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Modulation of exocrine pancreatic secretion by leptin through CCK_1-receptors and afferent vagal fibres in the rat

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Abstract

In this report, we determined whether leptin could modify the exocrine pancreatic secretion of anaesthetized rats in vivo. Intravenous injection of recombinant murine leptin resulted in a time- and dose-dependent stimulation of exocrine pancreatic secretion, maximally observed with 30 nmol/kg of leptin. This stimulation of pancreatic water, bicarbonate, and protein output was abolished by atropine, hexamethonium, L364,718 ([3*S*(−)-*N*-(1,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine]), a cholecystokinin CCK_1 receptor antagonist or perivagal capsaicin pretreatment, but unaffected by the CCK_2 receptor antagonist L365,260 ([3*R*(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3yl)-*N*′-(3-methylphenyl)urea]). In addition, the physiological dose of 3 nmol/kg leptin, ineffective per se, potentiated the secretory effect of 45 pmol/kg of cholecystokinin octapeptide (CCK-8) on exocrine pancreatic secretion. Furthermore, intraperitoneal leptin induced a rapid increase in plasma CCK levels in vivo in the rat. In conclusion, exogenous leptin can modulate exocrine pancreatic secretion through mechanisms involving CCK_1 receptors and capsaicin-sensitive afferent fibres in the rat. Whether this may have a physiological relevance in the postprandial regulation of exocrine pancreatic secretion and thus in nutrient digestion will require further investigations.

Keywords: Leptin; Vagus nerve; Afferent fibres; Cholecystokinin; Pancreatic secretion

1. Introduction

Leptin, the product of the *ob* gene, is predominantly produced by and secreted from adipose tissue (Zhang et al., 1994) but also at lower levels by other organs such as the gastric mucosa (Bado et al., 1998). It controls adiposity by acting on leptin receptors in the brain to modulate food intake and energy metabolism (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). The leptin receptor (Ob-R) is a member of the gp130 family of cytokine receptors (Tartaglia et al., 1995). Several isoforms that result from alternative splicing of the *db* gene were described (Lee et al., 1996): short isoforms or Ob-R_S (for Ob-Ra, -c, -d, -f) and a long isoform (Ob-Rb) with a full-length 302 amino acid carboxy-terminal domain which contains binding motifs required to activate the JAK/STAT (Janus Kinase/Signal Transducers and Activators of Transcription) signalling pathway (reviewed by Tartaglia, 1997). A high density of Ob-Rb was reported in hypothalamic neurones which was initially believed to be restricted to this location (Lee et al., 1996; Tartaglia et al., 1995). Leptin receptors are also present in various other tissues, including Langerhans islets of the endocrine pancreas, especially in β- and δ-cells (see review by Kieffer and Habener, 2000), in the mucosa of stomach (Goiot et al., 2001; Mix et al., 2000; Sobhani et al., 2000), small intestine (Buyse et al., 2001a; Morton et al., 1998), and on neuronal structures of rat pancreas (Sha and Szurszewski, 1999).

While insulin is adipogenic and increases the production of leptin by adipose tissue (Bradley and Cheatham, 1999; Leroy et al., 1996; Saladin et al., 1995), leptin suppresses insulin secretion in isolated rat islets (Emilsson et al., 1997; Kieffer et al., 1997; Kulkarni et al., 1997), providing the basis for bidirectional feedback loop between β-cells and adipose tissue. The effect of leptin on β-cells is probably both direct on the ob receptors of these cells (Emilsson et

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al., 1997) and through the autonomic nervous system because exogenous leptin increases sympathetic nerve activity in a variety of tissues (Haynes et al., 1997) and increases activity of gastric vagal afferents (Wang et al., 1997).

In addition, an exciting study by Sha and Szurszewski (1999) showed that leptin could modulate fast synaptic transmission in dog pancreatic ganglion neurones. Since it is unlikely that neural control of the pancreas could affect only the endocrine, but not the exocrine tissue, we investigated whether exogenous leptin displays effects on exocrine pancreatic function.

2. Materials and methods

2.1. Animals

Wistar male rats (Iffa-Credo, Les Oncins, F 69210 St. Germain l'Abresle), weighing 280–320 g were used. They were maintained in the laboratory on a standard rearing chow (A 113, autoclaved, UAR, F 91360 Villemoisson sur Orge), and housed at 21 °C on an 8 AM/8 PM light/dark cycle. All experiments were conducted in accordance with EEC regulations on animal management in research studies.

