascular tone in both the endocrine and exocrine parts of the pancreas, and may thereby influence the regulation of insulin secretion in rats.

Keywords: Capsaicin; Pancreatic islet; Insulin release; Blood flow; Pancreas; Peptidergic neuron

1. Introduction

The rat pancreas is richly innervated with sensory, neuropeptide-containing C-fibres (Sharkey et al., 1984; Sternini and Brecha, 1986; Su et al., 1987). Many of these nerves contain substance P and calcitonin gene-related peptide (CGRP), and are found also in association with the islets (Sundler and Böttcher, 1991). C-fibres have recently been shown to affect glucose homeostasis in mice. This was demonstrated by depleting neurotransmittors from sensory nerve fibres with the neurotoxin capsaicin, which led to a potentiation of the early insulin response to glucose and an improved glucose tolerance (Karlsson et al., 1992, 1994). The mammalian vascular system is also densely innervated with C-fibres (Barja et al., 1983; Furness et al., 1982) mediating vasodilation (Holzer, 1988). In mesenteric arteries, CGRP seems to be the most important neurotransmittor in this context (Holzer, 1988; Li et al., 1991; Manzini and Perretti, 1988), but other neuropeptides are probably also involved (Manzini et al., 1991).

Previous studies have shown that an altered insulin release may be associated with an enhanced islet blood flow (Jansson, 1994). It is, therefore, possible that the actions of vasoactive peptides, such as CGRP and other neuropeptides, on insulin release is connected to their influence on islet blood flow. The aim of the present study was to evaluate the importance of substance P- and CGRP-containing nerve fibres for the regulation of islet blood flow and glucose homeostasis. For this purpose we used capsaicin, the main pungent ingredient in red hot peppers. This is a well-known neurotoxin which acutely releases neuropeptides from C-fibres (for a review, see Holzer, 1988), and when given repeatedly irreversibly depletes substance P (Jessel et al., 1978) and CGRP (Sternini and Brecha, 1986; Su et al., 1987) from their nerve endings.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats were obtained from a local breeding colony (Biomedical Centre, Uppsala, Sweden).

* Corresponding author. Department of Medical Cell Biology, Biomedical Center, PO Box 571, S-751 23 Uppsala, Sweden. Tel.: +46 (0)18 174396; fax: +46 (0)18 556401.

PII S0014-2999(96)00492-X

The animals had free access to tap water and pelleted food (type R34; AnalyCen, Lidköping, Sweden) throughout the experimental period. The experiments were approved by the local animal ethics committee at Uppsala University.

2.2. Chemicals

The chemicals were purchased from Boehringer-Mannheim (Mannheim, Germany): collagenase from *Clostridium histolyticum*, type CLS (EC 3.4.24.3); HyClone (Cramlington, UK): fetal calf serum (FCS); Miles (Slough, UK): bovine serum albumin (BSA); Amersham International (Amersham, UK): D-[U-^{14}C]glucose; Sigma Chemicals (St. Louis, MO, USA): culture medium RPMI 1640, antimycin A, HEPES and capsaicin; Pharmacia Fine Chemicals (Uppsala, Sweden): Dextran T70; Novo Industries (Copenhagen, Denmark): a kit for immunoassay for insulin; New England Nuclear (Boston, MA, USA): hyamine hydroxide. Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide, crystalline 98%) was diluted in 80% (v/v) saline, 10% (v/v) ethanol and 10% (v/v) Tween 80 (14). The dilution medium is hereafter referred to as vehicle.

2.3. Measurements of blood glucose concentrations after a single bolus dose of capsaicin

A total of 6 animals, weighing approximately 350 g, were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight) and placed on a heated operating table. Capsaicin (4 μg/kg body weight) was then administered as an intravenous bolus injection. Blood glucose concentrations were measured in blood samples obtained from the cut tip of the tail with blood glucose reagent strips (ExacTech; Baxter Travenol Laboratories, Deerfield, IL, USA) immediately before and 3, 5, 10, 15, 30 and 60 min after the capsaicin injection.

