GLUCAGON-LIKE PEPTIDE-1 (GLP-1) AND SATIATION

ABHANDLUNG
zur Erlangung des Titels

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1 Summary

Signaling molecules that are released from several peripheral tissues, including the gastrointestinal tract, the pancreas and the adipose tissue, in response to a variety of physiological stimuli are involved in the control of food intake. One of these is glucagon-like peptide-1 (GLP-1), which is released by intestinal L-cells in response to preabsorptive nutrient stimuli. GLP-1 is rapidly degraded in the plasma by dipeptidyl peptidase-IV (DPP-IV), so that a steep concentration gradient exists, with highest concentrations in the lamina propria of the intestinal mucosa, intermediate levels in the hepatic portal vein (HPV), and lowest levels in the systemic circulation. GLP-1’s most extensively examined function is its incretin effect, i.e., the regulation of postprandial glucose metabolism via the stimulation of insulin secretion. Increasing evidence suggests that GLP-1 is also involved in the control of eating. At present, however, both the site of the GLP-1 receptors (GLP-1R) influencing eating and the physiological relevance of endogenous intestinal GLP-1 for eating are not clear. Moreover the pattern and magnitude of GLP-1 release in response to meals in rats are not known. These three questions are addressed in this thesis.

In the first set of experiments we investigated the site of action of peripheral GLP-1’s eating-inhibitory effect and the role of vagal afferent signaling in it. Because it has been demonstrated that vagal afferents terminating in the hepatic region are necessary for the full effect of GLP-1 on insulin secretion, we hypothesized that GLP-1’s eating-inhibitory effect might also depend on GLP-1R in the hepatic portal vein (HPV) or liver. We tested this hypothesis, first, by comparing the potencies of HPV and vena caval (VC) infusions of GLP-1 to inhibit spontaneous eating in rats, and, second, by comparing the effects of intraperitoneal (IP) and HPV infusions of GLP-1 on eating in neurally intact rats and rats with selective subdiaphragmatic vagal deafferentations. In the second set of experiments we addressed the physiological relevance of GLP-1 in the control of spontaneous eating. To this end, we intraperitoneally infused the GLP-1R antagonist exendin (9-39) [Ex (9-39)] during meals. In the third set of experiments we measured the effect of mixed-nutrient meals and HPV infusions of GLP-1 of on the levels of the active form of GLP-1 in the HPV and VC.
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Vehicle, 0.3, 1 and 3 nmol/kg body weight (BW) GLP-1 was infused into the HPV. One and 3 nmol/kg GLP-1 potently decreased ongoing meal size, but did not significantly affect cumulative food intake or later meals. When 1 nmol/kg BW GLP-1 was infused into either VC or HPV in rats prepared with double catheters, meal size was reduced similarly by the two infusions, suggesting that the eating-inhibitory effect of GLP-1 does not depend on GLP-1R in the HPV or liver. Next, we compared the effects of HPV infusions of Veh, 0.25, 0.5 and 1 nmol/kg GLP-1 on spontaneous eating in subdiaphragmatically deafferentated (SDA) and sham operated (Sham) rats. No differences were detected between the groups. Finally, we compared the effects of HPV or IP infusions of 10 nmol GLP-1 in the two surgical groups. HPV infusions of GLP-1 again reduced ongoing meal size significantly in SDA rats, but identical IP GLP-1 infusions had no significant effect in SDA rats. This suggests that IP, but not HPV, GLP-1 may act on GLP-1R in the lamina propria of the intestinal mucosa to initiate a vagal afferent signal, whereas HPV GLP-1 may act directly in the brain to inhibit eating.

In the second set of experiments, rats with IP catheters were used. First we tested Veh, 2.5, 5 and 10 nmol/kg GLP-1. Only 10 nmol/kg reduced ongoing meal size; both 5 and 10 nmol/kg reduced ongoing meal duration. In the next experiments we tested IP infusions of Ex (9-39) during the second spontaneous nocturnal meal, similar to a situation in which others reported that Ex (9-39) increased eating. Neither 10 nor 30 nmol/kg Ex (9-39) reliably stimulated eating in our tests, however. As a positive control, we IP infused 10 nmol/kg GLP-1 alone or together with 30 nmol/kg Ex (9-39), and observed that Ex (9-39) significantly blocked the satiating effect of GLP-1. Thus, IP Ex (9-39) reaches the receptors mediating the satiating action of exogenous GLP-1, but is not able to antagonize any action of endogenous GLP-1. These findings suggest that endogenous GLP-1 is not relevant for satiation under our test conditions.

In the final set of experiments we investigated the effect of ingestion of a 3 g mixed-nutrient meal after 5 h food deprivation on active GLP-1, glucose and insulin levels sampled simultaneously from HPV and VC catheters. HPV GLP-1 levels were significantly increased at 6 and 15 min after meal onset. In contrast, VC GLP-1 levels were not significantly increased at any time. Glucose and insulin levels increased more in the HPV than in VC and remained elevated throughout the 25 min test. Last,
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HPV infusion of 1nmol/ kg BW GLP-1 was done after 3 h food deprivation, with food withheld during the test, and VC blood samples were taken. HPV GLP-1 infusion increased VC GLP-1 levels more than did the meal. Insulin levels peaked at 6 min, and glucose levels were decreased 6 and 10 min after GLP-1 infusion. These results demonstrate that nutrients rapidly elicit intestinal GLP-1 release and that GLP-1 action on insulin is limited at basal glucose values. Thus, GLP-1 is released at a time that is relevant for satiation. In addition, that HPV GLP-1 infusion increased VC GLP-1 levels is consistent with the possibility that HPV GLP-1 acts in the brain to inhibit eating.

In summary we found that HPV, VC and IP GLP-1 infusion during spontaneous meals in rats reduce ongoing meal size, consistent with a role of GLP-1 in satiation. Intravenous GLP-1 infusions may have acted on GLP-1R in the brain to inhibit eating, whereas IP GLP-1 seems to have activated receptors on vagal afferents terminating in the lamina propria of the intestinal mucosa, near the site of release of endogenous intestinal GLP-1. Finally, although GLP-1 is released rapidly after meal onset, in time to participate in satiation, we were not able to obtain evidence for a satiating action of endogenous GLP-1 using GLP-1R antagonism. The mechanisms and physiological relevance of intestinal GLP-1 in satiation during spontaneous meals in rats require further investigation.
2. Zusammenfassung


In der ersten Reihe von Experimenten untersuchten wir, wo GLP-1 wirkt, um die Nahrungsaufnahme zu hemmen sowie die Rolle von vagalen Afferenzen bei diesem Effekt. Weil gezeigt wurde, dass vagale Afferenzen aus der Gegend der Leberpforte für die volle Ausprägung des GLP-1-Effekts auf die Insulinfreisetzung notwendig sind, vermuteten wir zunächst, dass auch der Hemmeffekt von GLP-1 auf die Nahrungsaufnahme von den GLP-1R im Bereich der Leberpforte vermittelt wird. Wir überprüften diese Hypothese, indem wir zuerst den Einfluss von GLP-1-Infusionen in die HPV und die Vena Cava (VC) auf das Fressverhalten von Ratten verglichen. Anschließend führten wir intraperitoneale (IP) und HPV-Infusionen von GLP-1 bei neural intakten Ratten und Ratten nach selektiver subdiaphragmatischer vagaler Deafferentation (SDA) durch. In der zweiten Experimentalreihe untersuchten wir die physiologische Relevanz von GLP-1 bei der Kontrolle des spontanen Verzehrs. Dazu infundierten wir den GLP-1R-Antagonisten Exendin (9-39) (Ex (9-
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39)) während Mahlzeiten IP. In der dritten Experimentalreihe bestimmten wir den Effekt von Mahlzeiten mit gemischten Nährstoffgehalt und von GLP-1-Infusionen in die HPV auf die Konzentrationen der aktiven Form von GLP-1, sowie von Insulin und Glucose in der HPV und VC.

Vehikel (Veh), 0.3, 1 and 3 nmol/kg Körpergewicht (KG) GLP-1 wurden in die HPV infundiert. Ein und 3 nmol/kg GLP-1 reduzierten die Mahlzeitgrösse signifikant, beeinflussten aber die kumulative Nahrungsaufnahme oder die Grösse und Dauer nachfolgender Mahlzeiten nicht. Die Infusionen von 1 nmol/kg KG GLP-1 in die VC oder HPV von Ratten mit Kathetern in beiden Blutgefässen reduzierten die Mahlzeitgrösse gleichermassen. Dies lässt vermuten, dass der hemmende Effekt von GLP-1 auf den Verzehr nicht von GLP-1R in der HPV oder in der Leber abhängt. Als Nächstes verglichen wir den Effekt von HPV-Infusionen von Veh, 0.25, 0.5 and 1 nmol/kg GLP-1 bei spontan essenden Ratten nach SDA oder Scheinoperation (Sham). Dabei wurde kein Unterschied zwischen den Gruppen gefunden. Schliesslich verglichen wir den Effekt von HPV und IP-Infusionen von 10 nmol/kg KG GLP-1 bei in denselben zwei Operationsgruppen. GLP-1-Infusionen in die HPV reduzierten wieder die laufende Mahlzeit bei SDA-Ratten, während die gleichen Infusionen IP bei diesen Ratten keinen Effekt hatten. Das lässt vermuten, dass IP aber nicht HPV appliziertes GLP-1 auf GLP-1R in der Lamina Propria der Dünndarmmukosa wirkt und diese vagalen afferenten Signale auslöst, während HPV appliziertes GLP-1 vermutlich direkt im Gehirn den Verzehr hemmt.

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aber den Effekt von körpereigenen GLP-1 nicht antagonisieren. Dies spricht dafür, dass GLP-1 unter diesen Bedingungen für die physiologische Sättigung nicht relevant ist.

In der letzten Experimentalreihe untersuchten wir, wie sich die Aufnahme einer 3 g-Mahlzeit aus gemischten Nährstoffen nach 5 h Futterentzug auf die Konzentrationen von aktivem GLP-1, Glucose und Insulin in der HPV und VC auswirkt. Die GLP-1 Konzentrationen in der HPV waren 6 und 15 min nach Mahlzeitbeginn signifikant erhöht. Im Gegensatz dazu waren die GLP-1 Konzentrationen in der VC zu keinem Zeitpunkt signifikant verändert. Die Glucose- und Insulinkonzentrationen waren in der HPV höher als in der VC und während der gesamten 20 min des Versuchs erhöht. Schließlich untersuchten wir den Einfluss einer HPV-Infusion von 1 nmol/kg KG GLP-1 nach 3 h Futterentzug ohne Zugang zu Futter während des Versuchs. Die Blutproben wurden dabei nur aus der VC entnommen. HPV-Infusionen von GLP-1 erhöhten die GLP-1-Konzentrationen in der VC deutlich mehr als dies nach einer Mahlzeit in der HPV der Fall war. Die Insulinkonzentration erreichte ihren Höchstwert 6 min nach Infusionsbeginn und die Glucosekonzentration war 6 und 10 min nach Beginn der Infusion reduziert. Diese Resultate zeigen, dass Nährstoffe zu einer raschen Freisetzung von intestinalen GLP-1 führen und dass der Effekt von GLP-1 auf die Insulinfreisetzung bei basalen Glucosewerten limitiert ist. Ferner wird GLP-1 während der Mahlzeit zu einer Zeit freigesetzt, die für die Sättigung relevant ist. Zusätzlich unterstützt der Befund, dass die GLP-1-Konzentration in der VC nach Infusion von GLP-1 in die HPV erhöht war, die Möglichkeit, dass in die HPV infundiertes GLP-1 direkt im Gehirn wirkt, um den Verzehr zu hemmen.

Insgesamt stellten wir fest, dass HPV, VC oder IP-Infusionen von GLP-1 während spontanen Mahlzeiten bei Ratten die Mahlzeitgrösse reduzieren. Dieser Befund ist mit einer Rolle von GLP-1 bei der Sättigung vereinbar. Intravenöse Infusionen von GLP-1 scheinen dabei den Verzehr über eine Wirkung auf GLP-1R im Gehirn zu hemmen, während IP-Infusionen von GLP-1 dies vermutlich über Rezeptoren auf vagalen Afferenzen tun, die in der Lamina Propria der Darmmokosa enden, d.h., nahe dem Ort der Freisetzung von körpereigenem GLP-1. Obwohl GLP-1 nach Beginn der Mahlzeit rasch freigesetzt wird und somit an der Sättigung beteiligt sein könnte, waren wir letztlich nicht in der Lage, mit dem Einsatz eines GLP-1R-
Zusammenfassung

Antagonisten Beweise für eine Sättigungswirkung von körpereigenem GLP-1 zu finden. Der Mechanismus und die physiologische Relevanz von intestinalem GLP-1 für die Sättigung während spontaner Mahlzeiten bei Ratten brauchen deshalb noch weitere Abklärung.
3. General Introduction

Eating in all mammals is organized in meals, which are the product of four different functional processes: 1) hunger, in which foods are acquired and selected and eating is initiated, 2) maintenance of eating, in which intra-meal signals increase or decrease the rate of eating, 3) satiation, which causes eating to end, and 4) postprandial satiety stage, which inhibits eating during the intermeal interval (65;82;106).

The brain controls the onset and termination of meals in response to a number of signals that reflect, among others, gastrointestinal nutrient content and metabolic state. These signals include gastrointestinal and pancreatic hormones and circulating metabolites that change in response to various mechanical and chemical stimuli related to the ingested food (2;21;50;114;171). These meal-related signals are modulated by so-called “adiposity signals” that reflect the size of the body’s fat stores (166;201). The interaction of homeostatic and non-homeostatic mechanisms therefore controls energy intake and, hence, contributes to the maintenance of stable body weight (2;20;201).

This thesis concerns the physiology of one proposed mechanism of satiation, GLP-1 released from the intestines during meals. This Introduction provides a general overview of the physiology of eating to place the studies in context and provides a specific introduction of the evidence implicating GLP-1 in satiation.