2.2. Pancreatic fistula

After an overnight fast, the rats were anesthetized with a mixture of xylazine and ketamine (13 and 100 mg/kg body weight) intramuscularly (i.m.), respectively, and an acute pancreatic fistula was prepared, with total bile derivation and separate collection of pure pancreatic secretion by a method using continuous dilution of the juice (Rozé et al., 1975). This experimental model was established to measure the pure pancreatic water and bicarbonate secretions, which are much weaker than that of bile, and to collect undegradated pancreatic enzymes. Briefly, a 0.8-mm (outside diameter (o.d.)) needle connected to a 15-cm-long Silastic® catheter was introduced in the upper common bile duct and ligated as close as possible to the liver to allow bile derivation. The lower bile–pancreatic duct was connected through another 0.8-mm (o.d.) needle and a 1-cm-long Silastic® catheter to a T-shaped glass piece connected by Tygon® tube (0.51-mm internal diameter (i.d.)) to a reservoir and a peristaltic pump allowing permanent infusion of a diluting fluid, using a volume flow of 4 ml/20 min. The pancreatic juice was diluted in this fluid as the secretion proceeded, and collected with a fraction collector. The system was devised in such a way that internal pressure exerted by the apparatus in the lumen of the lower bile–pancreatic duct was slightly negative at about −2 cm of water.

Central temperature of the rats was controlled at 38 ± 0.5 °C by a heating device and pancreatic secretion was collected as 20-min samples. In collected samples, we determined sodium by flame photometry, bicarbonate by an autoanalyser photometric method (Chariot et al., 1976), and total protein by UV absorption at 280 nm. Since sodium is secreted in the pancreatic juice at a constant concentration, near to sodium plasma concentration, measuring sodium output is equivalent to measuring the volume of secreted pancreatic juice (Rozé et al., 1975). The average of the data obtained on the two first 20-min samples collected between $t = 40$ min and $t = 0$ was considered as an estimate of basal pancreatic secretion.

This pharmacological model may be criticised because bile and pancreatic juice are diverted. It is known that in many experimental conditions, diverting pancreatic juice and bile from the duodenum in rats increases pancreatic juice flow rate and protein output. However, in this particular setup and time conditions, the stimulating effect of diverting bile and pancreatic juice is small enough to be neglected. This is shown on the control infusions of vehicle (Fig. 1) which do not demonstrate important variations of basal pancreatic secretion over 3 h. We have also previously shown in a similar model that infusing saline or bile + pancreatic juice (1 ml/h) in the duodenum for 3 h did not produce an important reduction of basal pancreatic juice output (Nagain et al., 1995). In addition, Li and Owyang (1993) have used a very similar model to show the relationship between cholecystokinin (CCK) stimulation and cholinergic mechanisms in the rat pancreas.

The effect of leptin was first studied on basal secretion after the intravenous (i.v.) injection of different doses (3, 10, 30, and 100 nmol/kg). Plasma leptin levels obtained following these i.v. doses were measured in order to be compared with postprandial levels. For this purpose, rats that had been starved for 18 h were anaesthetised with ethylurethane (1.2 g/kg, i.m., Prolabo, Paris, France). A short polyethylene catheter (0.58 mm i.d. × 0.965 mm o.d., length 15 mm; Clay adams PE 50, Parispany, NY) was inserted into a carotid artery and secured by a double ligature around the polyethylene part of the catheter. The rats were injected with 250 UI/kg heparin through the catheter and the carotid was immediately clamped.

The receptors involved in the leptin effect were studied by associating to leptin i.v. injections of the following antagonists: L364,718 ([3*S*(−)-*N*-(1,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine]), a CCK_1-receptor antagonist, or L365,260 ([3*R*(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3yl)-*N*′-(3-methylphenyl)urea]), a CCK_2-receptor antagonist (0.5 mg/kg i.v., 5 min prior to the administration of leptin), the muscarinic receptor antagonist atropine (100 μg/kg, i.v., followed by the infusion of 100 μg/kg h for 3 h), and the nicotinic receptor antagonist hexamethonium (6.7 mg/kg, i.v., followed by 6.7 mg/kg h for 3 h). These doses of anticholinergics have been validated as blocking the neurally induced pancreatic secretion in awake rats (Chariot et al., 1990). It was also verified in the present study that this dose of atropine could suppress totally the effect of 450 pmol/kg of cholecystokinin (CCK) on exocrine pancreatic secretion.