2.4. Blood flow measurements after a single bolus dose of capsaicin

A total of 11 animals weighing approximately 350 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (see above), heparinized and placed on an operating table maintained at body temperature. Polyethylene catheters, with an inner diameter of approximately 0.30 mm, were inserted into the ascending aorta (via the right common carotid artery) and into the left femoral artery. The catheter in the ascending aorta was used to continuously register the mean arterial blood pressure with a pressure transducer (PDCR 75/1; Druck, Groby, UK) connected to a recorder. The blood pressure was allowed to stabilize for 15 min after insertion of the catheters. The animals were then injected intravenously with either capsaicin (4 μg/kg body weight), or the vehicle.

The blood perfusion of the whole pancreas and the islets was measured 30 min later with a microsphere technique, as previously described in detail (Jansson and Hellerström, 1981, 1983). Briefly, approximately 150 000 non-radioactive microspheres with a diameter of 10 μm (NEN-Trac; Du Pont Pharmaceuticals, Wilmington, DE, USA), suspended in 0.2 ml of saline, were injected via the catheter in the ascending aorta. Starting 5 s before the microsphere-injection, and continuing for 60 s, an arterial reference blood sample was collected at a rate of approximately 0.50 ml/min from the femoral artery. The exact withdrawal rate was in each case confirmed by weighing the sample. Arterial blood samples for the analysis of serum glucose concentrations, with an automated glucose oxidase technique (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA, USA) were then obtained. The pancreas and adrenal glands were removed, blotted and weighed and the microsphere contents of these organs and the pancreatic islets were determined separately, as previously described (Jansson and Hellerström, 1981). The number of microspheres in each of the adrenal glands was estimated in order to confirm that a complete mixture of the microspheres in the arterial circulation had taken place. If the microsphere content differed > 10% between the two glands, the animals were excluded from the study. The organ blood flow values were calculated according to the formula: $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} = organ blood flow (ml/min), Q_{ref} = withdrawal rate of the reference sample (ml/min), N_{org} = number of microspheres present in the organ, N_{ref} = number of microspheres present in the reference sample.

2.5. Blood flow measurements after repeated capsaicin treatments

Animals weighing approximately 250 g were anesthetized with diethyl ether and then given either saline, the vehicle for capsaicin or capsaicin at increasing concentrations subcutaneously on 4 consecutive days. The anesthesia was maintained approximately 15 min after administration of any of the test substances. The doses of capsaicin were: day 1, 50 mg/kg body weight; day 2, 100 mg/kg body weight; day 3, 200 mg/kg body weight; and day 4, 400 mg/kg body weight. This repeated treatment with capsaicin depleted peptidergic sensory nerve fibres of their transmittor substances (Jessel et al., 1978). Blood glucose concentrations were measured with blood glucose reagent strips (ExacTech) immediately before the subcutaneous injections on days 1–4, and on days 5, 7 and 9 after the 1st day of administration. On day 9, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (see above), heparinized and placed on a heated operating table. The blood perfusion of the whole pancreas and the islets was measured with the microsphere technique described above, and blood samples for analysis of blood glucose concentrations with blood glucose reagent

strips (ExacTech) were obtained before the animals were killed.

2.6. Morphological studies after repeated capsaicin treatments

A piece of the pancreas (≈ 100 mg) together with ≈ 10 mm of the duodenum, from each of the animals given capsaicin or vehicle on 4 consecutive days, was placed in ice cold 10% (v/v) paraformaldehyde for 24 h. The pieces were then rinsed and dehydrated, embedded in paraffin, cut in 7-μm sections and mounted on glass slides. These sections were immunohistochemically stained for substance P with a peroxidase-antiperoxidase (PAP) technique (Erlandssen et al., 1975). The primary antibody against substance P was purchased from Euro Diagnostica (Malmö, Sweden), whereas secondary antibodies and the PAP complex were from Dakopatts (Glostrup, Denmark). The immunostained sections were lightly counterstained with hematoxylin. These preparations were evaluated for the presence of substance P-positive nerve fibres in the pancreas by an observer unaware of the origin of the sections. The duodenal specimen of control animals was used as a standard to evaluate the accuracy of the staining.