3.1 Central nervous system control of eating

The brain integrates all peripheral signals to control eating. These can either be nerve signals or metabolites and hormones acting directly on the brain. Centrally acting hormones are either transported across the blood brain barrier (BBB) via specific transport mechanisms (12;31;47;82) or enter via the circumventricular organs (CVO), where the BBB is permeable. Several areas in the hindbrain and the forebrain are involved in the processing of these peripheral signals. In particular, several hypothalamic nuclei, such as the arcuate nucleus (Arc), paraventricular nucleus (PVN), ventromedial nuclei (VMH), dorsomedial nucleus (DMH), and the lateral hypothalamic area (LHA), and the nucleus tractus solitarii (NTS) in the hindbrain play...
important roles in the control of eating (18;20;20;46;113;178). Two groups of Arc neurons are involved in the integration of adiposity signals and the control of eating: Anabolic (eating-stimulatory) neurons expressing Agouti-related peptide (AgRP) and neuropeptide Y (NPY), and catabolic (eating-inhibitory) neurons expressing proopiomelanocortin (POMC), which yields alpha-melanocyte-stimulating hormone (αMSH) (82;114;171). These two groups of Arc neurons project to other hypothalamic nuclei, including the PVN, LHA, VMH and DMH (82;173) and to the NTS in the brainstem (173). The PVN contains neurons that are suppressed by NPY and stimulated by αMSH and that release anorexigenic neurotransmitters. Thus, the PVN seems to integrate mainly “catabolic information” (124;136), whereas the LHA seems to integrate mainly “anabolic information” (136). These hypothalamic nuclei, project to many other forebrain and hindbrain areas including the NTS and the dorsal motor nucleus of the vagus (DMV) in the hindbrain (19;82).

In addition to the descending projections from the hypothalamic nuclei, the NTS also receives neural and humoral peripheral signals, i.e., neural signals through cranial nerves (including the facial, glossopharyngal, and vagus nerves), or splanchnic nerves, and humoral signals, in the form of hormones and metabolites acting directly in the NTS or in the area postrema (AP), which then projects to the NTS (167). The AP is a CVO (47) and contains receptors for various hormones. Thus, circulating hormones and other substances may easily access receptors in the AP and indirectly activate NTS neurons (82). The hormones originate mainly from the gastrointestinal (GI) tract. GI hormones can act directly on the brain, i.e., in an endocrine fashion, or can trigger peripheral afferent nerve signals, mainly in the vagus nerve, either at remote sites that they reach via the blood, i.e., in an endocrine fashion, or locally, near their site of release, i.e., in a paracrine fashion. (138;192). Taste signals also enter the NTS, via the facial and glossopharyngal nerves (151).

An interesting model is the chronic decerebrate (CD) rat. In CD rats the forebrain is disconnected from the hindbrain at the level of the midbrain, so that only olfactory flavor signals reach the forebrain (174). Other sensory inputs and the neurons controlling the mouth are disconnected from the forebrain. Nevertheless, CD rats eat when liquid food is placed into their mouth and show almost normal eating responses to eating-inhibitory signals such as intraperitoneally (IP) injected cholecystokinin (CCK) (70) and glucagon-like peptide-1 (GLP-1) (78;79). This
indicates that the hindbrain is sufficient to mediate these effects. The end of a meal is reached as soon as the rat stops swallowing and lets the fluid drip out of its mouth. However, CD rats do not start eating autonomously; rather, food must be administered orally by the experimenter (69). Also, meal size in CD rats is not altered by previous fasting (91). In summary, these findings indicate that the brainstem is sufficient to mediate satiation, but that sensations such as hunger and appetite as well the reaction to food deprivation require forebrain structures (92).

### 3.2 Hormones involved in the control of eating

Hormones play an important role in the control of eating. Their release and plasma concentration reflect either nutritional status in relation to meals or adiposity status (31;201). Criteria for deciding whether a hormone is physiologically relevant for eating, are: 1) Appropriate receptors for the hormone are located at the site of action; 2) the administration of a dose that mimics the natural release pattern affects eating either alone or together with other internal or external stimuli; 3) the removal of the hormone or its receptor prevents the effect on eating, but after the replacement with a physiological dose of the hormone the effect is normalized; 4) the administration of an antagonist reaching the receptor at the site of action leads to the opposite effect (61). In addition, the release of acute satiation hormones, which contribute to meal termination, must occur during meals.

#### 3.2.1 Adiposity signals

The plasma concentrations of hormones that reflecting adiposity, i.e. are proportional to fat mass, are thought to be adiposity signals. Examples include leptin, amylin and insulin (99). These hormones are not necessarily released by fat cells themselves. While leptin is released by adipocytes (111), insulin and amylin are released by the beta-cells in the pancreas (202). Mice, rats and humans missing either leptin or its receptor develop severe obesity, which is mainly due to overeating (17;34;55;206). Also, leptin administration reduces (30;35;73;157) and leptin receptor antagonism increases eating (205). Leptin seems to reduce eating by decreasing meal size (48;57;90). Leptin crosses the BBB via a specific transport mechanism (13), and it acts in the hypothalamus, in particular in the Arc and in the DMH, to
influence food intake (13;82). It has also been shown, however, that leptin also acts
directly in the NTS.

The basal plasma levels of insulin and amylin are increased in overweight
animals. Both hormones also increase in response to meals and may be acute
satiation signals as well as an adiposity signal (202). The physiological relevance of
insulin’s role as an adiposity signal is supported by studies showing that mice lacking
insulin receptors in the CNS are obese compared to wild-type mice, that the receptor
deficient females, but not the males, overeat (82;204), that chronic infusions of small
doses of insulin targeted into the VMH decreased eating and body weight, and that
similar infusions of an insulin antibody increased food intake and body weight (122).
Insulin is able to cross the BBB by a receptor-mediated mechanism, and similar to
leptin it acts in the Arc to decrease food intake (82;150).

Peripherally and centrally acutely and chronically administered amylin
reduces food intake dose-dependently, but the lowest effective dose increases
plasma amylin to twice the endogenous levels (117;118;120;159;202). Nevertheless
the blockade of amylin receptors in the AP increases eating, supporting a
physiological relevance of amylin in the regulation of food intake (117;130;159).

3.2.2 Hormones involved in the control of meal size.

Several hormones that are released from several organs in the gut are
involved in the acute regulation of eating (31;37). In contrast to adiposity signals,
rapid changes in secretion of these hormones that occur in relation to individual
meals are thought to signal the brain. This section introduces three examples,
ghrelin, cholecystokinin (CCK), and pancreatic glucagon.

Meal-related release of GI peptides is caused by chemical and mechanical
stimuli and therefore reflects nutritional status (37). As described above, GI peptides
may act in an endocrine fashion by entering the blood stream and acting directly in
the brain or by acting locally, for example, on vagal afferents. Thus, vagally
transmitted signals can arise from GI peptides prior to their entering the blood
circulation through paracrine mechanisms, i.e., by activating receptors on afferent
nerve fibers near the site of release (12;21;37;50;51;138;201).
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Ghrelin is the only GI peptide that appears to signal hunger: Plasma ghrelin concentrations increase during fasting, and its administration increases food intake in animals and man. In particular, ghrelin may prepare the organism for the intake of a larger amount of food (37;44;63;189). Ghrelin presumably affects food intake by a direct effect in the brain (7), and not via activation of vagal afferents. Nevertheless, the physiological relevance of the eating-stimulatory effect of ghrelin remains unclear (170).

CCK is released by duodenal I-cells shortly after nutrients, in particular fats and proteins, reach the duodenum (45). CCK-8 and CCK-33, two isoforms of CCK (31), reduce food intake most potently when injected IP (61;63). Animals lacking functional CCK-1 receptors tend to increase their food intake and develop obesity (14;24;63;132;133). Also, pharmacological antagonism of peripheral CCK-1 receptors increases food intake (14;63;134) supporting the physiological relevance of CCK in the control of food intake. CCK-8 and CCK-33 probably affect food intake via different mechanisms: As CCK-33, but not CCK-8, decreased food intake when given into the HPV, CCK-33 probably affects food intake by activation of receptors on vagal afferent fibers terminating in the hepatic portal area (14;36;61;63;68;147), whereas CCK-8 presumably acts on vagal afferents terminating in the lamina propria of the intestinal mucosa (23).

Glucagon is primarily released by the alpha-cells of the pancreas in response to low blood glucose levels. However, glucagon release is also stimulated during eating, in particular in response to protein intake (202). Parenteral glucagon administration inhibits (60;203) and glucagon receptor antagonism by specific antibodies stimulates eating (107), supporting the physiological relevance of glucagon’s satiating effect. Glucagon probably affects eating by activating receptors on vagal afferent fibers terminating in the hepatic region (61;63;202).

3.3 GLP-1

Glucagon was already described by using bioassays in the early 1920s. In the late 1960s it was observed that glucose triggers the release of substances that are very similar to glucagon. These substances were called “glucagon-like” (190;193). One of these substances was later described to be glucagon-like peptide-
1 (GLP-1) (16). GLP-1 is a hormone derived from the pro-glucagon gene that also encodes other hormones, such as glucagon, glycentin and oxyntomodulin. The post-translational processing of the pro-glucagon is mediated by different enzymes and is tissue-specific (11). As mentioned above, GLP-1 is not just released in the periphery but also in the brain.

3.3.1 Central GLP-1

GLP-1 in the brain is released solely from neurons in the NTS (82;86;194). GLP-1 neurons project to many brain areas, including the other structures of the dorsovagal complex, the thalamus, the pituitary, the hypothalamic PVN and DMH, and the central nucleus of the amygdala (129;163;194). The GLP-1 receptors (GLP-1R) in these areas may be affected by peripheral as well as by central GLP-1. Peripheral GLP-1 may enter the brain by passing the BBB through CVO (145), although an active transport process has also been described (93). GLP-1R are widely distributed across the entire brain. Although earlier data suggested central GLP-1 mediated aversions, such as some forms of illness anorexia, the physiological relevance of central GLP-1 in controlling normal eating is suggested by the demonstrations that the administration of GLP-1 into the 3rd ventricle potently inhibited, whereas administration of the GLP-1R antagonist Ex (9-39) stimulated eating (125;188). Central GLP-1 also decreases blood glucose levels, but the effects of GLP-1 on food intake and glucose metabolism seem to be mediated by different hypothalamic nuclei (162). That is, the Arc seems to mediate the effect of GLP-1 on glucose metabolism, whereas the PVN seems to mediate its satiating effect (162).

3.3.2 Peripheral GLP-1

Peripheral (or intestinal) GLP-1 is released in response to nutrient stimulation by the L-cells, which are most common in the ileum in rats and humans (27;29;40;52;86;86), but are also dense in the proximal colon in some species, including humans (29;52;97). After its release into the lamina propria of the intestinal mucosa, GLP-1 enters either intestinal capillaries, which drain into the HPV (38) or into the lymph. GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-IV), resulting in a short biological half-life in blood of 1-2 min as measured in
humans (40). Because of this rapid degradation, a steep concentration gradient is observed, with the highest GLP-1 concentration in the intestinal capillaries close to the site of release, intermediate levels in the HPV, and almost no active GLP-1 in the general circulation after passage through the liver (180). Therefore, intestinal GLP-1 presumably exerts its effects mainly via receptors prior to the liver (40;86). GLP-1’s incretin effect seems to be physiologically relevant. It has been demonstrated that GLP-1: 1) increase insulin release (10;49;103), 2) decrease hepatic gluconeogenesis by inhibiting glucagon secretion (112) and 3) have a long-term protective effect on pancreatic islets (146;156;182). Also, the parenteral administration of GLP-1 antagonists exendin (9-39) (Ex (9-39)) or des-His(1),Glu(9) exendin-4 (dHEx)) decreased insulin release (59;98;191), and GLP-1R-deficient mice display hyperglycemia and low plasma insulin levels (169). The therapeutic use of GLP-1 or its analogs to treat type II diabetes takes advantage of these effects. Long-acting GLP-1 analogs and substances which block GLP-1 degradation are currently among the most promising antidiabetic treatments (66). One advantage of this approach is that patients treated with such drugs do not experience hypoglycemic states because GLP-1 needs a certain blood glucose level to exert its action. In addition, GLP-1 seems to have cardio-protective effects and does not appear to loose much of its weight-reducing effect due to the reduction of food intake with chronic administration (95). In human patients it has been observed that diabetes improves significantly after bariatric surgery (28) even before weight loss occurs (149;164). Best effects are observed after a roux-en-y gastric bypass (RYGB) (28;186). RYGB combines a restricted stomach volume and the restriction of a short segment of proximal intestine (186). The positive effect on diabetic state is related to elevated GLP-1 levels in the plasma of human patients (26;28;100;101;104;110;135;154;186;196). The elevated plasma GLP-1 levels are not observed when only the stomach volume is restricted (186). Thus this increase is probably due to the accelerated nutrient contact to the intestinal L-cells (186).

### 3.3.3 Effect of GLP-1 on food intake

In humans, non human primates, rats, mice and many other species GLP-1 the GLP-1R agonist Exendin (4-39) (Ex-4) have been shown to reduce food intake when given acutely or chronically, via IV, IP, subcutaneously, or ICV routes
General Introduction

(1;32;43;56;78;96;108;121;155;162;168;185;188;199). GLP-1 administered ICV. (125;185;188) or IV (32;96) seems to inhibit food intake most potently while subcutaneous (155) application only gives minor effects. The mechanisms of the eating-inhibitory effect remain unclear. However, it has been excluded that this effect is caused by inhibited gastric emptying (32) or aversion (121).

None of the studies cited above, however, tried to mimic the physiological release pattern of GLP-1 in rats. Thus, at least one of the criteria for the physiological relevance of GLP-1 has not been confirmed in rats. Moreover, so far there is only a single report of increased in food intake after peripheral administration of Ex (9-39), and this effect was only seen in a rather unusual feeding regimen (200). In another experiment in which Ex (9-39) was administered peripherally, no significant effect on food intake occurred (32;96) As reviewed above, however, central administration of Ex (9-39) increases eating (125;188). Finally, GLP-1R knock-out animals do not display obesity or a change in eating behavior (169). Taken together, these studies leave in question whether peripheral GLP-1 fulfills the antagonism criterion for physiological relevance. All in all, therefore, the physiological relevance and mechanisms of peripheral GLP-1’s satiating action are still unclear and needs further research.
4 Aim of this Thesis

Despite the strong evidence for the physiological relevance of GLP-1’s effect on glucose metabolism, its role in the control of eating remains unclear. Several open questions in this context warrant investigation: 1) It is unclear how exogenous GLP-1 affects food intake when administered at a physiologically relevant time point during spontaneous meals in rats. 2) The short-term changes in the plasma concentration of GLP-1 in response to a mixed-nutrient meal in the rat are not known. 3) It is not clear where peripherally administered exogenous GLP-1 or endogenous intestinal GLP-1 act to reduce food intake. And 4) in order to critically examine the physiological relevance of GLP-1’s eating-inhibitory effect, the effect of pharmacological GLP-1R antagonism on eating should be investigated under nearly physiological conditions, i.e., in relation to spontaneous meals in freely eating animals.