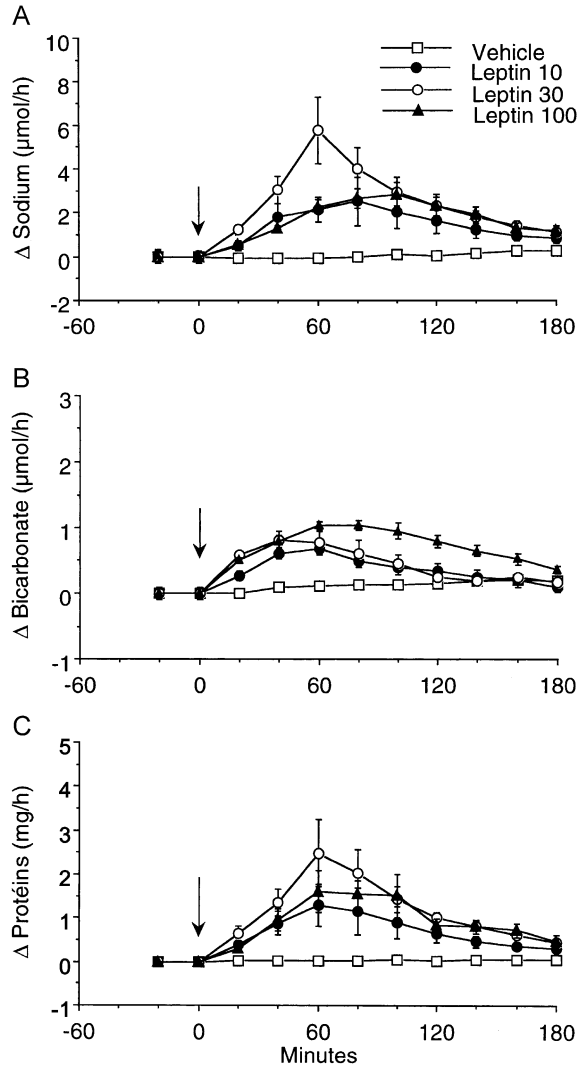

Fig. 1. Time-course of sodium (top), bicarbonate (middle), and total protein (bottom) output in pancreatic juice following the bolus i.v. injection of 10–100 nmol/kg of leptin or vehicle (control rats) at time zero (arrow). The data are variations (△) over the basal level, measured in 20-min samples of pancreatic juice. Mean ± S.E.M. in groups of five to six rats per dose (note that sodium output is a reflect of juice volume output).

Participation of vagal afferents in the leptin effect was studied by injecting exogenous leptin in rats which had been pretreated with perivagal capsaicin 10–12 days before (Raybould et al., 1990). After anesthesia with ketamine (100 mg/kg. i.p.) plus atropine sulphate (1 mg/kg), the cervical vagal trunks were exposed. A small piece of gauze soaked in 1% capsaicin in 90% olive oil plus 10% Tween 80 was applied for 30 min to each vagal nerve. Vehicle alone was applied to controls. The rats were allowed to recover. Ten days later, the effectiveness of the destruction of vagal afferent fibres by capsaicin was checked as previously by determining the effect of the synthetic octapeptide CCK-8 (4 µg/kg i.p) on 1-h food intake.

Finally, possible interaction between leptin and exogenous CCK was studied by determining a dose–response curve to CCK-8 boluses (45–1350 pmol/kg) on pancreatic secretion, and by injecting in association a subliminal dose of leptin (3 nmol/kg) and a liminal dose of CCK-8 (45 pmol/kg).

Leptin blood levels was determined after i.v. injection through the femoral artery of saline (vehicle) or recombinant murine leptin (3–30 nmol/kg). Blood samples were collected at 20–180 min following 30 nmol/kg and at 60 min following the other doses. To compensate for blood loss, an equal volume of Hæmaccel® (Behring, Marburg, Germany) was injected through the carotid catheter after each blood sample was taken. Blood was centrifuged at 3000 × *g* for 10 min and plasma was collected and stored at − 20 °C until leptin assay. Leptin was quantified using the mouse leptin RIA kit from Linco Research (St. Charles, MO) with a detection limit of 0.2 ng/ml.

In another set of experiments, anaesthetized rats received intraperitoneal saline, or recombinant murine leptin (6 nmol/kg), blood samples were collected as above at various times over 1 h, and plasma CCK was determined by radioimmunoassay.

2.2.1. Blood sampling

A short polyethylene catheter (0.58-mm internal diameter × 0.965 mm-outside diameter, length 1.5 cm) (Becton Dickinson, Parsippany, NJ) was inserted into a carotid artery and secured by a double ligature around the polyethylene part of the catheter. The rat was immediately injected with 250 IU/kg heparin through the catheter and the carotid was immediately clamped. Blood samples (0.5 ml) were collected in tubes containing EDTA. To compensate for blood loss, an equal volume of Hæmaccel® (Fresenius Kabi France, Sevres, France) was injected through the carotid catheter after the removal of each blood sample. During the experiments, normal body temperature was maintained with a heating device. Blood samples were centrifuged at 10,000 × *g* for 3 min, plasma was removed and speed-Vac-concentrated after an ethanol extraction, and stored at − 20 °C until CCK radioimmunoassay.

2.2.2. Radioimmunoassay of CCK

CCK was determined according to the procedure described by Rehfeld (1998) and modified by Dauge et al. (1999). Briefly, a C-terminal anti-CCK antibody (generously provided by Professor J. Rehfeld, Copenhagen, Denmark) was incubated at 4 °C for 4 days with CCK-8 (Sigma, St. Louis, MO) dilutions used as a standard or with plasma samples and ^{125}I CCK8 (Amersham Pharmacia Biotech, Piscataway, NJ) in RIA buffer (20 mM barbital buffer, 0.6 mM thiomersal, and 0.11% BSA v/v, pH 8.4). Bound and free fractions were separated by absorbing the free ^{125}I CCK8 onto active dextran T70-coated charcoal (4 and 40 g/l, respectively) in RIA buffer containing 10% filtered horse serum. Radioactivity in the bound fraction was measured with a gamma counter. Under these conditions, the detection limit was 0.5 pg of CCK.