2.7. Islet isolation and culture

Pancreatic islets were isolated from rats by a collagenase digestion procedure (Sandler et al., 1987). Groups of 150–200 islets were pre-cultured free-floating for 4–5 days in medium RPMI 1640 supplemented with 10% (v/v) fetal calf serum at 37°C in an atmosphere of humidified air + 5% CO_2. Medium was changed every 2nd day. Then, islets in groups of 80 were transferred to new culture dishes containing 2.25 ml RPMI 1640 + 0.25 ml fetal calf serum without or with addition of 0.25 μM capsaicin, 2.5 μM capsaicin or the corresponding amount of vehicle for these two capsaicin concentrations, and maintained for 4 days. In these experiments, capsaicin was firstly dissolved in the vehicle and prepared as a stock solution of 30 mM and then diluted with saline to a concentration of 0.25 or 2.5 μM during vigorous shaking. In this way, the concentration of the vehicle in these experiments was minimized and, therefore, also any unspecific effects of the vehicle on β-cell function. The culture medium was exchanged after 2 days. The vehicle solution added was diluted with 9 vols. of RPMI 1640 + 10% fetal calf serum. Samples of the culture medium were collected for measurement of medium insulin concentration with radioimmunoassay (Heding, 1972) after the 4-day culture period.

2.8. Islet glucose-stimulated insulin release and islet contents of insulin and DNA and medium insulin concentration

Triplicate groups of 10 islets were transferred to sealed glass vials containing 0.25 ml Krebs-Ringer bicarbonate buffer supplemented with 2 mg/ml bovine serum albumin and 10 mM HEPES. The islets were incubated at 37°C in a gas phase of 95% O_2 + 5% CO_2 at 1.7 mM glucose for 1 h. Then, the incubation medium was gently removed and replaced by medium supplemented with 16.7 mM glucose, and the incubation was continued for another hour. The incubation media were collected and frozen at −20°C prior to RIA for insulin (Heding, 1972). After the incubations, the islets were harvested and pooled in groups of 30 and homogenised in 0.2 ml redistilled water. A fraction of the homogenate was mixed with acid ethanol and insulin was extracted overnight at 4°C. The insulin concentration of the extract was measured by radioimmunoassay (Heding, 1972) Another fraction of the aqueous homogenate was used for DNA measurement (Hinegardner, 1971).

2.9. Islet glucose oxidation rate

Groups of 10 islets in triplicate were incubated in 100 μl Krebs-Ringer bicarbonate buffer, supplemented with D-[U-^{14}C]glucose and non-radioactive glucose at a concentration of 16.7 mM in glass vials at 37°C in an atmosphere of 95% O_2 and 5% CO_2 (Andersson and Sandler, 1983). After 90 min, the incubation was interrupted and 0.05 mM antimycin A added. $^{14}CO_2$ liberated was trapped in hyamine hydroxide and the radioactivity measured by liquid scintillation.

2.10. Statistical analysis

Values are expressed as mean ± S.E.M. When multiple comparisons between data were performed, analysis of variance (ANOVA) and Fisher's protected least-square difference (PLSD) test were used. When only two groups were compared, probabilities (P) of chance differences

Table 1
Serum glucose concentrations, mean arterial blood pressure, whole pancreatic and islet blood flow values in anesthetized adult male Sprague-Dawley rats 30 min after administration of a single bolus dose of vehicle or capsaicin (4 μg/kg body weight)

Treatment	Vehicle (5)	Capsaicin (6)
Serum glucose concentration (mmol/l)	7.0 ± 0.6	7.2 ± 0.9
Mean arterial blood pressure (mm Hg)	108 ± 5	85 ± 5 [a]
Pancreatic blood flow (ml/min × g pancreas)	0.52 ± 0.04	0.23 ± 0.01 [b]
Islet blood flow (μl/min × g pancreas)	50 ± 9	15 ± 2 [a]
Islet blood flow (% of pancreatic blood flow)	8.9 ± 0.8	7.0 ± 1.0

All values are mean ± S.E.M. for the number of animals shown within parentheses. [a] $P < 0.01$ and [b] $P < 0.001$ when compared with the vehicle-treated animals, using Student's unpaired two-tailed t-test.