We designed several experiments to examine these questions: In a first series of experiments we tried to mimic the physiological, meal-contingent release of GLP-1 by short, intrameal infusions of GLP-1 into the HPV via chronic HPV catheters. Then, to identify the site of action of peripheral GLP-1, we performed two experiments. A) To address the question of a possible hepatic portal site of action, we used animals with chronic VC and HPV catheters, and compared directly the effects on eating of short, intrameal VC and HPV GLP-1 infusions. B) Further, to test whether abdominal vagal afferent signaling is involved in mediating GLP-1’s eating-inhibitory effect, we compared the effect of short, intrameal HPV and IP infusions of GLP-1 in rats after subdiaphragmatic vagal deafferentation (SDA) or sham-surgery (Sham). In a second series of experiments, we first established the effect of short, meal-contingent IP infusions of GLP-1 on eating, and then examined the effect of GLP-1R antagonism on eating and on the satiating effect of exogenous GLP-1 by IP infusing Ex (9-39) alone or together with GLP-1 under the same conditions. In a last series of experiments we measured plasma levels of GLP-1, glucose and insulin in the HPV and VC during and after a mixed-nutrient meal. Finally, we infused into the HPV a dose of GLP-1 that reliably inhibited eating and assessed GLP-1’s effect on GLP-1, glucose and insulin concentrations in the VC.
5 Intrameal Hepatic-Portal and Intraperitoneal Infusions of Glucagon-Like Peptide-1 (GLP-1) Reduce Spontaneous Meal Size in the Rat via Different Mechanisms.

Based on Ruttimann et al. Endocrinology, 150:1174-1181, 2009

5.1 Introduction

GLUCAGON-LIKE PEPTIDE-1 (7–36)-amide (GLP-1) is a gut hormone synthesized and released in response to intraluminal nutrient stimuli by the L-cells of the distal ileum and colon, and a brain neurotransmitter synthesized and released by neurons in the nucleus tractus solitarii (NTS) (86). GLP-1 appears to act at a number of sites to elicit a variety of physiological or pharmacological effects (86). Both peripheral and central GLP-1 have been implicated in the control of eating (37;119;180). Rather little is known, however, about the physiological significance, sites of action, or neural mechanisms of GLP-1’s actions on eating.

GLP-1 released from the intestinal L-cells enters the systemic circulation rapidly via the intestinal capillaries draining into the hepatic portal vein (HPV) (87) and more slowly via the lymph (38). GLP-1 is quickly degraded by DPP-IV in the intestinal capillaries and in the liver, so that following release from intestinal L-cells, the largest GLP-1 concentrations occur in the intestinal submucosal extracellular space, intermediate levels are found in the HPV, and comparatively small concentrations occur in the systemic circulation (87). Unfortunately, as yet there are only few data regarding prandial concentrations of GLP-1 in the systemic circulation and none in the HPV or brain. GLP-1 may act at a variety of sites to inhibit eating, as GLP-1 receptors are expressed in several loci that have been implicated in the control of eating, including the arcuate (Arc) and paraventricular (PVN) nuclei of the hypothalamus (67;128) as well as in several peripheral sites, including the pancreatic islets (71), most of the gastrointestinal tract (86), and vagal afferent fibers terminating in the HPV (10;191).

In humans, oral loads of carbohydrates or fats increased systemic plasma GLP-1 levels within 5-15 min (39;195) and intravenous infusion of physiological doses of GLP-1 during meals were sufficient to increase the perception of satiety
and to reduce food intake (15;56;72). In rats the situation is less clear. Systemic plasma GLP-1 increases within 15 min after intragastric administration of Ensure (Abbott Laboratories, Abbott Park, IL, USA) (38) or triglyceride emulsions (88). Neither systemic plasma levels of GLP-1 after orally ingested meals nor prandial levels of GLP-1 in the HPV, however, have been reported in rats. Furthermore, as yet there are no published reports that acute GLP-1 antagonism during meals can increase meal size. Thus, whether GLP-1 has a normal physiological satiating action in rats remains uncertain.

Injections of GLP-1 into the lateral, third or fourth cerebral ventricles, or direct injections into the paraventricular hypothalamic nucleus (PVN) have also been reported to inhibit eating in rats (79;162;185), and GLP-1 receptors are expressed in these areas (67;128), suggesting that intestinal GLP-1 might act directly in the brain to elicit satiation. Again, however, it is not known whether, or in what amount, peripheral GLP-1 reaches these receptors or how concentrations after central injections and during meals compare. Turton et al., (188), however, have reported that intracerebroventricular administration of the GLP-1 antagonist Ex (9-39) does increase eating, encouraging the view that GLP-1 may act on central GLP-1 receptors to produce satiation.

Lesion studies have contributed only limited knowledge about the potential site of GLP-1’s satiating actions. Abbott et al. (1) reported that intraperitoneal (IP) injection of 100 nmol/kg GLP-1 did not significantly reduce food intake in rats with bilateral abdominal vagotomy, suggesting that IP injected GLP-1 acts locally in the gut to initiate a neural signal relayed to the brain via vagal afferents to inhibit eating. However, total subdiaphragmatic vagotomy ablates vagal efferents as well as vagal afferents, which is well known to have marked adverse effects on gastrointestinal motility (102). Total vagotomy has also been reported to reduce endogenous GLP-1 release (153). Both of these effects might alter the eating-inhibitory effect of peripherally administered GLP-1.

In view of the available data concerning GLP-1’s potential role in meal-ending satiation, the present experiments had three goals: First, to test whether brief, remotely controlled, meal-contingent, intrameal GLP-1 infusions affect meal patterns in spontaneously eating rats.
This has not been examined previously, and such tests have face validity as models of voluntary natural eating, and in several instances have revealed phenomena not apparent in tests of scheduled test meals in food-deprived animals (e.g. (64;183)). Second, to investigate the site of action of exogenous GLP-1 by comparing the effects of HPV, vena caval (VC) and IP GLP-1 infusions; and third, to investigate further the role of the vagus nerve in peripheral GLP-1’s eating-inhibitory effect using rats with subdiaphragmatic vagal deafferentations (SDA) (143).

5.2 Materials and methods

5.2.1 Animals and housing

Male Sprague–Dawley rats (Charles River; Sulzfeld, Germany; body weight (BW) 200-230g on arrival) were individually housed in a temperature-controlled (21 °C) colony room with a 12/12h dark/light cycle with the lights off at 1100. The rats had ad libitum access to water and ground rat chow (No. 3433, Provimi Kliba NAFAG, Kaiseraugst, Switzerland). Rat chow was left in an open bin for at least 1 d prior to use to prevent the availability of fresh chow from disrupting spontaneous eating patterns. Rats were adapted to housing conditions for 10 d before onset of experiment. All procedures were approved by the Canton of Zurich Veterinary Office.

5.2.2 Catheter implantation

Catheters were sterilized in ethylene oxide before use and implanted using sterile techniques. The custom-made headsets described before (26;28) were used except in the SDA tests, in which headsets made from 20 G (0.90 x 40 mm) surgical stainless-steel tubing (Sterican; Braun, Melsungen, Germany) bent into U-shapes were. Catheters consisted of 27 cm silastic tubing (Gore W.L., Newark, DE, USA; ID 0.508 mm, OD 0.914 mm). Connections with the headsets were shielded with 2.5 cm pieces of silicon tubing (ID 1.02 mm, OD 2.18 mm). The catheters were then led through a folded 2.5 x 3 cm square of polypropylene surgical mesh (Marlex; Bard Implants, Billerica, MA, USA) to improve adhesion to the skin and fascia.
For infection prophylaxis and analgesia, rats were pretreated with 4 mg/kg trimethoprim / 20 mg/kg sulfadoxin (Borgal 24%, Intervet, Shering-Plough Animal Health, Kenilworth, NJ), subcutaneously a few hours before surgery and anesthetized by IP injection (1.0 ml/kg) of 80 mg/kg ketamine (Ketasol-100, Dr. E. Gräub AG, Bern, Switzerland) and 4.0 mg/kg xylazine (Rompun, Bayer, Leverkusen, Germany). Atropine (0.05 mg/kg, Sintetica, Mendrisio, Switzerland) was injected subcutaneously immediately before surgery. Catheter headsets were led subcutaneously from 2-cm midline interscapular incisions to puncture wounds 1 cm rostral to the incision and exteriorized. The proximal ends were led subcutaneously from the neck to 5-cm midline laparotomies. HPV catheters were inserted into the ileocolic vein, advanced into the HPV (172;183) so that it ended 1-2 mm distal to the gastroduodenal vein, and anchored with silk suture (Silkam, 3/0, Braun, Melsungen, Germany) and Histoacryl glue (Braun, Melsungen, Germany). In one group of animals both HPV and VC catheters were implanted during the same surgery. Inferior VC catheters were implanted just rostral to the renal veins, as described by Kaufman (94), and the tips advanced 3-4 cm so that it lay near the junction of the hepatic vein. In some SDA animals, HPV catheters were implanted at the time of SDA surgery, and IP catheters 7 wk later. IP catheters ended in the peritoneal cavity, and were anchored to the serosa on the left side of the abdominal wall with silk sutures.

Skin and muscle were closed with resorbable sutures (3-0 Vicryl, Ethicon, Norderstedt, Germany). Four mg/kg trimethoprim / 20 mg/kg sulfadoxin (Borgal 24%, Intervet, Shering-Plough Animal Health, Kenilworth, NJ) and 5 mg/kg carprofen (Rimadyl, E. Gräub, Bern, Switzerland) were injected subcutaneously on the day after surgery. Catheters were flushed regularly with 0.2 ml 0.9% sterile saline and filled with 80 µl heparinized saline (100 IU heparin/ml saline; Heparin, Braun, Melsungen, Germany) between tests.
5.2.3 Subdiaphragmatic vagal deafferentation (SDA)

Rats received 4 mg/kg trimethoprim / 20 mg/kg sulfadoxin SC and 0.05 mg/kg atropine (Sintetica, Mendrisio, Switzerland) prior to surgery, were anesthetized with 5% isoflurane (Attane, Minrad Inc., Buffalo, NY, USA) in oxygen, and anesthesia was maintained with 2-3% isoflurane in 1:1 oxygen:N\textsubscript{2}O. SDA consists of a transection of the left dorsal vagal rootlets and dorsal (left) esophageal vagal trunks, resulting in complete subdiaphragmatic vagal deafferentation, while sparing about half of the abdominal vagal efferents (143). The sham procedure consisted of similarly exposing the vagal rootlets and abdominal vagus, but not further manipulating them. Rats were nursed with special diets for 1 wk postsurgery (7).

SDA were verified functionally and histologically (7). The functional test was lack of cholecystokinin (CCK) satiation, which depends on abdominal vagal afferent fibers (132;177;197). Four-h food-deprived rats were IP injected 4 µg/kg CCK-8 (Bachem, Bubendorf, Switzerland) or saline according to a crossover design. In sham-operated rats, CCK-8 reduced 30 min food intake 40-68 %. Therefore, the inclusion criterion for SDA rats was a <30% reduction in food intake. To test retrograde labeling of vagal motor neurons in the dorsal motor nucleus of the vagus (DMX) and anterograde labeling of vagal afferents in the NTS (142;148;177;197) rats were IP injected with 2 mg fluorogold (Fluorochrome, Denver, CO, USA) in 1 ml of saline. Two d later, the left nodose ganglion was exposed via a ventral midline neck incision, a glass micropipette was inserted into the ganglion nerve using a micromanipulator, 1.5 µl of 2% wheat germ agglutinin– horseradish peroxidase (HRP; Vector Laboratories, Burlingame, CA, USA) in saline was pressure injected (PicoSpritzer 3; Parker Instrumentation, Fairfield, NJ, USA) over 5–8 min, and the wound was closed. Rats were treated postoperatively as before, 2 d later anesthetized with pentobarbital (Nembutal; Abbott) intravenously, and perfused and processed as before (7). An observer blind to the rat’s surgery and behavioral data counted fluorogold-labeled neurons in the DMX and examined the HRP labeling in all NTS sections that included the area postrema (AP). Neurons projecting from this region of the DMX are contained in all branches of the abdominal vagus (142). The inclusion criteria for SDA rats were: 1) for anterograde labeling (7;143;148) that the number of labeled cells in the right DMX be <3% of the number in the left DMX; 2)
for retrograde labeling (74;143;197) that the left DMX contain some retrograde labeling as a positive control; and 3) that neither the left nor the right NTS contain any labeled fibers. Only rats that met all three criteria were included in the analysis.

5.2.4 Test Procedures

After recovery from surgery, rats were placed in custom-made, open-topped acrylic infusion cages (37x21x41 cm) with stainless-steel grid floors. A 60 W red incandescent light bulb provided dim illumination during the dark phase, and a radio music station was played continuously to mask extraneous noise. Ground chow was available ad libitum in food cups accessible via a 5 cm tunnel 5 cm above the cage floor. The cups were mounted on electronic balances (Mettler PM 3000, Greifensee, Switzerland) interfaced with a computer (Olivetti M 300, Nuremberg, Germany) in an adjacent control room, and a custom-designed program (VZM; Krügel, Munich, Germany) recorded the weights of the food cups every 30 s. Video cameras (VSS 3440; Philips, Amsterdam, The Netherlands) permitted continuous observation of the rats.

At 0830 h catheters were connected to infusion pumps (A99; Razel, Stamford, CT, USA) via two segments of polyethylene tubing (0.76 mm ID, 1.22 mm OD; Portex, Hythe, UK). The lower segment of tubing was sheathed with a stainless-steel spring fixed to a swivel joint 45 cm above the cage floor, thus allowing the rats to move freely. The infusion pumps were operated by remote control from the control room. Infusions (0.2 ml/min) started 2–3 min after the onset of the first spontaneous nocturnal meal and lasted for 5 min unless stated otherwise. If a meal was under way at dark onset (1100 h), the infusion was done during the next meal. The criteria for meal onset were a 0.2 g decrease in the food cup weight and visual verification of eating. Catheters were detached at 1500 and rats were weighed and food cups refilled between 1700-1900 h.

Within-subjects crossover designs were used in each experiment. GLP-1(7-37)-amide (Bachem, Bubendorf, Switzerland) was dissolved in PBS (Gibco, Basel, Switzerland) with 1% BSA (Sigma, Buchs, Switzerland); control infusions were vehicle. Infusions were given in random order on consecutive days. Pilot experiments (data not shown) were used to determine GLP-1 doses that were near-
Effect of Short Intrameal Infusions of GLP-1 on Spontaneous Meal Size

threshold and moderately supra-threshold and to insure that infusion of such doses did not have carry-over effects on subsequent days’ eating under our conditions.

Catheter patency was tested after experiments by infusing 0.8 ml/kg of a mixture of ketamine (26 mg/kg) and xylazine (0.9 mg/kg). Rats that did not loose muscle tone completely within 1 min were excluded from analysis.