2.3. Drugs

Recombinant murine leptin was purchased from R&D Systems (Abingdon, UK), atropine sulphate, hexamethonium bromide, and capsaicin from Sigma, and CCK octapeptide from Neosystem (Strasbourg, France); L364,718 and L365,260 were gifts from Dr. R.M. Freidinger (Merck, Westpoint, PA). Atropine and hexamethonium were dissolved in 0.9% saline and L364,718 and L365,260 were dissolved in dimethylsulfoxide and diluted 1/100 in 0.9% saline.

2.4. Statistics

Results are expressed in the text and the figures as means ± one standard error of the mean ± S.E.M. in groups of six to eight rats. The responses are expressed as the amount of pancreatic secretion produced over the basal level (□), and shown either as the time-related variation of outputs measured in 20-min samples of pancreatic juice, or as the integrated response over the basal level. The integrated response was measured during 3 h following leptin or CCK injection. Statistical analysis was performed by analysis of variance, followed when significant ($P < 0.05$) by a multigroup comparison test.

3. Results

3.1. Effect of leptin on basal pancreatic secretion

Leptin injected as an i.v. bolus (3–100 nmol/kg) produced a dose-related increase of all the measured variables of pancreatic secretion (Fig. 1). The dose 3 nmol/kg had no significant effect and is not shown in Fig. 1. The response was progressive and the peak response was observed about 60 min after leptin injection; secretion returned to the basal level after 3 h. The dose–response was biphasic: the maximal response was observed after 30 nmol/kg of leptin for sodium (reflecting the water flow) and protein output, while the response to 100 nmol/kg tended to be smaller (Fig. 1A and C). However, the bicarbonate response was less clearly dose-related between 10 and 100 nmol/kg (Fig. 1B), and no decreased response was observed after the largest dose of 100 nmol/kg.

3.2. Mechanism of leptin effect on basal pancreatic secretion

Adding either of the cholinergic antagonists atropine or hexamethonium totally suppressed the pancreatic response to the maximally effective dose of 30 nmol/kg of leptin (Fig. 2).

The effect of leptin was also suppressed by the CCK_1-receptor antagonist L364,718, while the CCK_2-receptor antagonist L365,260 had no effect (Fig. 2). The mechanism of the leptin effect appeared identical for all the variables measured, i.e. on the secretion of water and bicarbonate, and of exocrine enzymes by the pancreas.

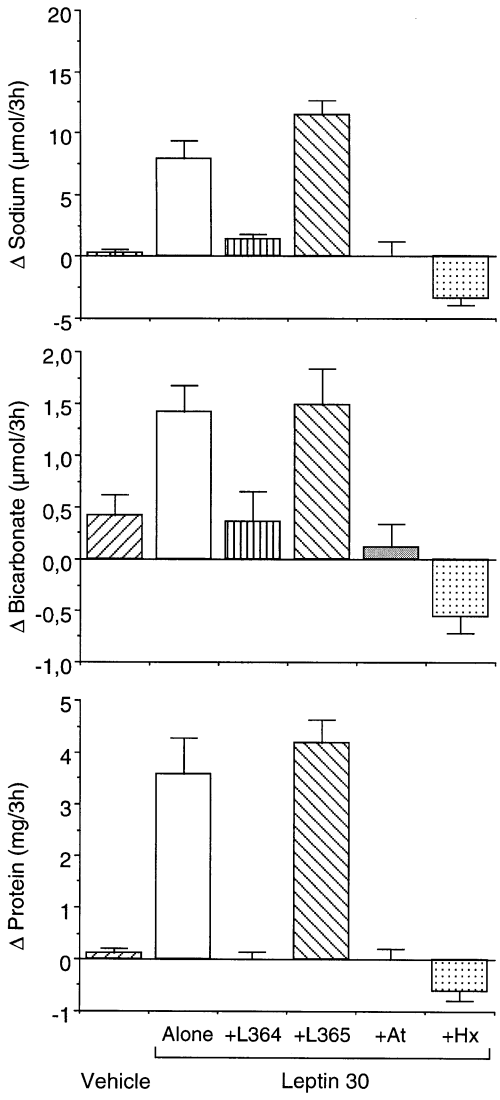

Fig. 2. Leptin effect on pancreatic secretion involves CCK-1 and cholinergic receptors. The effect of 30 nmol/kg of leptin (alone) was suppressed by L364,718 (L364, 0.5 mg/kg, i.v.), by atropine (At, 100 µg/kg + 100 µg/kg h, i.v.), and by hexamethonium (Hx, 6.7 mg/kg + 6.7 mg/kg h, i.v.), but not decreased by L365,260 (L365, 0.5 mg/kg, i.v.). The results are expressed as the integrated output over the basal level during the 3 h following time zero. Mean ± S.E.M. in groups of five to six rats.

3.3. Leptin after perivagal capsaicin

Perivagal capsaicin did not affect by itself the basal pancreatic secretion, as shown in Table 1, which reports the average values observed in the capsaicin-treated groups and in control groups. However, the effect of the maximally effective dose of 30 nmol/kg of leptin was totally suppressed in capsaicin-treated rats, while it remained complete in sham-treated animals (Fig. 3).