Table 2
Blood glucose concentrations, mean arterial blood pressure, whole pancreatic and islet blood flow values in anesthetized adult male Sprague-Dawley rats after repeated capsaicin treatment

Treatment	Saline (8)	Vehicle (6)	Capsaicin (6)
Blood glucose concentration (mmol/l)	4.1 ± 0.2	4.2 ± 0.8	3.8 ± 1.2
Mean arterial blood pressure (mm Hg)	105 ± 5	101 ± 5	102 ± 6
Pancreatic blood flow (ml/min × g pancreas)	0.55 ± 0.06	0.21 ± 0.07 [e]	0.83 ± 0.08 [c,e]
Islet blood flow (μl/min × g pancreas)	34 ± 4.3	19 ± 4.9	46 ± 9.6 [a]
Islet blood flow (% of pancreatic blood flow)	6.3 ± 0.4	8.5 ± 0.5 [d]	5.3 ± 0.8 [b]

All values are mean ± S.E.M. for the number of animals shown within parentheses. [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ vs. the vehicle-treated animals, whereas [d] $P < 0.05$ and [e] $P < 0.01$ vs. the saline-treated rats, using ANOVA and Fisher's protection least-square difference (PLSD) test.

were calculated with Student's paired or unpaired two-tailed *t*-test.

3. Results

The mean arterial blood pressure was reduced by a single bolus dose of capsaicin (Table 1), but was not affected at the time of the blood flow measurements in animals given repeated treatments (Table 2). In the animals given a bolus dose of capsaicin, a marked reduction in both whole pancreatic blood flow and islet blood flow was observed, whereas the fraction of whole pancreatic blood flow diverted through the islets was unaffected by this procedure (Table 1). In the rats depleted of neuropeptides in their afferent C-fibres by repeated treatment with capsaicin, both pancreatic and islet blood flow were increased compared with vehicle-treated rats, whereas fractional islet blood flow was decreased (Table 2). Light microscopy of the slides immunohistochemically stained for the presence of substance P showed no such nerve fibres in pancreata from animals repeatedly treated with capsaicin, whereas such nerve fibres were seen in all glands from vehicle-treated rats (Fig. 1). The vehicle itself decreased pancreatic