5.2.4.1 Satiating effect of HPV GLP-1.

To test whether intrameal HPV infusion of GLP-1 has an acute satiating effect during the first spontaneous nocturnal meal, rats (n = 14, BW, 343-403 g at test onset), received doses of 0 (vehicle), 0.33, 1.0, or 3.0 nmol/kg BW GLP-1, prepared as described above.

5.2.4.2 Comparison of HPV and VC GLP-1.

To test whether GLP-1 acts in the liver to inhibit eating during the first spontaneous nocturnal meal, rats (n = 16, BW, 280-402 g at test onset) received HPV or VC infusions of 0 or 1.0 nmol/kg BW GLP-1.

5.2.4.3 SDA Experiment.

The necessity of vagal afferent signaling for the eating-inhibitory effects of HPV and IP GLP-1 was tested. First, 7 sham-operated and 9 SDA rats (BW, 306-418 g at test onset) received HPV infusions of 0, 0.25, 0.5 and 1.0 nmol/kg BW GLP-1 during the first spontaneous meal of the dark phase. The GLP-1 doses were modified slightly from those used in the first tests in an attempt to obtain a graded dose-response relation, as described below. Seven weeks later, 6 sham-operated and 9 SDA rats (BW 422-603 g at test onset) were equipped with additional IP catheters and, after recovery, received IP or HPV infusions of 0 or 10 nmol/kg BW GLP-1 (2.5 min infusions at 0.2 ml/min by each route in random order on 4 consecutive days). The larger dose was selected on the basis of pilot tests and the literature (22;37), which suggested that GLP-1 doses sufficient to inhibit eating when infused intravenously would not suffice when administered IP.
5.2.5 Data analysis

Meals were defined as food removals ≥ 0.1 g, with the interval between removals ≤ 15 min. Sizes and durations of spontaneous meals during the test period, intermeal intervals (IMI, duration between the end of one meal and the beginning of the next), and first-meal satiety ratios (meal size/subsequent intermeal interval) were analyzed with repeated-measures ANOVA (SAS 0.1.3, SAS Institute, Cary, NC, USA) and Bonferroni-Holm (83) pairwise comparisons. Data sets that did not meet the ANOVA criteria of normality and equal variance were transformed to logarithms for ANOVA. To identify outliers, data were converted to standard scores using the medians and median absolute deviates x 1.48 (which estimates the standard deviation), and values > 2.57 (i.e., P < 0.01) were excluded. Differences were considered significant when P < 0.05.
5.3. Results

5.3.1 Satiating effect of HPV GLP-1.

HPV infusions of 1.0 and 3.0, but not 0.33, nmol/kg GLP-1 reduced the size of the first spontaneous dark-phase meal compared to vehicle infusions ($F_{3/39} = 9.19$, $P < 0.001$) (Fig. 1). No significant dose-response relation was detected; i.e., the effects of infusions of 1.0 and 3.0 nmol/kg GLP-1 did not differ reliably ($P > 0.05$). Meal duration was also reduced by HPV GLP-1 (Table 1). The IMI after the first meal, the corresponding satiety ratio, the size of the second meal, and cumulative food intakes 2, 4, 6 and 22 h after the beginning of the first meal were not significantly affected by any dose of GLP-1 (Table 1). First meal size and meal duration data are from 11 rats that passed the catheter patency test. The number of data points for IMI and second meal size on Figure 1 is smaller than for first meal size because some rats did not consume a second meal before the infusion pumps were detached.

Table 1: Effects of intrameal HPV GLP-1 infusions during the first spontaneous meal of the dark on meal duration, satiety ratio, and cumulative food intake.

<table>
<thead>
<tr>
<th>GLP-1 dose (nmol/kg)</th>
<th>0</th>
<th>0.33</th>
<th>1.0</th>
<th>3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal duration (min)</td>
<td>9 ± 1.1</td>
<td>8 ± 1.3</td>
<td>5 ± 0.5*</td>
<td>4 ± 0.4*</td>
</tr>
<tr>
<td>Satiety ratio (min/g)</td>
<td>47.9 ± 6.2</td>
<td>55.6 ± 6.0</td>
<td>66.2 ± 9.7</td>
<td>51.6 ± 14.0</td>
</tr>
</tbody>
</table>

Cumulative food intake (g)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0 ± 0.2</td>
<td>3.6 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>6.7 ± 0.3</td>
<td>6.7 ± 0.1</td>
<td>6.5 ± 0.3</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>10.3 ± 0.5</td>
<td>11.1 ± 0.4</td>
<td>11.1 ± 0.4</td>
<td>10.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>25.4 ± 0.7</td>
<td>23.5 ± 0.3</td>
<td>23.7 ± 1.0</td>
<td>22.9 ± 1.2</td>
</tr>
</tbody>
</table>

Meal durations are medians ± SEM from 11 rats. * Different from 0 value, Bonferroni-Holm test (83) after significant ANOVA, $F_{3/22} = 5.47$, $P < 0.01$. 
Figure 1: Intrameal hepatic portal vein (HPV) infusions of 1 or 3 nmol/kg GLP-1 reduced the size of the first spontaneous dark-phase meal compared to vehicle infusions (F$_{3.29}$ = 9.19, p < 0.001) (A). The duration of the following intermeal interval (IMI) (F$_{3.21}$ = 2.08, P > 0.05) (B), and the second meal size (F$_{3.22}$ = 1.55, P > 0.05) (C) were not significantly affected by GLP-1 infusions. Values are means ± SEM of 11 (A) or 10 (B, C) rats. *P < 0.05 vs 0 dose, Bonferroni-Holm test (83) after significant ANOVA.
5.3.2 Comparison of HPV and VC GLP-1.

Both HPV and VC GLP-1 infusions of 1 nmol/kg GLP-1 reduced the size of the first spontaneous dark-phase meal, with no significant difference between the effects of HPV and VC infusion detected (Fig. 2). The ANOVA results for the main effects of GLP-1, route of administration, and their interactions were $F_{1/22} = 46.34$, $p < 0.0001$, $F_{1/22} = 1.59$ and 1.68, $Ps > 0.05$, respectively. Neither HPV nor VC GLP-1 infusion significantly affected other spontaneous eating parameters or cumulative food intake (data not shown). Data are from 12 rats that passed the catheter patency test.

![Figure 2](image)

**Figure 2:** Intrameal vena cava (VC) and hepatic portal vein (HPV) infusions of 1 nmol/kg GLP-1 reduced the size of the first spontaneous dark-phase meal similarly. Values are means ± SEM from 12 double-catheterized rats. *P < 0.05 vs 0 dose, Bonferroni-Holm test (83) after significant ANOVA.
5.3.3 SDA.

Data from 3 SDA rats had to be excluded because 2 failed the HPV catheter patency test and one failed the two criteria for complete SDA. HPV infusions of 0.5 and 1.0, but not 0.25, nmol/kg GLP-1 reduced the size of the first spontaneous dark-phase meal in both sham-operated and SDA rats, and no significant differences in the effects of GLP-1 in the two surgical groups were detected (Fig. 3). The ANOVA results for the main effects of GLP-1, surgical group, and their interaction were $F_{3/33} = 7.60, P < 0.001$, $F_{1/12} = 0.11, P > 0.05$, and $F_{3/33} = 0.07, P > 0.05$, respectively. Other spontaneous eating parameters and cumulative food intake were not affected significantly in either surgical group (data not shown).

![Figure 3](image)

**Figure 3:** Intrameal hepatic portal vein (HPV) infusions of 0.5 or 1.0 nmol/kg GLP-1 reduced the size of the first spontaneous dark-phase meal similarly in rats after subdiaphragmatic vagal deafferentation (SDA, $n = 7$) or sham surgery (Sham, $n = 7$). *$P < 0.05$ vs 0 dose, Bonferroni-Holm test (83) after significant ANOVA.
Effect of Short Intrameal Infusions of GLP-1 on Spontaneous Meal Size

spontaneous dark-phase meal in both sham-operated and SDA rats (Fig 3). In contrast, IP infusions of 10 nmol/kg GLP-1 reduced first spontaneous dark meal size in sham-operated rats, but not in SDA rats, with the difference in GLP-1’s effects significantly different between the two surgical groups (Fig. 4). The ANOVA results for the main effects of GLP-1, surgical group, and their interaction were \( \left( F_{2/18} = 20.53, P < 0.0001; F_{1/13} = 0.04, P > 0.05; F_{2/18} = 4.24, P < 0.05 \right) \), respectively. Again, other spontaneous eating parameters and cumulative food intake were not affected significantly in either surgical group (data not shown).

**Figure 4:** Intrameal intraperitoneal (IP) infusion of 10 nmol/kg GLP-1 reduced the size of the first spontaneous dark-phase meal in sham-operated rats (Sham, n = 6), but not in rats with subdiaphragmatic vagal deafferentation (SDA, n = 9). In contrast, hepatic portal vein (HPV) infusions of this dose of GLP-1 similarly reduced meal size in both surgical groups. *P*<0.05 vs vehicle; + *P* < 0.05, (vehicle – IP GLP-1) difference in sham-operated rats vs. (vehicle – IP GLP-1) difference in SDA rats; Bonferroni-Holm tests(83) after significant ANOVA.
5.4 Discussion

Here we tested for the first time the effects of intrameal peripheral infusions of GLP-1 during spontaneous meals in rats. Our data demonstrate that brief, intrameal peripheral infusions of GLP-1 selectively reduce the size of ongoing meals in rats. In addition, HPV and VC GLP-1 infusions reduced meal size similarly, suggesting that GLP-1’s eating-inhibitory effect does not originate in the hepatic portal area or in the liver. Finally, SDA eliminated the eating-inhibitory effect of IP, but not of HPV, infusions of GLP-1, indicating that circulating GLP-1 does not require vagal afferent signaling to inhibit eating and that different sites and mechanisms mediate the satiating actions of IP and HPV GLP-1.

Independent of whether infused HPV, VC, or IP, intrameal administration of 0.5 – 10 nmol/kg GLP-1 potently and selectively reduced the size of the ongoing meal without affecting the size of subsequent meals or the duration of the subsequent IMIs and, hence, meal frequency. Cumulative food intake was also unaffected. These results extend earlier reports of reduced food intake in response to IP (1;199) and intravenous (32;108) GLP-1 by showing that GLP-1 can reduce the size of ongoing meals, i.e., elicit satiation.

Our GLP-1 infusion procedure was designed to model only the initial secretion of GLP-1 during meals, without considering continued postprandial secretion. Intrameal plasma levels of GLP-1 have not been reported, and we were technically unable to match the plasma levels of GLP-1 after either HPV or VC infusions to the levels during meals. Therefore, whether the satiating effects of GLP-1 we report here represent a physiological action is uncertain. Nevertheless, it is interesting to note that the threshold HPV GLP-1 dose under our conditions, which appeared to be just under ~0.5 nmol/kg, is small in comparison to doses used in most studies of peripheral GLP-1’s acute effect on food intake (1;37;79;108;180;185;188). Chelikani et al. (32), however, reported that the threshold dose for a reduction of 1-h cumulative food intake (their first
Effect of Short Intrameal Infusions of GLP-1 on Spontaneous Meal Size

measurement) during continuous intrajugular GLP-1 infusion was between 5 and 17 pmol/kg-min, or about 0.3 - 1 nmol/kg, which is similar to our results.

It has also been proposed that GLP-1 might act as an “across meal satiating signal” (168). As we did not test infusions of GLP-1 that continued during the IMI, our data do not address this hypothesis and there are few relevant data in the literature. Intramuscular injections of the long-lasting GLP-1 agonist exendin-4 (Ex-4) had a selective effect on meal size in a 6-h test in rhesus monkey (168). On the other hand, Chelikani et al. (32) reported that 3-h intrajugular infusions of GLP-1 reduced meal frequency in addition to meal size. In this study, however, all of the effective doses reduced meal size before they affected meal frequency. Thus, further work on this hypothesis is clearly warranted.

SDA attenuated the satiating effect of IP GLP-1, but did not affect the satiating effect of HPV GLP-1. This indicates that the former, but not the latter, effect depends on abdominal vagal afferent signaling and suggests that GLP-1 inhibits eating after HPV and IP administration by activating at least partly different mechanisms. The failure of conservatively verified SDA to attenuate the satiating effect of HPV GLP-1 across a wide dose-range (0.5 – 10 nmol/kg) strongly indicates that vagal afferent signaling is not involved in this action of GLP-1. In contrast, our finding that SDA blocked the satiating effect of IP infused GLP-1 confirms and extends previous reports that the eating-inhibitory effects of IP GLP-1 in rats (1) or Ex-4 in mice (184) were reduced by complete subdiaphragmatic vagotomy or by subcutaneous capsaicin administration, respectively. However, both these procedures are less selective than SDA: systemic capsaicin causes degeneration of vagal and non-vagal small diameter unmyelinated sensory neurons (58), and total subdiaphragmatic vagotomy destroys vagal efferents in addition to afferents and, hence, leads to substantial side effects (102) due to disturbances in gastrointestinal motility and secretion. Moreover, vagal afferents and efferents have been implicated in the meal-related release of endogenous GLP-1 (153), suggesting that complete subdiaphragmatic vagotomy could change the meal-induced release of endogenous GLP-1 in a way that may
influence the effects of exogenous GLP-1. In contrast, SDA surgery has few side effects because it leaves about 50% of the vagal efferents intact. The animals are able to eat solid chow in normal amounts, although their eating rate is sometimes slightly reduced, they have similar spontaneous meal sizes as intact or sham-operated rats, and they maintain nearly normal body weights (495 ± 10 and 510 ± 21 g for SDA and sham rats at the end of the experiments).

The failure of IP GLP-1 to reduce meal size in SDA rats demonstrates that IP administered GLP-1 requires intact vagal afferents to inhibit eating and, together with the observation that HPV GLP-1 still reduced meal size in SDA rats, strongly suggests that it acts on GLP-1 receptors near the site of release in the intestines to do so. Endogenous GLP-1 released from the L-cells diffuses into the lamina propria and enters the lymph or capillaries. Dipeptidyl peptidase IV (DPP-IV) is expressed on the capillary walls and immediately begins to degrade absorbed GLP-1 (75); indeed, this process is so efficient that only 25% of the absorbed GLP-1 is estimated to reach the liver intact (86;87). Prior to absorption into the capillaries and the onset of degradation, however, endogenous GLP-1 should be present in higher concentrations (87) and could act on GLP-1 receptors on vagal afferent endings in the lamina propria (22;137) to trigger vagal afferent signaling and satiation. IP GLP-1 may therefore mimic a physiological paracrine action of GLP-1 released from the intestines during meals. Our results suggest that IP GLP-1 reaches these intestinal receptors, which is not obvious because IP-administered substances are usually supposed to be absorbed into more superficial intestinal capillaries rather than the deeper lamina propria. Thus, evaluation of the hypothesis that IP administered GLP-1 models a physiological paracrine action of endogenous GLP-1 requires further investigations. Particularly useful would be tests of local administration of the GLP-1 receptor antagonist Ex (9-39), as has been done with the CCK-r receptor antagonist devazepide in rats (36).