Table 1
Average basal values in capsaicin-treated groups and related control groups

	Sodium (μmol/h)	Bicarbonate (μmol/h)	Protein (mg/h)
Vehicle	4.25 ± 0.27	1.80 ± 0.07	0.83 ± 0.05
Leptin alone	4.73 ± 0.22	1.86 ± 0.08	0.90 ± 0.08
Sham capsaicin + leptin	4.69 ± 0.43	1.84 ± 0.09	0.89 ± 0.11
Capsaicin + leptin	4.46 ± 0.50	2.03 ± 0.13	0.84 ± 0.12

Mean ± S.E.M.; $n = 6$ to 8 rats per group; no significant difference between groups by ANOVA.

3.4. Interaction of leptin and exogenous CCK

Since leptin had been currently injected as i.v. boluses, we decided to also inject CCK-8 as boluses, and a dose–response curve was first drawn. As shown in Fig. 4, a small effect was obtained with the dose of 45 pmol/kg of CCK-8; the response increased with dose up to 450 pmol/kg, which was the maximally effective dose in this model in the conditions used, and a submaximal response was obtained with 1350 pmol/kg of CCK-8, thus reproducing the biphasic dose–response curve usually elicited by CCK. In addition, as shown in Fig. 4, the effect of atropine was checked on CCK-8 stimulation, and the effect of 450 pmol/kg CCK-8 was totally blocked by atropine, indicating that in the conditions used, the effect of CCK-8 indeed occurred through the neural pathway described by Li and Owyang (1993).

The interaction of leptin with exogenous CCK-8 was studied by i.v. injecting together 3 nmol/kg of leptin (a dose

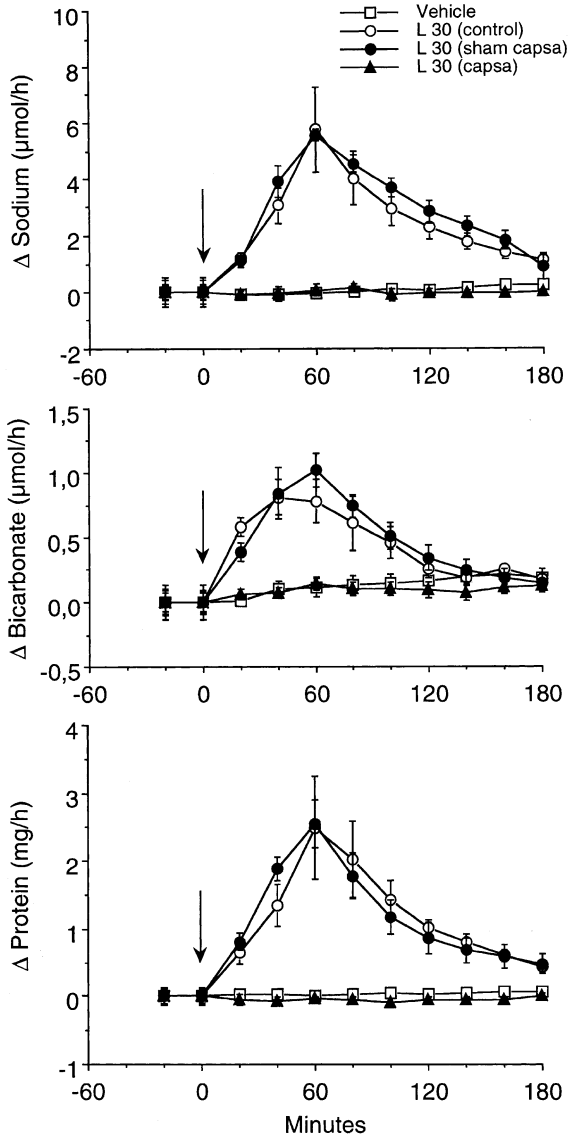

Fig. 3. The effect of leptin was suppressed by perivagal capsaicin. The effect of 30 nmol/kg of leptin was identical in sham-treated (L30 sham capsa) and in control rats (L30 control, while it was totally suppressed in capsaicin (capsa) treated rats). Mean ± S.E.M. in groups of five to six rats.