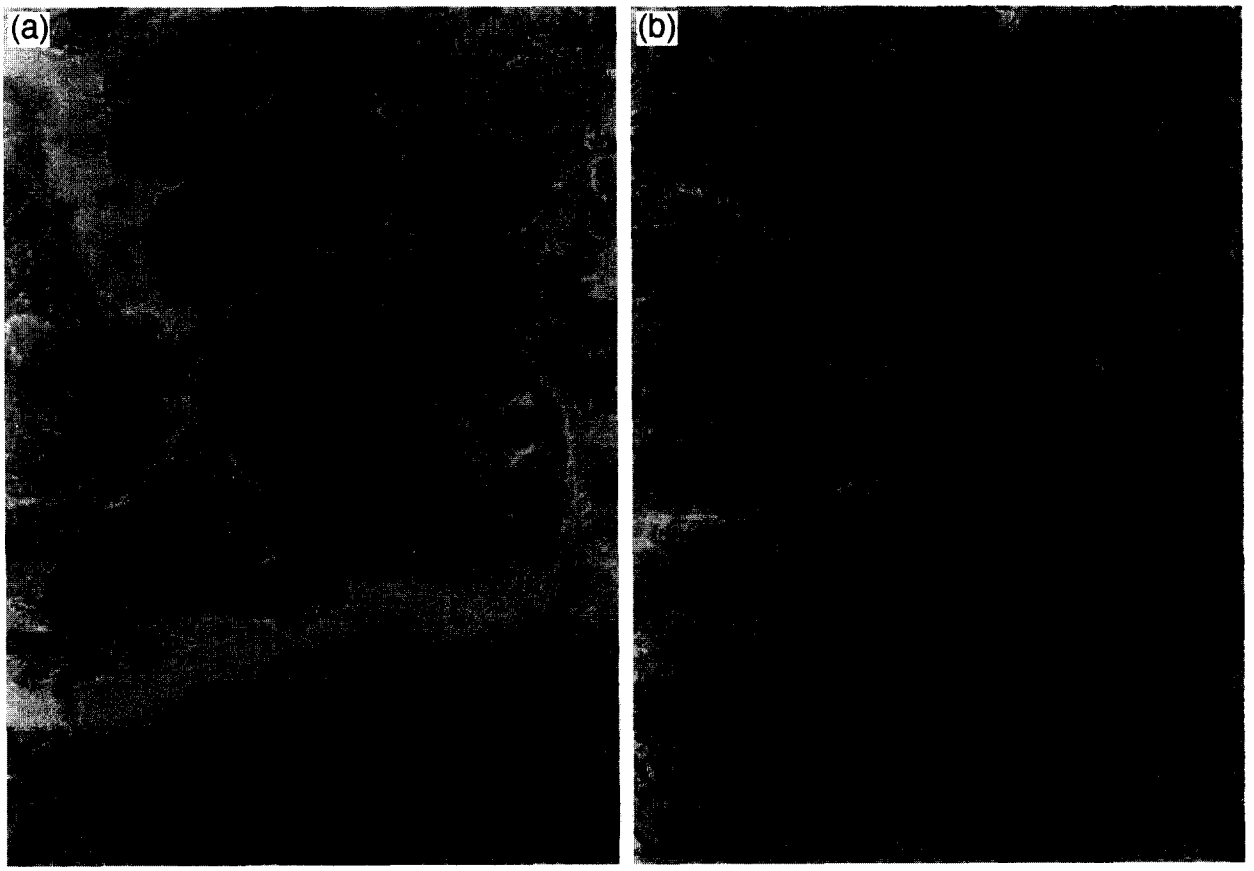

Fig. 1. Light micrographs of pancreata from rats treated with multiple injections of capsaicin (a) or vehicle (b) subcutaneously. The sections were stained for the presence of substance P-positive nerve fibres by indirect immunocytochemistry. The pancreatic islets from vehicle-trated rats demonstrate staining for substance P (arrows), whereas this is absent in capsaicin-treated rats. Magnification 1200 × .

Table 3
Long-term effects in vitro of capsaicin on rat pancreatic islet function

	Control	Vehicle	0.25 mM Capsaicin	2.5 mM Capsaicin
DNA content (mg DNA/10 islets)	0.38 ± 0.02	0.34 ± 0.02	0.38 ± 0.02	0.35 ± 0.03
Insulin content (ng insulin/10 islets)	602 ± 61.2	690 ± 123	672 ± 111	576 ± 55.2
Medium insulin accumulation (ng insulin/75 islets × 48 h)				
Days 0–2	5433 ± 651	6750 ± 415 [a]	6938 ± 678	6282 ± 306
Days 2–4	7258 ± 1032	6873 ± 841	7250 ± 1025	7773 ± 1298
Insulin release (ng insulin/10 islets × 60 min)				
1.7 mM glucose	8.5 ± 2.0	8.3 ± 1.1	9.0 ± 1.4	6.8 ± 1.5
16.7 mM glucose	38.8 ± 9.8	38.7 ± 12.1	41.8 ± 10.1	46.1 ± 10.6
Glucose oxidation rate (pmol/0 islets × 90 min)	761 ± 86.5	637 ± 50.5	677 ± 66.8	669 ± 64.4

Values are mean ± S.E.M. for 6 experiments. [a] $P < 0.05$ vs. control islets cultured in the presence of medium RPMI 1640 + 10% fetal calf serum alone, using Student's paired t-test.

blood flow, while islet blood flow was not affected to the same extent, thereby causing an increased fractional islet blood flow when compared with saline-treated rats (Table 2).

Capsaicin given as a single bolus dose in 6 animals did neither cause any marked changes in the blood glucose concentrations during the following 60 min, nor were the blood glucose concentrations affected by repeated treatment with capsaicin (data not shown). Likewise, at the time of the blood flow measurements after a single dose or repeated capsaicin treatments the circulating glucose concentrations did not differ between capsaicin- and vehicle-treated rats (Tables 1 and 2).

Culture of rat islets for 4 days with addition of the vehicle or of 0.25 or 2.5 μM capsaicin did not significantly alter the islet DNA or insulin content (Table 3). The medium insulin accumulation was not affected by capsaicin or the vehicle, with the exception of a slight increase during the first 2 days in islets cultured with addition of vehicle alone (Table 3). When the islet responsiveness to glucose was tested after the culture period, both the basal insulin release at 1.7 mM glucose and the stimulated insulin release at 16.7 mM glucose were similar in islets cultured with either the vehicle or with 0.25 or 2.5 μM capsaicin added (Table 3). Neither was the islet glucose oxidation rate affected in any of the experimental groups after culture (Table 3).