Our HPV infusions were designed to model the meal-induced release of endogenous GLP-1 into the HPV (86;87) and to target GLP-1 receptors in the
hepatic portal area or liver (10;86;191). The similar satiating effects of HPV and VC GLP-1 infusions suggests, however, that GLP-1 did not act on these receptors to reduce meal size. At first glance, the similar satiating effects of HPV and VC GLP-1 infusions are surprising as only about 50% of GLP-1 entering the liver is supposed to reach the general circulation because of efficient DPP-IV-mediated GLP-1 degradation in the liver (86;87). On the other hand, it appears to be consistent with the lack of a clear dose-relationship for the satiating effect of GLP-1 in our experiments. In some other studies the eating-inhibitory effects of peripheral GLP-1 or Ex-4 were dose-related (32;168). We can only speculate about the reasons for this discrepancy. As these other studies employed continuous GLP-1 infusions (32) or intramuscular Ex-4 injections (168), it is possible that longer-term GLP-1 receptor activation is necessary to reveal a dose-dependent effect.

The similar satiating effects of HPV and VC GLP-1 infusions, together with the fact that the effect of HPV GLP-1 infusions did not depend on vagal afferent signaling, suggest that intravenously infused GLP-1 reduces meal size by acting in the brain. Alternatively, it is possible that that spinal visceral afferents in the hepatic portal area contributed to the satiating actions of HPV- or VC- infused GLP-1, although to our knowledge spinal visceral afferents have not been directly implicated in any actions of GLP-1. Consistent with the possibility of a central action, circulating GLP-1 apparently passes the blood-brain barrier by simple diffusion both in circumventricular organs (145) and elsewhere (93). Previously, GLP-1 was suggested to act in the Arc to reduce food intake (41), whereas more recent findings implicate the PVN (162) and the caudal brainstem (79) in the eating-inhibitory effect of GLP-1. Thus, further studies are necessary to determine where in the brain circulating GLP-1 acts to reduce meal size.

The relationship between GLP-1’s satiating action and its other putative physiological actions remain unclear. HPV GLP-1 infusion has been shown to increase hepatic-branch vagal afferent activity (141), and activation of hepatic-portal GLP-1 receptors appears to contribute to the effect of GLP-1 on peripheral
glucose handling (191) and to the synergistic effect of GLP-1 and glucose on pancreatic insulin release (10). Our findings suggesting that hepatic and hepatic-portal GLP-1 receptors are not involved in the satiating effect of GLP-1 therefore dissociate GLP-1’s satiating effect from these metabolic effects. A similar dissociation between GLP-1’s central effects on metabolism and eating was recently reported by Sandoval et al. (162), who demonstrated that injection of GLP-1 into the hypothalamic Arc nucleus affected glucose metabolism and insulin release, whereas injection of GLP-1 into the PVN reduced food intake.

Endogenous GLP-1 is also considered to be a major contributor to the “ileal brake”, i.e., to the mechanism through which nutrients in the distal part of the small intestine slow gastric emptying (84). In line with this physiological function, intravenous GLP-1 (32) and IP Ex-4 (184) administration has been shown to inhibit gastric emptying, and intrajugular infusion of the GLP-1 receptor antagonist Ex (9-39) increased intestinal motility, indicating inhibition by endogenous GLP-1, in both rats and humans (84;187). Both intrajugular infusion (32) and intracerebroventricular injection (8) of GLP-1 have been shown to inhibit eating in rats sham-feeding with open gastric cannulas, suggesting that GLP-1 does not require the presence of food in the stomach to inhibit eating. More recently, Strubbe et al. (55) reported that increased endogenous GLP-1 following intragastric administration of GLP-1 secretagogues also inhibited sham feeding and that infusion of a GLP-1 antibody reduced the satiating effect of GLP-1, but not its effect on gastric emptying. Together, these findings suggest that the satiating effect of peripheral GLP-1 that we report here does not depend on an inhibition of gastric emptying or on the accumulation of ingested food in the stomach. This, however, does not preclude the possibility that GLP-1 might synergize with signals related to gastrointestinal motility to control eating.

In sum, the major findings of this study were 1) that peripherally infused GLP-1 specifically reduces spontaneous meal size in rats, 2) that the effect of IP, but not HPV GLP-1 requires vagal afferent signaling, and 3) that intravenous infusions of GLP-1 do not inhibit eating via hepatic portal or hepatic GLP-1
receptors, but may act directly on the brain. Whether the satiating effect of IP or intravenously administered GLP-1 reflects a physiologic satiating action of endogenous GLP-1 merits further studies.
6 GLP-1 Antagonism with Exendin (9-39) Fails to Increase Spontaneous Meal Size in Rats

Based on Ruttimann et al, Physiol. Behav. 100(4):291.6, 2010

6.1 Introduction

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is a gut-brain peptide. In the gut, GLP-1 is mainly released by L-cells in the distal small intestine in response to luminal stimulation by carbohydrates [11, 85, 86] and fats [4, 38, 88]. In the brain, GLP-1 is expressed in neurons of the NTS [89]. GLP-1R are located in various brain regions, including several loci that are implicated in the control of eating, such as the NTS, AP, and the Arc and the PVN [67, 128]. The presence of GLP-1R in the nodose ganglion [137] and the NTS fits findings suggesting the presence of GLP-1R on abdominal vagal afferents [e.g. [10,161]]. GLP-1R are also expressed widely in the gastrointestinal tract [86], in the pancreatic islets [71] and in other peripheral organs (see [86]). Central or peripheral GLP-1R activation has been shown to cause a variety of effects, including an increase in insulin release and a reduction of food intake [37, 87, 119, 161].

Intestinal GLP-1 is implicated in satiation because exogenous GLP-1 or the GLP-1R agonist Ex-4 specifically reduced meal size in the rat [32, 161] and in non-human primates [168]. Also, in humans, GLP-1 doses that led to increases in circulating GLP-1 within the physiological range inhibited eating and increased satiety ratings [15, 72], suggesting that the satiating effect of GLP-1 could be physiologically relevant. So far, however, only a few studies have critically examined the physiological role of peripheral GLP-1 in satiation by attempting to acutely antagonize endogenous intestinal GLP-1, and the results are conflicting: Williams et al. [200] reported that intraperitoneal (IP) administration of the GLP-1R antagonist Ex (9–39) increased food intake in the middle of the dark phase, i.e. at a time when food intake is low, and antagonized the satiating effect of voluntarily consumed food or glucose solution and of intragastrically infused glucose. In
contrast, Kim et al. [96] reported that hepatic portal vein (HPV) or jugular vein infusion of the GLP-1R antagonist [desHis1,Glu8] Ex-4 for 40 min either in the middle of the light phase or at dark onset did not affect food intake. In these studies, however, the animals were food deprived for several hours prior to dark onset and the antagonists were not administered in direct relation to the meals, both of which may have had metabolic or endocrine effects that influenced GLP-1’s satiating action.

Here we examined the physiological role of endogenous intestinal GLP-1 in the control of meal size by testing brief, remotely controlled IP infusions of Ex (9–39) in ad libitum-fed rats during spontaneous dark-phase meals. We have previously reported that under these conditions both HPV and IP infusion of GLP-1 reduced spontaneous meal size [161]. We conducted three experiments. First we IP infused various doses of GLP-1 to identify the lowest dose of exogenous GLP-1 with a reliable satiating effect under our test conditions. Second, we tested the effect on eating of two doses of Ex (9–39). Finally, we tested the efficacy of Ex (9–39) to antagonize the satiating effect of 89 exogenous GLP-1.

6.2 Materials and methods

6.2.1 Subjects and housing

Male Sprague-Dawley rats (Crl:CD®(SD) Charles River; Sulzfeld, Germany; body weight (BW) 150–180 g on arrival) were individually housed in a temperature-controlled (21±2 °C) colony room kept on a 12/12 h dark/light cycle with the lights off at 1100. The rats had ad libitum access to water and ground chow (No. 3433, Provimi Kliba NAFAG, Kaiseraugst, Switzerland). Chow was left in an open bin for at least 1 d prior to use to prevent the disruption of normal spontaneous eating patterns by the availability of fresh chow. All procedures were approved by the Canton of Zurich Veterinary Office.
The catheters were made of 22 cm silastic tubing (W.L. Gore, Newark, DE, USA; ID 0.508 mm, OD 0.914 mm) and implanted after 14 d of adaptation to the housing conditions. The catheters were sterilized in a disinfectant solution (Kodan–Tinktur forte farblos; Schluer & Mayr GmbH, Norderstedt, Germany) for 30 min, thoroughly rinsed with sterile 0.9% saline, and implanted using sterile techniques. The proximal ends of the catheters were fitted with infusion ports made from 3 cm lengths of 20 G (0.90×40 mm) surgical stainless-steel tubing (Sterican; Braun, Melsungen, Germany) and bent into U-shapes. Both ends of the infusion ports were polished. The connections between catheters and infusion ports were shielded with 2.5 cm pieces of heavier silicon tubing (ID 1.02mm, OD 2.18mm). The infusion ports were fixed to a 2.5×3 cm oval of polypropylene surgical mesh (Marlex; Bard Implants, Billerica, MA, USA). For infection prophylaxis and analgesia, rats received subcutaneous injections of 4 mg/kg trimethoprim and 20 mg/kg sulfadoxin (Borgal 24%, Intervet, Shering-Plough Animal Health, Kenilworth, NJ, USA) and 0.05 mg/kg atropine (Sintetica, Mendrisio, Switzerland) a few hours and 15 min prior to surgery, respectively. Anesthesia was introduced with 5% isoflurane (Attane, Minrad Inc., Buffalo, NY, USA) in oxygen and maintained with 2–3% isoflurane in 1:1 O2:N2O. The proximal ends of the catheters with the infusion ports and the surgical mesh were led subcutaneously from 2 cm midline incisions caudal to the scapula to cutaneous puncture wounds near the base of the neck, where the infusion ports were exteriorized. The surgical mesh (see above) ensured adhesion to skin and fascia, thus anchoring the infusion ports in place. The distal ends of the catheters were led subcutaneously to the abdomen and into the peritoneal cavity via 5 cm midline parotomies, where they were anchored to the serosa on the left side of the abdominal wall with silk sutures. Skin and muscle were closed with resorbable sutures (muscle: 3–0, skin: 5–0 Vicryl, Ethicon, Norderstedt, Germany). Four mg/kg trimethoprim and 20 mg/kg sulfadoxin (Borgal 24%) and 5 mg/kg carprofen (Rimadyl, E. Gräub, Bern, Switzerland) were injected subcutaneously on the day after surgery. Catheters were flushed with 0.5 ml 0.9% solution.
sterile saline 3 x/wk (Monday, Wednesday, and Friday) throughout. Post-mortem visual examination confirmed that all catheters remained fully patent throughout the experiments.

6.2.3 Test Procedures

Adaptation to the test procedures started after a 7 d postsurgical recovery period. Rats were placed in custom-made, open-topped acrylic infusion cages (37×21×41 cm) with stainless-steel grid floors. A red incandescent light bulb provided dim illumination during the dark phase, and a radio tuned to a music station was played continuously to mask extraneous noise. Ground chow was available ad libitum in food cups placed under a 39 cm 2 hole at the end of a niche in the cage wall that was 4.6 cm wide, 7 cm deep, and 29.5 cm high, with the base 5 cm above the cage floor. The cups were mounted on electronic balances (Mettler PM 3000, Greifensee, Switzerland) interfaced with a computer (Olivetti M 300, Nuremberg, Germany) in an adjacent control room, and a custom-designed program (VZM, Krügel, Munich, Germany) recorded the weights of the food cups every 30 s. Video cameras (VSS 3440; Philips, Amsterdam, The Netherlands) permitted continuous observation of the rats. At 0830 h the infusion ports of the catheters were connected to infusion pumps (A99; Razel, Stamford, CT, USA) via two segments of polyethylene tubing (0.76 mm ID, 1.22 mm OD, Portex, Hythe, UK). The lower segment of tubing was sheathed with a stainless-steel spring fixed to a swivel joint 45 cm above the cage floor, thus allowing the rats to move freely. The infusion pumps were operated by remote control from a different room. To reduce possible adhesion of the peptides to the tubing we incubated the infusion lines with the test solutions prior to the experiments and refilled them with fresh solutions for the infusions. The infusions (0.2 ml/min) started 2–3 min after the onset of the first or second (see below) spontaneous nocturnal meal and lasted for 2.5 min, resulting in a total infusion volume of 0.5 ml. The dead space of the catheters inside the animals (approximately 20 μl or 4% of the infused volume) was neglected for the dose calculations. If a meal was under way at dark onset (1100
Effect of Short Intrameal Infusions of GLP-1 R Antagonist Ex (9-39) on Meal Size

h), the infusion was done during the next meal. The criteria for meal onset were a 0.2 g decrease in the food cup weight and visual verification of eating. Catheters were detached and flushed with 0.5 ml 0.9% sterile saline at 1500, and rats were weighed and foodcups refilled between 1700 and 1900 h. Experiments started 15 d after surgery. Within-subjects crossover designs were used in each of them. GLP-1 (7–36-amide) or exendin (9–39) (Ex (9–39)), (both from Bachem, Bubendorf, Switzerland) was dissolved in PBS (Gibco, Basel, Switzerland) with 1% BSA (Sigma, Buchs, Switzerland); control infusions were vehicle. Infusions were given in random order on consecutive days.

6.2.3.1 Effects of IP GLP-1 on eating

To test whether IP infusion of GLP-1 dose-dependently decreases spontaneous meal size, rats (n = 15, BW: 367-451 g at test onset), received doses of 0 (vehicle), 2.5, 5, or 10 nmol/kg BW GLP-1 during the first spontaneous nocturnal meal.

6.2.3.2 Effects of IP Ex (9-39) on eating

To test whether IP infusion of GLP-1 dose-dependently decreases spontaneous meal size, rats (n=15, BW: 367–451 g at test onset), received doses of 0 (vehicle), 2.5, 5, or 10 nmol/kg BW GLP-1 during the first spontaneous nocturnal meal.