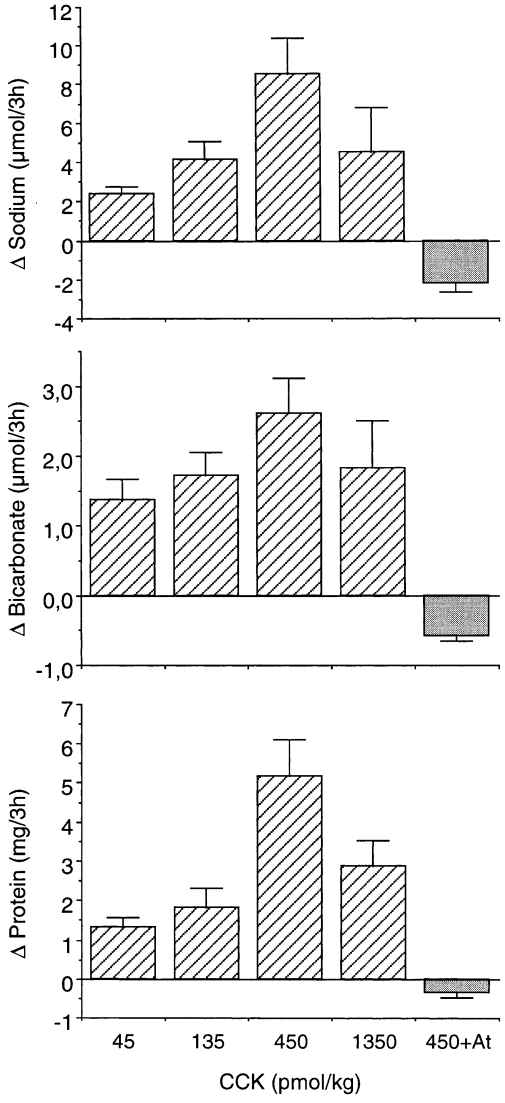

Fig. 4. Responses to CCK boluses and the effect of atropine. CCK was injected at time zero and the secretion was integrated over 3 h. The pancreatic response increased with the dose up to 450 pmol/kg, and decreased thereafter. Atropine (At, 100 μg/kg + 100 μg/kg h, i.v.) totally suppressed the effect of 450 pmol/kg of CCK. Mean ± S.E.M. in groups of five to six rats.

which had no effect per se on pancreatic secretion) and 45 pmol/kg of CCK-8 (a dose producing a slight secretory effect). In these conditions (Fig. 5), the association of CCK with leptin induced a secretory effect which was significantly larger than the effect of CCK alone, thus indicating a potentiation of the CCK effect by leptin.

3.5. Plasma levels of leptin

To determine whether the leptin dose injected with CCK-8 had some relevance to physiological situations, we measured the time course of the plasma level of leptin following the i.v. injection of 30 nmol/kg of leptin in six rats. The results were as follows: basal level (time 0): 0.5 ± 0.2 ng/ml; 20 min: 14.2 ± 1.9; 40 min: 14.1 ± 1.3; 60 min: 10.2 ± 0.8; 120 min: 7.9 ± 1.1; 180 min: 8.0 ± 0.8 ng/ml. We also measured plasma leptin 60 min after injecting 3, 10, 20, and 30 nmol/kg of leptin, and found the following regression line: plasma leptin (ng/ml) = 0.29 + 5.65 log dose (nmol/kg), which gives plasma levels around 3 ng/ml for a leptin dose of 3 nmol/kg.

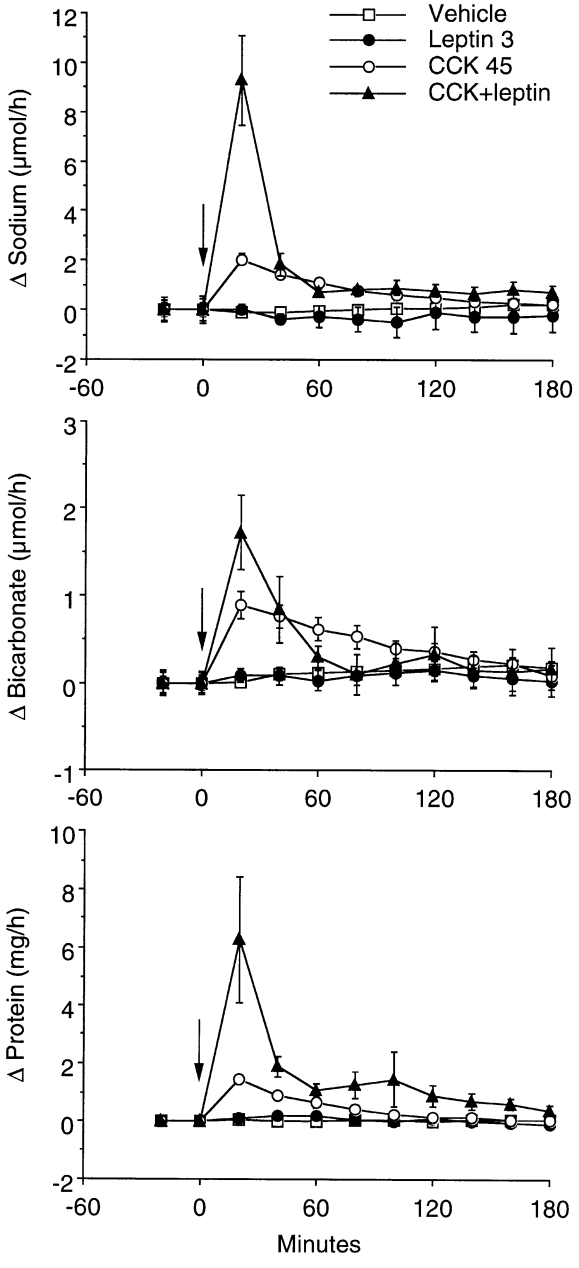

Fig. 5. Potentiation of the CCK effect by leptin. CCK (45 pmol/kg) were injected at time zero, either alone, or associated with the subliminal leptin dose of 3 nmol/kg. The peak effect of CCK was significantly larger in the presence of leptin. Mean ± S.E.M. in groups of five to six rats.