4. Discussion

The demonstration of a decreased mean arterial blood pressure after acute capsaicin administration confirms previous observations in rats (Donnerer and Lembeck, 1982, 1983; Makarara et al., 1967). This effect is probably due to a systemic vasodilatation mediated by the acutely released neuropeptides. Both pancreatic and islet blood flow were decreased after a bolus dose of capsaicin. This is in contrast to the findings in several other organs where capsaicin is a potent vasodilator, e.g. skin (Jancso et al., 1968), airway mucosa (Lundberg and Saria, 1983), ureter (Saria et al., 1983) and intestines (Rozsa et al., 1984). Capsaicin may, however, cause vasoconstriction in some blood vessels, such as the cat middle cerebral artery (Duckles, 1986) and dog mesenteric artery and renal artery (Toda et al., 1972). It has, therefore, been proposed that capsaicin also has a direct contractile effect on vascular smooth muscle in some arteries (Duckles, 1986).

An approximately 20% decrease in mean arterial blood pressure, as seen in the present study, is not enough to cause any change in the blood flow to the splanchnic vasculature (cf. Kvietys et al., 1982; Jansson, 1992), although a redistribution within the splanchnic organs may occur. Indeed, the capsaicin-induced decrease in pancreatic and islet blood flow could be explained by such a redistribution of blood to other splanchnic organs, or by a specific intra-pancreatic effect mediated by the released neuropeptides. In a previous study, administration of CGRP decreased both whole pancreatic and islet blood flow to the same extent as seen after acute administration of capsaicin in the present study (Svensson et al., 1994). The acute vascular effects of capsaicin on the pancreas may, therefore, have been mediated mainly through CGRP. The effects of substance P on pancreatic and islet blood flow are unknown. Also other vasoactive neuropeptides present in pancreatic peptidergic nerve fibres are affected by capsaicin, such as VIP (Hökfelt et al., 1980) and cholecystokinin (Jancso et al., 1981; Schultzberg et al., 1982), and may be of importance in this context.

In the animals treated with capsaicin dissolved in vehicle on 4 consecutive days, an increase in both pancreatic and islet blood flow was observed, whereas fractional islet blood flow was decreased, when compared with only vehicle-treated rats. That a depletion of nerve transmittors had occured in the nerve fibres was confirmed by the absence of substance P-immunoreactive nerves in the pancreata of the rats treated with capsaicin. These combined observations after a single dose and repeated treatments with capsaicin suggest that neuropeptide-containing C-fibres normally modulate blood flow through both the

exocrine and endocrine parts of the rat pancreas. The net effect of the neurotransmittors in these nerve fibres seem to be to decrease the blood perfusion to the whole gland.

Acetylcholine-induced endothelium-dependent relaxation in the mesenteric vascular bed has been shown to depend on the presence of peptidergic nerves (Scott et al., 1992). Furthermore, substance P- and CGRP-containing nerve fibres are abundant in pancreatico-duodenal grafts of rats (unpublished observation), although both pancreatic blood flow and islet blood flow are increased in the transplanted pancreas compared with the native gland (Jansson, 1994). The increased blood perfusion of the graft, which is seen also in other transplanted tissues (Hendersson et al., 1989; Perry et al., 1986; White et al., 1981), probably reflects a lack of exogenous innervation with an associated loss of vasomotor tone (cf. Hendersson et al., 1989). The present substance P- and CGRP-containing nerves would, therefore, depend on modulating influences from other nerve fibres, e.g. sympathetic or parasympathetic nerves, to exert their flow-decreasing effects on pancreatic circulation. An alternative explanation is that the maintenance of an inhibitory vasomotor tone within the pancreas demands innervation with extrinsic peptidergic nerve fibres.

The reason for the less pronounced effects on islet blood flow compared with whole pancreatic blood flow after depletion of neuropeptides in the pancreas is unknown. However, it supports previous observations that the blood perfusion is differently regulated in the endocrine and exocrine parts of the pancreas (Jansson, 1994).

Repeated treatment with the vehicle for capsaicin decreased both the whole pancreatic blood flow and the islet blood flow when compared with the corresponding animals treated with only saline. Since the vehicle for capsaicin was given in the same amounts to the capsaicin-treated group as to the group treated with only vehicle, it seems nevertheless reasonable to assume that repeated treatments with capsaicin itself increases both whole pancreatic and islet blood flow, probably via depletion of neurotransmittors from nerve fibres.

Since capsaicin did not affect islet β-cell function in vitro, it argues in favour of the view that the changes in islet blood flow observed after capsaicin treatment are mediated by the nerve system and not at the β-cell level.