6.2.3.3 Effect of IP Ex (9-39) on the satiating effect of IP GLP-1

To test whether intrameal IP infusions of Ex (9–39) antagonize the effect of simultaneously infused GLP-1, rats (n=15, BW: 334–472 at test onset) received IP infusions of vehicle, 10 nmol/kg BW GLP-1,30 nmol/kg Ex (9–39) or 10 nmol/kg BW GLP-1 mixed together with 30 nmol/kg BW Ex (9–39) during the first spontaneous nocturnal meal.
Effect of Short Intrameal Infusions of GLP-1 R Antagonist Ex (9-39) on Meal Size

6.2.4 Data analysis

Meals were defined as food removals ≥0.2 g, with the interval between removals ≤15 min. Sizes and durations of spontaneous meals during the test period, intermeal intervals (IMI, duration between the end of one meal and the beginning of the next), first meal satiety ratios (subsequent intermeal interval/meal size) were analyzed with repeated-measures ANOVA (SAS 9.1.3, SAS Institute, Cary, NC, USA) and planned Bonferroni–Holm [83] post-hoc comparisons. Data sets that did not meet the ANOVA criteria of normality and equal variance were transformed to inverses for analysis. Data from the two trials in which the effect of Ex (9–39) on eating was tested were analyzed with the paired Student's t-test. To identify outliers, data were converted to standard scores using the medians and median absolute deviates × 1.48 (which estimates the standard deviation), and values N2.57 (i.e., p=0.01) were excluded. Differences were considered significant when p=0.05.
6.3 Results

6.3.1 Effects of IP GLP-1 on eating

Intrameal IP infusions of 10, but not 5 or 2.5, nmol/kg BW GLP-1 reduced the size of the first spontaneous nocturnal meal compared to vehicle infusions (F3/36=3.38, pb0.005). Both 5 and 10 nmol/kg IP 223 GLP-1 reduced the duration of the first meal (F3/32=5.68, pb0.05) (Fig. 1). Five nmol/kg GLP-1 also increased the satiety ratio (F3/22=4.11, pb0.05) (Table 1). The IMI between the first and second meals, the size of the second meal, and cumulative food intake 2, 4, 6 and 20 h after the beginning of the first meal were not significantly affected by any dose of GLP-1 (Table 1). Two outliers were excluded from the meal size analysis. Some additional data points are missing from other meal parameter analyses because of technical problems with the scales.
Table 1: Effects of intrameal IP GLP-1 infusions during the first spontaneous nocturnal meal on intermeal interval, satiety ratio, second meal size and cumulative food intake

<table>
<thead>
<tr>
<th>GLP-1 dose (nmol/kg)</th>
<th>0</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intermeal interval</strong> (min)</td>
<td>145 ± 23</td>
<td>111 ± 29</td>
<td>168 ± 27</td>
<td>139 ± 18</td>
</tr>
<tr>
<td>Satiety ratio (min/g)</td>
<td>36.6 ± 6.1</td>
<td>32.6 ± 9.8</td>
<td>51.3 ± 5.5*</td>
<td>41.7 ± 5.5</td>
</tr>
<tr>
<td>2nd meal size (g)</td>
<td>2.6 ± 0.7</td>
<td>3.5 ± 0.8</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.5</td>
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<table>
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<tr>
<th><strong>Cumulative intake</strong> (g)</th>
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<tr>
<td>2h</td>
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<tr>
<td>4h</td>
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<tr>
<td>6h</td>
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<tr>
<td>20h</td>
</tr>
</tbody>
</table>

Intermeal interval, satiety ratio and 2nd meal size data are from only 12 rats (vs. 1st meal data of 15 rats in Figure 1) because some rats did not consume a second meal before the infusion pumps were detached. *Different from control (0 value), Bonferroni-Holm test (83) after significant ANOVA ($F_{3/22} = 4.11; P < 0.05$) (see text for details).
Effect of Short Intrameal Infusions of GLP-1 R Antagonist Ex (9-39) on Meal Size

Figure 5: Intrameal IP infusions of GLP-1 reduced the size (A; 10 nmol/kg BW GLP-1) and duration (B; 5 and 10 nmol/kg GLP-1) of the first spontaneous nocturnal meal compared with vehicle infusions. *P < 0.05, **P < 0.01, Bonferroni-Holm test (83) after significant ANOVA. See text for further details.
6.3.2 Effects of IP Ex (9-39) on eating

In both experiments the sizes of the first nocturnal meals were between 2.3 and 2.6 g for all treatments and not significantly different ($t_{12} = 1.80$ and $1.83$, respectively, both $Ps > 0.05$). Intrameal IP infusions of 10 or 30 nmol/kg BW Ex (9-39) during the second nocturnal meal did not significantly affect the size of that meal (Figure 6) (10 nmol/kg: $t_{12} = 1.78$, $p = 0.10$; 30 nmol/kg: $t_{13} = 1.73$, $p = 0.11$), the subsequent IMI (10 nmol/kg: $t_{11} = 0.24$, $p = 0.82$; 30 nmol/kg: $t_{9} = 1.32$, $p = 0.22$) or subsequent meal size (10 nmol/kg: $t_{9} = 1.13$, $p = 0.29$; 30 nmol/kg: $t_{8} = 0.86$, $p = 0.41$) and cumulative intake (all $Ps > 0.05$). Both 10 and 30 nmol/kg BW Ex (9-39) decreased the duration of the second meal (10 nmol/kg: $t_{12} = 2.27$, $p = 0.04$; 30 nmol/kg: $t_{9} = 2.87$, $p = 0.02$). Thirty, but not 10, nmol/kg BW Ex (9-39) increased the satiety ratio (10 nmol/kg: $t_{9} = 1.50$, $p = 0.17$; 30 nmol/kg: $t_{9} = 2.87$, $p = 0.02$) (Table 3).
### Table 3: Effects of intrameal IP Ex (9-39) infusions during the second spontaneous nocturnal meal on meal duration, intermeal interval, satiety ratio, third meal size and cumulative food intake

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td></td>
<td>Ex (9-39) (nmol/kg)</td>
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<td>Ex (9-39) (nmol/kg)</td>
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<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Intermeal interval (min)</td>
<td>87 ± 11</td>
<td>89 ± 14</td>
<td>51 ± 6</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>Satiety ratio (min/g)</td>
<td>35.0 ± 4.0</td>
<td>43.6 ± 3.4</td>
<td>25.2 ± 3.0</td>
<td>45.8 ± 5.5*</td>
</tr>
<tr>
<td>3rd Meal size (g)</td>
<td>1.9 ± .2</td>
<td>1.6 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Cumulative intake (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2h</td>
<td>3.1 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>4h</td>
<td>6.0 ± 0.2</td>
<td>5.5 ± 0.4</td>
<td>6.5 ± 0.1</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>6h</td>
<td>8.7 ± 0.3</td>
<td>9.6 ± 0.4</td>
<td>10.5 ± 0.6</td>
<td>9.8 ± 0.7</td>
</tr>
<tr>
<td>20h</td>
<td>20.8 ± 0.4</td>
<td>19.9 ± 20.3</td>
<td>21.4 ± 0.6</td>
<td>21.6 ± 0.7</td>
</tr>
</tbody>
</table>

Intermeal interval, satiety ratio, and 3rd meal size data are from 15 rats for 10 nmol Ex (9-39) and 14 rats for 30 nmol Ex (9-39) because some rats did not consume a third meal before the infusion pumps were detached.

* Different from control (0 value), paired Student’s t-test, P < 0.05 (see text for details).
Figure 6: Intrameal IP infusions of 10 (A, C) or 30 (B, D) nmol/kg BW Ex (9-39) did not affect the size of the second nocturnal meal compared with vehicle fusions, but reduced meal duration. *P < 0.05, Student’s t-test. See text for further details.
6.3.3 Effect of IP Ex (9-39) on the satiating effect of IP GLP-1

Intrameal IP infusion of 10 nmol/kg BW GLP-1 alone decreased the size ($F_{3/39} = 4.36, P < 0.01$) and duration ($F_{3/37} = 2.96, P < 0.05$) of the first meal. IP infusion of 30 nmol/kg Ex (9-39) alone again did not affect any meal parameter or cumulative food intake (data not shown), but antagonized the effects of 10 nmol/kg GLP-1 on first meal size (Figure 7), i.e. the difference between GLP-1 and vehicle treatment was greater than the difference between Ex (9-39) and Ex (9-39) plus GLP-1 treatment ($P < 0.02$).
Effect of Short Intrameal Infusions of GLP-1 R Antagonist Ex (9-39) on Meal Size

Figure 7: Intrameal IP infusions of 30 nmol/kg Ex (9-39) antagonized the satiating effect of exogenous 10 nmol/kg BW GLP-1, but failed to affect meal size when infused alone (A). Meal duration was significantly reduced by GLP-1, and this effect was not significantly affected by Ex (9-39) (B). *P < 0.05, **P < 0.01, Bonferroni-Holm test (83) after significant ANOVA. * P < 0.05 for A
6.4 Discussion

This study examined the physiological relevance of intestinal GLP-1 for satiety. One crucial criterion for the physiological relevance of a peptide’s satiating effect is that specific antagonism at the site of its action has the opposite effect on eating as the peptide does (63;175), i.e., if endogenous intestinal GLP-1 is a necessary part of satiation, GLP-1R antagonism at the site of action should increase meal size. In our experimental model of remotely controlled IP infusions during spontaneous meals in non-deprived rats, Ex (9-39) blocked the satiating effect of simultaneously infused exogenous GLP-1, but did not increase meal size when infused alone. Thus, the study failed to establish this criterion of physiological relevance for intestinal GLP-1.

As previously reported for HPV and IP GLP-1 infusion (161), IP administered GLP-1 selectively reduced the size and duration of the ongoing spontaneous meal without affecting subsequent meal parameters or cumulative food intake, indicating that exogenous GLP-1 specifically affects meal termination. A specific role of GLP-1 in meal termination is consistent with results from several previous tests of GLP-1 or Ex-4 done in a variety of conditions (32;139;168). Also, as in our previous experiments with meal-contingent HPV GLP-1 infusion (161), intrameal IP GLP-1 infusions did not produce a significantly dose-related satiating effect. While this is consistent with a role of GLP-1 in satiation during spontaneous eating, it does not support a role for GLP-1 in the graded satiating effect of food (123). We can only speculate about the reasons for the lack of a clear dose-related satiating effect of GLP-1 in the present study: 1) The threshold dose for IP GLP-1 to inhibit eating under our experimental conditions appeared to be between 5 and 10 nmol/kg BW. Therefore, more and higher GLP-1 doses might be needed to reveal a significantly dose-related effect of GLP-1 on meal size. 2) GLP-1 may only have a permissive, and not a dose-dependent effect on satiation. 3) GLP-1 synergizes with glucose to stimulate insulin release (10), and preliminary data of ours suggest that it may also synergize with glucose to inhibit eating (Rüttimann et al., unpublished).
Effect of Short Intrameal Infusions of GLP-1 R Antagonist Ex (9-39) on Meal Size

If so, a concomitant increase in the plasma glucose concentration may be necessary to reveal the full satiating potential of GLP-1 and, hence, to observe a dose-related effect on meal size. Because exogenous GLP-1 lowers glucose levels very efficiently, this increase in plasma glucose may not have occurred here, which may have masked a graded effect of exogenous GLP-1 on eating. 4) Longterm-term GLP-1R activation may be necessary for a clear dose-dependent effect of GLP-1 on eating. In support of this assumption, two studies in which dose-dependent eating-inhibitory effects of GLP-1R activation were reported both involved prolonged receptor activation, either by continuous infusions of GLP-1 for several hours (32) or by injection of a long-acting GLP-1R agonist (108). Whether endogenous GLP-1 produces such prolonged receptor activation is unknown.

The physiological site of action of intestinal GLP-1 to inhibit eating is still uncertain. GLP-1R in the brain (85;145;161) or in the periphery (103), in particular on abdominal vagal afferents (1;161;184), have been implicated in the mediation of the eating-inhibitory effect of intestinal GLP-1. Although some evidence suggests that IV infused GLP-1 acts in the brain to induce satiation (96), IP infused GLP-1 presumably acts in the abdomen to inhibit eating because its effect was abolished by subdiaphragmatic vagal deafferentation (161) and by IP, but not intracerebroventricular, administration of Ex (9-39) (200). The brain is also unlikely to be the major site for the satiating effect of endogenous intestinal GLP-1 because most endogenous intestinal GLP-1 is degraded by DPP-4 before reaching central receptors (86). The most likely peripheral site of action appears to be GLP-1R on vagal afferents terminating in the lamina propria of the intestinal mucosa.

Significant amounts of IP administered GLP-1 are presumably absorbed from the peritoneal cavity into the intestines because of their large surface area. GLP-1 may accumulate in the lamina propria of the mucosa, where it is protected from DPP-4 degradation (38).

Interestingly, Lo et al. (115) recently reported that IP injections of CCK produced high lymphatic CCK concentrations without altering lymph flow. It is reasonable to assume that this also holds for IP administered GLP-1. As lymphatic
Effect of Short Intrameal Infusions of GLP-1 R Antagonist Ex (9-39) on Meal Size

centations reflect mucosal interstitial fluid peptide concentrations, these findings support the idea that IP administered GLP-1 acts locally in the intestine to inhibit eating. Endogenous intestinal GLP-1 may act there as well because GLP-1R on vagal afferents terminating in the lamina propria of the intestinal mucosa encounter high concentrations of endogenous GLP-1 released from L-cells. A similar intestinal paracrine action has been implicated in the satiating effect of CCK (36;175).

Ex (9–39) reversed the eating-inhibitory effect of exogenous GLP-1, but did not stimulate eating when administered alone. The latter finding is consistent with previous reports that IV administration of the GLP-1R antagonist [desHis1,Glu8] Ex-4 did not increase food intake (95) and that IP injection of Ex (9–39) done at dark onset did not increase food intake (200). The reversal of exogenous GLP-1’s satiating effect by Ex (9–39) indicates that after IP administration Ex (9–39) reached the receptors through which IP administered GLP-1 induces satiation early enough and in sufficient quantity to block this effect. Therefore, the most parsimonious interpretation of the failure of Ex (9–39) alone to alter food intake is that endogenous GLP-1 is not necessary for satiation under these conditions. Alternative explanations are that Ex (9–39) can block only pharmacological effects of GLP-1 under our conditions or that the dose of the antagonist that we used was too low to block the effect of the endogenous peptide, i.e. that a higher dose of Ex (9–39) may be necessary to increase normal meal size than to block the satiating effect of exogenous GLP-1. That hormone antagonists block the eating-inhibitory actions of exogenous hormones more effectively than they block the actions of endogenous hormones has been reported in tests of CCK1 receptor antagonism (63).