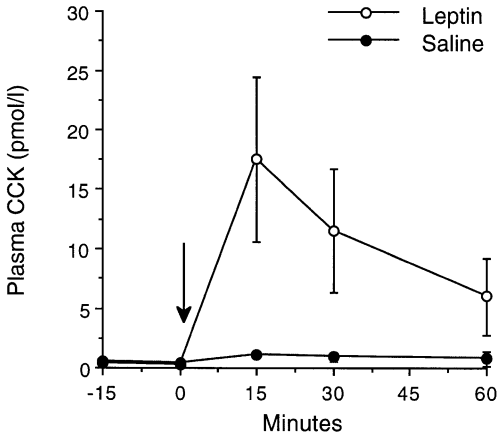

Fig. 6. Changes in plasma CCK levels after intraperitoneal injection of 6 nmol/kg leptin. Blood was collected at various times through a carotid catheter. Results are expressed as plasma CCK concentration in pM and each point corresponds to the mean ± S.E.M. of eight rats in each group. Arrow indicates the start of injection.

3.6. Leptin increases plasma CCK levels in vivo in the rat

Intraperitoneal bolus injection of leptin (6 nmol/kg) resulted in a rapid increase in plasma CCK levels (Fig. 6). This increase was maximal 15 min after the injection of leptin (17.5 ± 6.9 vs. 1.1 ± 0.2 pM; $P < 0.01$) and gradually decreased thereafter to reach a value of 6.0 ± 3.2 pM at 60 min.

4. Discussion

Regulation of endocrine pancreatic function by leptin has been extensively investigated (see Kieffer and Habener, 2000) due to the essential function of insulin in regulating adipose tissue, and to the presence of leptin receptors on Langerhans islets. Leptin directly inhibits insulin secretion in vitro as well as in vivo in leptin-deficient, *ob*/*ob* mice (Emilsson et al., 1997; Kieffer et al., 1997). Up to now, however, only a single in vivo study has showed that leptin attenuated basal and sham-feeding stimulated pancreatic protein output in dogs, a process mainly involving neural mechanisms (Konturek and Konturek, 2000). In the pancreatic tumour AR4-2J cells in vitro, leptin was reported to reduce the sensitivity of CCK-induced amylase release, an

effect which was paradoxically associated with an enhanced intracellular calcium mobilisation response stimulated by CCK (Harris et al., 1999). Together, these data suggest that exogenous leptin could modulate exocrine pancreatic secretion.

The present in vivo study demonstrates that exogenous recombinant murine leptin significantly stimulated exocrine pancreatic secretion in rats. Venous injection of bolus doses of leptin induced a long-lasting (about 3 h) stimulation of water, bicarbonate, and protein (i.e. exocrine enzymes) in pancreatic secretion. The maximal effect (observed after 30 nmol/kg of leptin, and calculated from the detailed data used in Figs. 1 and 4) represented about 50% of pancreatic response to the maximally active dose of 450 pmol/kg of CCK-8 in the same model. The pancreatic response to leptin was moderately dose-related between 10 and 100 nmol/kg, and the response to 100 nmol/kg was slightly smaller than that to 30 nmol/kg, so that the dose relationship to leptin was to some extent biphasic, resembling that of CCK. We have no clear explanation for these discrepancies with the previous in vivo study of Konturek and Konturek (2000) in dogs, but it is likely that the model, the experimental conditions, and the doses of leptin used in our study could account for these differences.

Measuring immunoreactive circulating leptin has indicated blood leptin varying from 3 ng/ml plasma leptin after 3 nmol/kg to 10 ng/ml after 30 nmol/kg. Postprandial levels from 3.7 to 9.8 ng/ml of leptin have been reported in the rat (Attoub et al., 1999; Sarraf et al., 1997; Wu-Peng et al., 1997), so that the leptin levels producing a direct pancreatic effect in the present study generate similar circulating levels of leptin to the postprandial rise in plasma leptin in the rat. Thus, we suggest that leptin may be involved in the physiological regulation of pancreatic secretion. The mechanism is also likely to be complex since the maximal pancreatic response (to 30 nmol/kg of leptin) did not parallel the immunoreactive circulating leptin level: 3 h after leptin injection, the pancreatic response returned to basal, while the leptin blood level remained elevated at 8 ng/ml.

Efficient digestion of meal nutrients relies for a great part upon pancreatic enzyme and bicarbonate secretions, which are controlled by intricated hormonal and neural mechanisms. Neural control depends mainly on vagal cholinergic and noncholinergic stimulation (Chariot et al., 1990), while among the several hormones that stimulate (CCK, secretin, and gastrin-releasing peptide) or inhibit (somatostatin, pancreatic polypeptide, and peptide YY) exocrine pancreatic secretion, CCK probably plays a central role. It may be appropriate to recall at this point that in pharmacological doses, CCK can act directly on pancreatic acinar cells, while in physiological doses, CCK stimulates a neural circuitry involving CCK_1 receptors of capsaicin-sensitive vagal afferent neurones, central synapses, and vagal cholinergic efferent fibres (Li and Owyang, 1993). We verified herein that in the conditions we used, the CCK effect was suppressed by atropine, and thus neurally mediated.