We conclude that peptidergic nerves have an important role for the maintenance of basal vascular tone in both the endocrine and exocrine parts of the pancreas, and may thereby influence regulation of insulin secretion in rats.

Acknowledgements

The skilled technical assistance of Birgitta Bodin, Eva Forsbeck, Astrid Nordin and Eva Törnelius is gratefully acknowledged. The study was supported by grants from the Swedish Medical Research Council (Nos. 12X-109, 12X-8273, 12P-9287 and 12P-10739), the Novo Nordic Fund, the Swedish Diabetes Association, the Family Ernfors Fund, the Juvenile Diabetes Foundation International and the Åke Wiberg Fund.

References

Andersson, A. and S. Sandler, 1983, Viability tests of cryopreserved endocrine pancreatic cells, Cryobiology 20, 161.

Barja, F., R. Mathison and H. Huggel, 1983, Substance P containing nerve fibers in large peripheral blood vessels of the rat, Cell Tissue Res. 229, 411.

Donnerer, J. and F. Lembeck, 1982, Analysis of the effects of intravenously injected capsaicin in the rat, Naunyn-Schmiedeberg's Arch. Pharmacol. 320, 54.

Donnerer, J. and F. Lembeck, 1983, Capsaicin-induced reflex fall in rat blood pressure is mediated by afferent substance P-containing neurones via a reflex centre in the brain stem, Naunyn-Schmiedeberg's Arch. Pharmacol. 324, 293.

Duckles, S.P., 1986, Effects of capsaicin on vascular smooth muscle, Naunyn-Schmiedeberg's Arch. Pharmacol. 333, 59.

Erlandssen, S.L., J.A. Parsons, D.E. Van Orden and L.S. Van Orden, 1975, A modification of the unlabeled antibody enzyme method using heterologous antisera for light microscopic and ultratsructural localization of insulin, glucagon anf growth hormone, J. Histochem. Cytochem. 23, 666.

Furness, J.B., R.E. Papka, N.G. Della, M. Costa and R.L. Eskay, 1982, Substance P-like immunoreactivity in nerves associated with the vascular system of the guinea pigs, Neuroscience 7, 447.

Heding, L.G., 1972, Determination of total serum insulin (IRI) in insulin-treated patients, Diabetologia 8, 260.

Hendersson, J.M., W.J. Millikan, M. Hooks, B. Noe, M.H. Kutner and W.D. Warren, 1989, Increased galactose clearance after liver transplantation: a measure of increased blood flow through the denervated liver?, Hepatology 10, 288.

Hinegardner, R.T., 1971, An improved fluorometric assay for DNA, Anal. Biochem. 39, 197.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonon gene-related peptide and other neuropeptides, Neuroscience 24, 739.

Hökfelt, T., O. Johansson, A. Ljungdahl, J.M. Lundberg and M. Schultzberg, 1980, Peptidergic neurones, Nature (London) 285, 515.

Jancso, N., A. Jancso-Gabor and J. Szolcsanyi, 1968, The role of sensory nerve endings in neurogenic inflammation induced in human skin and eye and paw of the rat, Br. J. Pharmacol. 33, 32.

Jancso, G., T. Hökfelt, J.M. Lundberg, E. Kiraly, N. Halasz, G. Nilsson, L. Terenius, J. Rehfeld, H. Steinbusch, A. Verhofstad, R. Elde, S. Said and M. Brown, 1981, Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptide and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin, and 5-hydroxytryptamine, J. Neurocytol. 10, 963.

Jansson, L., 1994, The regulation of pancreatic islet blood flow, Diabetes Metab. Rev. 10, 407.

Jansson, L., 1992, Whole pancreatic blood flow and islet blood flow in hypovolemic hypotension in rats, Eur. Surg. Res. 24, 291.

Jansson, L. and C. Hellerström, 1981, A rapid method of visualizing the pancreatic islets for studies of islet capillary flow using non-radioactive microspheres, Acta Physiol. Scand. 113, 371.

Jansson, L. and C. Hellerström, 1983, Stimulation by glucose of the blood flow through the pancreatic islets of the rat, Diabetologia 25, 45.

Jessel, T.M., L.L. Iversen and A.C. Cuello, 1978, Capsaicin induced depletion of substance P from primary sensory neurones, Brain Res. 152, 183.