Williams et al. (200) reported an increase in 1 h food intake after IP injection of 100 μg/kg BW Ex (9-39), a very similar dose to ours, within 1h after food access. There are several possible explanations for this discrepancy: First, the failure of Ex (9-39) to increase meal size under our conditions may be a pharmacokinetic artifact, i.e., it is possible that Ex (9-39) reached the receptors too
late or in too small a concentration to influence the effect of endogenous GLP-1. Williams et al. (200) IP injected Ex (9-39) as a bolus just prior to offering food, whereas we IP infused the Ex (9-39) over 2.5 min beginning only 2-3 min after meal onset, i.e., at a time when the release of endogenous GLP-1 may have begun. It is difficult to estimate the GLP-1 concentration at the mucosal receptors during the meal. D’Alessio et al. (38) reported, however, that intragastric liquid diet infusion led to a peak concentration of 300 pmol/kg GLP-1 in an intestinal lymph sample collected for 30 min after the intragastric load. As postprandial intestinal lymph flow rate is only about 4 ml/h (152), it is possible that the local GLP-1 concentration had already begun to increase within the first few min of meal onset, i.e., too early for Ex (9-39) infused under our conditions to compete with the endogenous GLP-1 for receptor sites in the intestinal lamina propria. Another possible explanation is related to the different experimental set ups. Williams et al. (200) reported that Ex (9–39) increased food intake in four tests: first, when 4 h food-deprived rats were injected with Ex (9–39) 8 h into the light phase; second, when 6 h food-deprived rats were intragastrically infused with glucose before Ex (9–39) injections in the middle of the light phase; third, when 4 h food-deprived rats were given 15min oral glucose preloads 15 min before Ex (9–39) injections at dark onset; and fourth, when rats were fasted for 4 h prior to dark onset, then re-fed for 1 h, and then injected with Ex (9–39). Clearly, none of these tests is similar to our tests of the first nocturnal meal. We did, however, try to mimic the fourth of Williams et al.’s (200) regimens by infusing the Ex (9–39) during the second dark phase spontaneous meal. Nevertheless, our animals were fed ad libitum throughout and, therefore, were perhaps in a different endocrine or metabolic state. We did not food deprive the rats because we wanted to test Ex (9–39) during spontaneous eating, when rats may display effects that may not be observed in fasted animals or in rats eating a scheduled meal. This might be true in particular for GLP-1 because fasting has been reported to decrease its satiating effect (199). Finally, it is worth noting in this context that the efficacy of CCK1 receptor antagonism to increase food intake also depends on the experimental paradigm used (63).
Ex (9-39) reduced rather than increased meal duration in two of the three tests when it was administered alone. This appears peculiar, in particular since meal duration was unchanged when Ex (9-39) and GLP-1 were administered together. As might be expected from the discrepant effects on meal size and duration, Ex (9-39) tended to increase the intrameal eating rate in these two tests (not shown), but this effect did not reach statistical significance. We can only speculate about the reasons for this puzzling result. Perhaps IP administered Ex (9-39) can antagonize GLP-1 actions in two different sites, i.e., on vagal afferents terminating in the lamina propria of the intestinal mucosa and in the HPV. The non-significant increase in eating rate may then reflect a residual effect of Ex (9-39) on intestinal vagal afferents that, for reasons discussed above, was too weak to increase meal size. In contrast, the reduction of meal duration by Ex (9-39) may be related to an antagonism of GLP-1’s metabolic effects mediated through vagal afferents terminating in the HPV (10). Further studies are necessary to clarify this point. The failure of Ex (9-39) to stimulate eating when administered alone also appears to be consistent with the facts that neither eating nor body weight changes were found in transgenic GLP-1R-deficient mice (169). However, negative findings in unconditional genetically modified animals are always questionable because the results may not reflect effects of the lack of the hormone itself, but may be a result of developmental compensation.

In conclusion, the main results of this study are: 1) Intrameal IP infusion of GLP-1 selectively reduced meal size, but no significant dose-related effect was detected. This is consistent with a role of GLP-1 in satiation but does not support a role of GLP-1 in the graded satiating effect of food. 2) Intrameal IP infusion of Ex (9-39) did not affect eating by itself under conditions when it did block the satiating effect of exogenous GLP-1. This questions the physiological role of endogenous GLP-1 in satiation under our conditions. Several explanations for these seemingly discrepant findings are possible. Therefore, further research into the mechanisms of the satiating effect of GLP-1 and its physiological relevance is warranted.
Hepatic portal vein (HPV) but not vena cava (VC) glucagon like peptide-1 (GLP-1) increases during a chow meal in rats.

Based on Ruttimann et al., in Preparation 2010

7.1 Introduction

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is a gut peptide and a gut peptide that is released in response to intestinal luminal nutrient stimulation (86). GLP-1R activation affects glucose metabolism by increasing insulin and inhibiting glucagon release from the pancreas (10;86;169), and by enhancing glucose uptake into hepatocytes and muscle cells (6;9;39). Genetic lack of the GLP-1R or pharmacological GLP-1R antagonism consistently leads to increases in plasma glucose and decreases in plasma insulin concentrations in response to meals (39;86;169;198), suggesting a physiological role of peripheral GLP-1 in glucose metabolism. GLP-1 also inhibits gastrointestinal motility and secretion (86) and is considered to be a major mediator of the “ileal break effect”, i.e., the inhibition of proximal gastrointestinal functions by the arrival of undigested nutrients in the ileum (86).

Finally GLP-1 decreases food intake after IV and IP administration (56;86;96;108;161;199;200). whether peripheral GLP-1 plays a physiological role in the control of eating remains uncertain, however, because the available data of GLP-1R antagonism studies are conflicting (96;199) and because GLP-1R deficient mice have normal food intake and body weight (169). Also, it is still unclear whether the often observed inhibition of eating in response to exogenous GLP-1 (1;32;56;78;79;86;96;161;199) reflects a physiological or a pharmacological effect.

In the gut, GLP-1 is produced mainly by intestinal L-cells, which have their highest density in the ileum, but are also found in other parts of the small and large intestines (97). Luminal carbohydrates and, in particular, fatty acids serve as major
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stimuli for GLP-1 release (41;54;165). Nutrient-induced GLP-1 release in humans has been described to be biphasic under some conditions (72). The release pattern in rats appears to be similar (11;38;88).

So far, however, plasma and intestinal lymphatic (38) GLP-1 concentrations in rats were measured only after intragastric (38;88) or intraintestinal (4) administration of liquid diets (4;38) or nutrients (4;38;88), and not in response to real, solid, mixed-nutrient meals. Once GLP-1 enters the blood vessels, it has a biological half life of only 1-2 min due to the rapid inactivation by DPP-IV (41). This leads to a steep concentration gradient of active GLP-1, with the highest levels in the lamina propria of the intestinal mucosa beneath the L-cells, intermediate levels in the HPV, and low levels in the general circulation after GLP-1 has passed the liver, where DPP-IV concentration is high (41).

Detailed short-term measurements of the GLP-1 release in response to meals and comparison of the endogenous levels with the levels found after GLP-1 administration would help to answer the question of whether the satiating effect of peripherally administered exogenous GLP-1 observed in several experiments (1;32;56;96;99;161;200) is physiologically relevant. For instance, we reported that remotely controlled intrameal HPV and IP infusions of GLP-1 in rats acutely and selectively reduced ongoing meal size and duration (161), but whether this effect is physiologically relevant is unclear. The site of GLP-1’s satiating action is also unknown. SDA which eliminates all vagal afferent signaling from below the diaphragm to the NTS (142), blocked the satiating effect of GLP-1 after IP but not after HPV infusion (161). Moreover, systemic GLP-1 infusions seem to inhibit eating as (161) or even more (96) potently as HPV infusions, suggesting that IV administered GLP-1 acts directly in the brain to inhibit eating. IP administered GLP-1, on the other hand, inhibits eating by acting peripherally (161;200), presumably through GLP-1R on intestinal vagal afferents (137). Intestinal GLP-1 released from L-cells into the lamina propria of the mucosa is either collected in small intestinal lymph vessels or transported by intestinal capillaries to the HPV (38;41). It is very difficult to collect intestinal lymph from freely moving rats during spontaneous
meals, and the slow rate of intestinal lymph flow, between 2 and 3 ml/h (77;116), makes it hard to detect dynamic changes in the GLP-1 concentration around meals and to judge the possible endocrine effects of the released hormone. We therefore performed two experiments in rats to 1) measure plasma GLP-1 levels in the HPV and in the inferior vena cava (VC) during and after a solid, mixed meal and 2) to measure the increase in systemic, i.e., VC, plasma GLP-1 concentration and the changes in plasma glucose and insulin levels in response to a dose of exogenous GLP-1 that reliably reduced meal size when infused into the HPV (161).

7.2 Material and methods

7.2.1 Animals and housing Male Sprague–Dawley rats (Crl:CD®(SD) Charles River; Sulzfeld, Germany; body weight (BW) 182-206 g on arrival) were individually housed in two temperature-controlled (22 ± 1 °C) colony rooms and kept on a 12/12 h dark/light cycle with the lights off at 08.00 or 12.00. Rats were housed in two separate rooms with staggered lighting cycles were used to ease performance of the experiments (see below). The rats had continuous ad lib access to water and rat chow (No. 3433, Provimi Kliba NAFAG, Kaiseraugst, Switzerland) except where noted otherwise. They were adapted to housing conditions for 10 d before the experiments began. All procedures were approved by the Canton of Zurich Veterinary Office.

7.2.2 HPV and VC catheter implantation

Catheters were implanted after 11 d of adaption to the housing conditions. The catheters were sterilized in a disinfectant solution (Kodan Tinktur forte farblos®; Schluer &Mayr GmbH, Norderstedt, Germany) for 30 min, then thoroughly rinsed with sterile 0.9% saline, and implanted using close to sterile techniques. The proximal ends of the catheters were fitted with blood sampling/infusion ports made from 3 cm long 22 G (0.70 x 30 mm) surgical
stainless-steel tubing (Sterican; Braun, Melsungen, Germany) bent into U-shapes. Both ends of the tubing were polished. Catheters consisted of 20 cm silastic tubing (Gore W.L., Newark, DE; ID 0.508 mm, OD 0.940 mm) attached to the subcutaneous part of the steel tubing. The connections of the steel tubing with the catheters were shielded with two layers of heavier silicon tubing (ID 0.305/OD 0.635 and ID 0.762/OD 1.651 mm for the inner and outer layer, respectively). The catheters were then led through a 2.5 x 3 cm oval of polypropylene surgical mesh (Marlex; Bard Implants, Billerica, MA, USA) and fixed to it with 4 silk sutures (Silkam, 5/0; Braun). The surgical mesh helped to improve adhesion to the skin and fascia, thus anchoring the blood sampling/infusion ports in place.

For infection prophylaxis, rats received subcutaneous injections of 4 mg/kg trimethoprim and 20 mg/kg sulfadoxin (Borgal 24%, Intervet, Shering-Plough Animal Health, Kenilworth, NJ, USA) and 0.05 mg/kg atropine (Sintetica, Mendrisio, Switzerland), a few hours and 15 min prior to surgery, respectively. Anesthesia was initiated with 5% isoflurane (Attane, Minrad Inc., Buffalo, NY, USA) in oxygen and maintained with 2-3% isoflurane in 1:1 oxygen:O\textsubscript{2}. The proximal ends of the catheters with the steel tubing and the surgical mesh were led subcutaneously from 2-cm midline incisions caudal to puncture wounds near the rostral end of the scapula, where the proximal ends of the steel tubing were exteriorized. The distal ends of the catheters were led subcutaneously from the neck to 5 cm midline laparotomies.

Both HPV and inferior VC catheters were implanted during the same surgery. Inferior VC catheters were inserted just rostral to the renal veins, as described by Kaufman (94), and the tips advanced so that they lay anterior to the junction of the hepatic vein. For the HPV catheter, the mesenteric vein was gently dissected free between the gastroduodenal and splenic veins, and the beveled catheter tip was inserted into the mesenteric vein and advanced 4-6 mm, so that it ended in the HPV 2 mm downstream of the junction of the gastroduodenal vein. The catheter was fixed to the mesenterium with silk suture (Silkam, 5/0; Braun). Skin and muscle were closed with resorbable sutures (muscle: 3-0, skin: 5-0 Vicryl,
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Ethicon). Four mg/kg trimethoprim and 20 mg/kg sulfadoxin (Borgal 24%, Intervet, Shering-Plough Animal Health) and 5 mg/kg carprofen (Rimadyl, E. Gräub, Bern, Switzerland) were injected SC on the day after surgery. Catheters were flushed regularly with 0.2 ml 0.9% sterile saline and filled with 100 µl heparinized saline (100 IU heparin/ml saline; Heparin, Braun) between tests.

7.2.3 Donor blood preparation

Thirteen rats of the same strain and sex were used as blood donors to replace the volume withdrawn during the repetitive blood samplings (see below). Donor blood was freshly prepared each day and stored on ice until use. The donor rats were anesthetized with 5% isoflurane (Attane, Minrad Inc.) in oxygen. After thoracotomy the right ventricle was punctured and 4.5 ml blood was withdrawn into each of two 5 ml syringes, each containing 250 IU Heparin in 0.5 ml saline, yielding a concentration of 25 IU /ml blood. Donor blood administration under comparable conditions did not affect metabolic measures or plasma corticosterone in a previous study of ours.

7.2.4 Test procedures

Six days after surgery, rats were placed in custom-made, open-topped acrylic infusion cages (length x width x height: 37 x 21 x 41 cm) with stainless-steel grid floors. A red incandescent light bulb provided dim illumination during the dark phase. The rats were evenly distributed into the two rooms (dark phase from 08.00-20.00 or 12.00-24.00, respectively) so that two sets of four rats each could be tested per day.

7.2.4.1 Effects of a Meal on HPV and VC Levels of GLP-1, Insulin and Glucose

Fourteen rats (BW 349-421 g at test onset) were used. On the day of the experiment food was withdrawn 1 h before dark onset. Three hours after dark onset
the blood sampling/infusion ports of both catheters were connected to polyethylene tubing (0.58 mm ID, 0.914 mm OD; Portex, Hythe, UK). The lower part of the tubing was sheathed with a stainless-steel spring, allowing the rats to move freely. Four h after dark onset, a baseline sample (0 min) was taken, after which the rats received a 3 g pellet of their standard chow. Blood samples were taken 3, 6, 10, 15 and 20 min after meal onset. At each time point blood was withdrawn simultaneously from the HPV and the VC (330 µl each) into syringes containing 5 µl DPP-IV inhibitor (Millipore, St. Charles, USA) and 11 µl EDTA. The contents of each syringe were mixed, transferred into an Eppendorf tube and put on ice. All samples were centrifuged (4 °C, 5000 × g, 8 min) immediately after the last blood withdrawal. Plasma (160 µl) was removed and stored at -70 °C. After each of the first four blood withdrawals, the catheters were flushed with 0.9% saline, and 330 µl donor blood was infused into the VC. After the last blood withdrawal on each experimental day, 600 µl donor blood was infused. Thereafter, the catheters were detached and food was returned.