Analysing the mechanism of leptin effect in the present experiments showed that leptin-induced pancreatic stimulation was suppressed by L364,718, a CCK_1, and the suppression of leptin-induced exocrine pancreatic secretion by capsaicin-induced inactivation of sensory nerves can be compared to previous data showing that exogenous leptin exerts a potent gastroprotective action depending upon intact sensory nerves in rat receptor antagonist, by capsaicin and by atropine. In addition, (Konturek et al., 2001a). The mechanism of leptin stimulation seems very similar to that of CCK stimulation. Leptin might either act like CCK and/or potentiate the effect of endogenously released CCK. Leptin receptors (long Ob-Rb and short Ob-Ra), isoforms, as well as the leptin-induced transcription factor STAT3 have been evidenced in the nodose ganglion in rat (Buyse et al., 2001b) and in man (Burdyga et al., 2002). In vagal trunks in the rat, the amounts of the leptin receptor immunoreactive proteins were modulated by feeding. Cholinergic vagal efferent cell bodies of the dorsal motor nucleus of the vagus express the functional Ob-Rb and STAT3 proteins (Buyse et al., 2001b; Mercer et al., 1998; Smedh et al., 1998). It is thus possible that leptin acts directly on receptors of the vagal system, especially on the afferent branches, which stay out of the blood–brain barrier. Alternatively, leptin might stimulate the release of CCK and can act through this mechanism. Indeed, in this study, we have clearly demonstrated that intraperitoneal leptin induces a rapid and substantial increase in plasma CCK levels, consistent with the expression of functional leptin receptors in

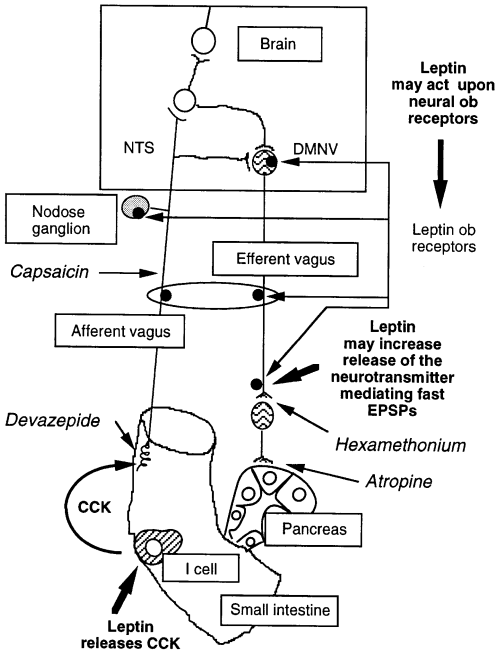

Fig. 7. Proposed mechanisms for the effect of leptin on exocrine pancreatic secretion in rats.

the small intestine (Buyse et al., 2001a; Lostao et al., 1998; Morton et al., 1998). These data together with previous reports showing that CCK increases mRNA and protein expression of gastric leptin (Bado et al., 1998; Brzozowski et al., 1999) suggest the existence of a positive feedback loop between CCK and leptin. The exact function of this loop requires further investigations.

We also observed that a small dose of leptin, which produced a physiological circulating blood level, and had no effect per se, potentiated the effect of a small dose of exogenous CCK-8. These data are very close to those results showing that leptin and CCK interact synergistically to induce an early inhibition of food intake, an effect further demonstrated to be critically dependent on CCK_1-receptor subtype and capsaicin-sensitive vagal fibres (Barrachina et al., 1997). They are also close to the suggestion that leptin can act synergistically with CCK to protect the pancreas against noxious agents and to maintain tissue integrity in rats (Konturek et al., 2001b).

Sha and Szurszewski (1999) have shown by intracellular recordings of dog pancreatic neurones that fast excitatory post-synaptic potentials induced by presynaptic stimulation in isolated pancreas, which were subthreshold in normal Krebs solution, reached threshold for firing action potentials in the presence of leptin. This effect was due to leptin action at presynaptic sites to facilitate release of the neurotransmitter mediating fast transmission (which was presumably acetylcholine), and shows that leptin receptors should indeed be present at presynaptic sites on efferent vagal neurones in the pancreas. This might satisfactorily explain how CCK-induced activation of afferent vagal, then of preganglionic efferent neurones innervating the pancreas, could lead to an increased post-ganglionic atropine-sensitive pancreatic response to CCK in the presence of leptin in our model. We have brought together these interpretations in the suggested mechanism schematised in Fig. 7. Leptin could both act directly on vagal receptors, and also release CCK and potentiate the effect of circulating CCK through neural mechanisms. However, the present experiments cannot determine which of these possible mechanisms was predominant in our experimental conditions.

In conclusion, leptin increases basal and CCK-stimulated exocrine pancreatic secretion in anaesthetised rats through reflex cholinergic stimulation involving capsaicin-sensitive vagal afferent fibres. This leptin effect seems to involve endogenously released CCK operating through CCK_1 receptors. Whether this may have a physiological relevance in the postprandial regulation of exocrine pancreatic secretion and thus in nutrient digestion will require further investigations.

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