Karlsson, S., F. Sundler and B. Ahrén, 1992, Neonatal capsaicin-treatment in mice: effects on pancreatic peptidergic nerves and 2-deoxy-D-glucose-induced insulin and glucagon secretion, J. Auton. Nerv. Syst. 39, 51.

Karlsson, S., A.J. Scheurink, A.N. Steffens and B. Ahrén, 1994, Involvement of capsaicin-sensititive nerves in regulation of insulin secretion and glucose tolerance in conscious mice, Am. J. Physiol. 267, R1071.

Kvietys, P.R., J.M. McLendon, G.B. Bulkley, M.A. Perry and D.N. Granger, 1982, Pancreatic circulation: intrinsic regulation, Am. J. Physiol. 242, G596.

Li, D.-S., H.E. Raybould, E. Quintero and P.H. Guth, 1991, Role of calcitonon gene related peptide in gastric hyperemic response to intragastric capsaicin, Am. J. Physiol. 261, G657.

Lundberg, J.M. and A. Saria, 1983, Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants, Nature (London) 302, 251.

Makarara, B., L. Györgi and J. Molnar, 1967, Circulatory and respiratory responses to capsaicin, 5-hydroxytryptamine and histamine in rats pretreated with capsaicin, Arch. Int. Pharmacodyn. 170, 39.

Manzini, S. and F. Perretti, 1988, Vascular effects of capsaicin in isolated perfused rat mesenteric bed, Eur. J. Pharmacol. 148, 153.

Manzini, S., M. Tramontana and F. Perretti, 1991, Efferent function of capsaicin-sensitive nerves and neurogenic vasodilation in rat mesenteric circulation, in: Sensory Nerves and Neuropeptides in Gastroenterology (Plenum, New York) p. 241.

Perry, R.R., B.L. Bass, J.W. Harmon and V.F. Garcia, 1986, Blood flow to transplanted fetal rat intestine, J. Surg. Res. 41, 627.

Rozsa, Z., G. Jancso and V. Varro, 1984, Possible involvement of capsaicin-sensitive sensory neurones in the regulation of intestinal blood flow in the dog, Naunyn-Schmiedeberg's Arch. Pharmacol. 326, 352.

Sandler, S., A. Andersson and C. Hellerström, 1987, Inhibitory effects of interleukin 1 on insulin secretion, insulin biosynthesis and oxidative metabolism of isolated rat pancreatic islets, Endocrinology 121, 1424.

Saria, A., J.M. Lundberg, X. Hua and F. Lembeck, 1983, Capsaicin induced substance P release and sensory control of vascular permeability in the guinea pig ureter. Neurosci. Lett. 111, 167.

Schultzberg, M., G.J. Dockray and R.G. Williams, 1982, Capsaicin depletes CCK-like immunoreactivity detected by immunohistochemistry but not that measured by radioimmunoassay in rat dorsal spinal cord, Brain Res. 235, 198.

Scott, T.M., K.H. Drodge and J. Foote, 1992, Peptidergic nerve involvement in the control of endothelium-dependent vascular relaxation, Artery 19, 211.

Sharkey, K.A., R.G. Williams and G.J. Dockray, 1984, Sensory substance P innervation of the stomach and pancreas, Gastroenterology 87, 914.

Sternini, C. and N. Brecha, 1986, Immunocytochemical identification of islet cells and nerve fibers containing calcitonin gene-related peptide-like immunoreactivity in the rat pancreas, Gastroenterology 90, 1155.

Su, H.C., A.E. Bishop, R.F. Power, Y. Hamada and J.M. Polak, 1987, Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas, J. Neurosci. 7, 2674.

Sundler, F. and G. Böttcher, 1991, Islet innervation, with special reference to neuropeptides, in: The Endocrine Pancreas, ed. E. Samols (Raven, New York) p. 29.

Svensson, A.M., S. Sandler and L. Jansson, 1994, Pancreatic islet blood flow in the rat after administration of islet amyloid polypeptide or calcitonin gene-related peptide, Diabetes 43, 454.

Toda, N., H. Usui, N. Nishino and M. Fujiwara, 1972, Cardiovascular effects of capsaicin in dogs and rabbits, J. Pharmacol. Exp. Ther. 181, 512.

White, T.P., L.C. Maxwell, D.M. Sosin and J.A. Faulkner, 1981, Capillarity and blood flow of skeletal muscle of cats, Am. J. Physiol. 241, H630.