7.2.4.2 Effects of HPV GLP-1 infusion on VC levels of GLP-1, insulin and glucose

Twelve rats (n = 12, BW 370-445 g at test onset) were used. On the day of the experiment food was withdrawn at dark onset. Two hours after dark onset the blood sampling/infusion ports of both catheters were connected to polyethylene tubing as described above. Three h after dark onset VC baseline (0 min) blood samples were taken, and immediately thereafter 1 nmol/kg BW GLP-1 (7-36)-amide (Bachem, Bubendorf, Switzerland) or vehicle was infused into the HPV (2.5 min, 0.4 ml/min). GLP-1(7-36)-amide was dissolved in PBS (Gibco, Basel, Switzerland) with 1% BSA (Cohn Fraction V, Sigma, Buchs, Switzerland). A within-subjects cross-over design with one intervening, no-infusion day was used. VC blood samples were taken at 3, 6, 10 and 25 min after infusion onset as described, except that blood was withdrawn only from the VC and no donor blood was infused.
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After the final blood withdrawal, the catheters were detached and food was returned.

7.2.5 Hormone and glucose analyses

Active GLP-1 in plasma was analyzed by GLP-1 (Active) ELISA kit (Linco Research Inc., St. Charles, MO, Cat. # EGLP-35K), and plasma insulin was determined by rat specific radioimmunoassay test kits (Linco Research Inc., Cat. # SRI-13K). The intra- and inter-assay coefficients of variation for the GLP-1 assay were 2.6 and 3.9%, respectively, and for the insulin assay were 4.3 and 8.7%. Plasma glucose was determined by a standard enzymatic method adapted for the Cobas Mira autoanalyzer (Roche, Basel, Switzerland)(105).

7.2.5 Statistical analysis

The data were analyzed with a 2 way (time x blood vessel) repeated-measures ANOVA (SAS 9.1.3, SAS Institute, Cary, NC, USA) and Bonferroni-Holm (83) planned comparisons for each time point between blood vessels and for each blood vessel between all time points and baseline. The AUC was calculated with respect to the baseline and analyzed with a paired Student’s t-test. To identify outliers, data were converted to standard scores using the medians and median absolute deviates x 1.48 (which estimates the standard deviation), and values > 2.57 (i.e., P < 0.01) were excluded. In the first experiment, data from three rats that did not finish their 3 g meals were not included. Differences were considered significant when P < 0.05.
7.3 Results

7.3.1 Effects of a meal on HPV and VC GLP-1, insulin and glucose

Eleven rats ate their three g meals in 5-6 min. Plasma GLP-1 concentrations changed significantly in response to the meal (main effect of time: $F_{5/60} = 4.02$, $P < 0.01$) and were generally higher in the HPV than in the VC (main effect of blood vessel: $F_{1/56} = 12.58$, $P < 0.01$), with a significant interaction between time and blood vessel ($F_{5/96} = 3.34$, $P < 0.05$, Figure 1). The planned comparisons revealed that HPV GLP-1 increased between 3 and 6 min after meal onset (3 min vs. 0 min: $P > 0.05$; 6 min vs. 0 min: $P < 0.01$) and returned to baseline between 15 and 20 min after meal onset (20 min vs. 0 min: $P > 0.05$). No significant changes in VC GLP-1 concentrations were detected. HPV GLP-1 concentrations were higher than VC concentrations at 6 and 15 min after meal onset (HPV vs. VC: $P < 0.01$), and the AUC for GLP-1 was greater in the HPV than in the VC (HPV: $33.6 \pm 16.9$ vs. VC: $23.1 \pm 10.0$, $t_8 = 2.81$, $p < 0.05$). Plasma insulin concentrations increased in response to the meal ($F_{5/60} = 19.77$, $P < 0.001$) and were generally higher in the HPV than in the VC ($F_{1/56} = 5.91$, $P < 0.05$), although the planned comparisons did not reveal a significant difference for any time point. Also, HPV and VC insulin concentrations increased similarly (time x blood vessel: $F_{5/96} = 1.34$, $P > 0.05$). HPV insulin concentrations were higher than at baseline at each time point (3 min vs. 0 min: $P < 0.01$; 6, 10, 15 and 20 min vs. 0 min: all $Ps < 0.001$). VC insulin concentrations were higher than baseline at 6, 10 and 15 min after meal onset (6 min vs. 0 min: $P < 0.01$; 10 and 15 min vs. 0 min: $P < 0.001$). The AUC for insulin was greater in the HPV than in the VC (HPV: $100.3 \pm 13.4$ vs. VC: $62.0 \pm 9.6$, $t_8 = 2.62$, $P < 0.05$). Plasma glucose concentrations increased in response to the meal ($F_{5/60} = 62.69$, $P < 0.001$) and were generally higher in the HPV than in the VC ($F_{1/56} = 39.88$, $P < 0.001$). HPV glucose concentrations increased more than VC concentrations (time x blood vessel: $F_{5/96} = 4.83$, $P < 0.05$) and did not return to baseline within 20 min (3, 6, 10, 15 and 20 min vs. 0 min: all $Ps < 0.001$). VC plasma glucose concentrations were lower than the corresponding HPV values at
Meal-Related Alterations of Endogenous GLP-1 Levels in HPV and VC

6, 10, 15 and 20 min (all Ps < 0.001) and were increased compared to meal onset at 6, 10, 15 and 20 min (all Ps < 0.001). The AUC for glucose was greater in the HPV than in the VC (VC; -43.1 ± 5.1; HPV; 88.5 ± 6.0; \( t_8 = 7.44, P < 0.01 \)).

Figure 8: Effects of a 3g chow meal in rats on plasma levels of active GLP-1 (A), glucose (B), and insulin (C) as well as on the area under the curve (AUC) for insulin (D) in the hepatic portal vein (HPV) and the vena cava (VC). The meal was given 4 h into the dark after 5 h of food deprivation. \( N = 12 \) for all measurements. *p<0.05 vs. baseline (0 min); + p<0.05 HPV vs. VC, Bonferroni-Holm test (83) after significant ANOVA. (VC: open circles; HPV: filled circles)
7.3.2 Effects of HPV GLP-1 infusion on VC GLP-1, insulin and glucose

HPV infusion of 1 nmol/kg BW GLP-1 increased ($F_{4/45} = 19.57$, $P < 0.001$) VC plasma concentrations of active GLP-1 3 min after infusion onset (3 vs. 0 min: $P < 0.01$; 3 min GLP-1 vs. Veh: $P < 0.01$), but no significant changes were detected at later time points (all $P$s > 0.05, Figure 2). VC GLP-1 concentrations did not change over time in Veh-treated rats. The AUC for GLP-1 was greater in GLP-1 than in Veh-treated rats (GLP-1: 2680 ± 817 vs. Veh: 42 ± 133, $t_{11} = 3.31$, $P < 0.01$). HPV GLP-1 infusion also increased ($F_{4/55} = 21.75$, $P < 0.001$) VC plasma insulin concentrations only at 3 min (3 vs. 0 min: $P < 0.001$; 3 min GLP-1 vs. Veh: $P < 0.001$). VC insulin concentrations did not change over time in Veh-treated rats. The AUC for insulin was greater in GLP-1 than in Veh-treated rats (GLP-1: 1.8 ± 0.6, vs. Veh: 0.0 ± 0.3, $t_{8} = 2.70$, $P < 0.05$). HPV GLP-1 infusion decreased ($F_{4/53} = 4.00$, $P < 0.05$) VC plasma glucose concentrations at 6 and 10 min after infusion onset compared to Veh and baseline (all $P$s < 0.05). The AUC for glucose did not differ between treatments (Veh: -3.2 ± 3.4, GLP-1: 0.0 ± 9.7, $t_{11} = 0.36$, $P > 0.05$).
Figure 9: Effects of a hepatic portal vein infusion (2.5 min, 0.4 ml/min) of GLP-1 (1 nmol/kg body weight) vs. vehicle on plasma levels of active GLP-1 (A), glucose (B), and insulin (C) as well as on the area under the curve (AUC) for insulin (D) in the vena cava (VC). The infusion was given 3 h into the dark after 3 h of food deprivation. N = 12 for all measurements. *p<0.05 vs. baseline (0 min); + p<0.05 vs. vehicle (Veh). Bonferroni-Holm test (83) after significant ANOVA.
7.4 Discussion

This study yielded two important findings: First, a regular chow meal acutely and transiently increased the plasma concentration of active GLP-1 in the (HPV), but not in the systemic circulation (VC). Second, HPV infusion of a dose of GLP-1 that reliably reduced meal size under comparable conditions (161) produced marked increases in systemic plasma concentrations of active GLP-1.

The increase in HPV active GLP-1 levels after the meal is the first report of a solid meal related change in circulating GLP-1 in rats. Previous reports of GLP-1 levels in rats involved intragastric (38;88) or intraintestinal (4) administrations of liquid diets (4;38) or nutrient solutions (88;4;38) and anesthetized animals, i.e., situations that do not necessarily mimic a solid, mixed-nutrient meal in undisturbed, awake animals. The lack of a significant increase in active GLP-1 in the systemic plasma extends the recent report of Shin et al. (171), who failed to see an increase in systemic GLP-1 level in rats fed a 5 ml meal of Intralipid (Abbot Laboratories, Columbus, OH).

In humans, meal-related changes in circulating GLP-1 have been reported (41;80;127), but these studies did not include GLP-1 measurements in the HPV. Also, we measured active GLP-1, which is important to note because in plasma GLP-1 is quickly degraded by DPP-IV (41;42;127). As a result, a significant part of circulating total GLP-1 in humans is deactivated GLP-1 (195), which is rapidly eliminated from plasma by renal filtration (76;127). Although circulating total GLP-1 is a reliable index of secreted hormone, it is not a useful measure of the circulating amount of active hormone.

That levels of active GLP-1 are higher in the HPV compared to the VC during the meal is to be expected and is consistent with another report of the effects of intragastric loads of fat (88). GLP-1 released from the intestinal L-cells is taken up by PP-IV located in the capillary endothelial cells begins immediately (75). HPV levels of active GLP-1 are therefore lower than levels in the interstitial fluid at
the site of release or in the lymph (41). Because the liver has a particularly high concentration of DPcapillaries (86;41;38) and lymph vessels (38) in the lamina propria, deactivation of GLP-1 by DP-IV (41), a major part of the remaining active GLP-1 is degraded there, so that much lower active GLP-1 levels are found in the general circulation than in the HPV (40;41).

The rapid release of GLP-1 during the meal seems to suggest this response is due to GLP-1 release from small intestinal L-cells triggered by a cephalic reflex, for which there is no evidence in humans or by a neuroendocrine reflex originating in the proximal small intestine (41;3;81). Another, not mutually exclusive possibility is, however, that L-cells in the duodenum and proximal jejenum (97) contributed. That HPV plasma glucose was increased already 3 min after meal onset documents that nutrients had reached the proximal small intestine early enough for such a direct stimulation to occur. This may in fact be particularly relevant in rats, which have a higher density of L-cells across the whole small and large intestine than humans or pigs (52). Measurements of GLP-1 levels in relation to meals in rats with selective lesions of vagal afferents or efferents (132) would help clarify this issue. We saw only one early peak of active GLP-1 in the HPV during the meal.

This appears to be consistent with reports of a single peak of circulating GLP-1 around 15 min after intragastric glucose infusion in rats (88) or after an oral glucose tolerance test in humans (80). A second peak of circulating GLP-1 in rats was observed around 4 h after the intragastric infusion of corn oil (88), i.e., under substantially different conditions and at a much later time point than our last measurement. We wanted to evaluate whether an endocrine effect of endogenous GLP-1 contributes to satiation and therefore focused on the duration of the meal, which under our conditions is usually between 6 and 10 min (161) and because the number of repetitive blood samples that can be taken in parallel from two blood vessels in a rat is limited. Our chow is low in fat, the most potent GLP-1 secretagogue (38;41;54;80). Shin at al. (171), however, failed to see an increase in systemic GLP-1 in the first 90 min after 5 ml meals of Intralipid, which is high in
both fat and sugar. Whether GLP-1 increased later under either their or our conditions warrants further research.

The abrupt decrease of HPV GLP-1 to baseline levels shortly after meal termination (while insulin remained elevated) was somewhat surprising because pre-absorptive nutrient stimuli were certainly still present in the lumen of the small intestine. In some previous rat (38;88) and human (80) studies transient increases in circulating GLP-1 were reported. However, these findings can not be directly compared with the present study because the authors used a different time frame, i.e., performed the first measurement later than in the present study (38;88), collected blood samples from blood vessels other than the HPV (80), did not use real meals as stimuli (80;80;38;32), or measured total rather than active GLP-1 (38). The rapid degradation of GLP-1 by capillary DPP-IV as well as the relatively small amount of fat that the 3 g chow meal contained may have contributed to the comparatively small and short increase in HPV active GLP-1 that we saw. Other factors may have also played a role. Somatostatin, for instance, which is also released during a meal by osmotic and acidic stimuli (158), powerfully inhibits GLP-1 secretion by a paracrine action (79).

Mimicking the real meal situation as closely as possible is important for the evaluation of the possible contribution of endogenous GLP-1 to satiation (1;32;56;99;96;161;299;15). Intestinal GLP-1 release is influenced by various meal-related stimuli (41;86). Furthermore, an important criterion for the physiological relevance of any effect is that the hormone is available at the time and presumed site of action (64). Thus, in order to contribute to satiation, GLP-1 has to be released during the meal. Here we observed that active GLP-1 levels in the HPV peaked 6 min after meal onset. Thus, the increase in GLP-1 in the HPV occurred in a time frame that would permit endogenous intestinal GLP-1 to contribute to satiation. In contrast, VC GLP-1 levels did not increase in relation to the meal. Together, these findings suggest that any contribution of endogenous GLP-1 to satiation under the present test conditions could not be due to a systemic
endocrine effect. If endogenous GLP-1 contributes to physiological satiation under these conditions, it has to be by a hepatic or pre-hepatic action.

An endocrine effect of GLP-1 on vagal afferents terminating in the HPV or the liver has been shown to be involved in the insulinotropic (=incretin) effect of GLP-1 (10). However, such an endocrine action is unlikely to contribute to the satiating effect of endogenous intestinal GLP-1 because we (161) and others (96) observed that HPV GLP-1 infusion failed to inhibit eating more potently than VC or jugular vein infusions. Also, subdiaphragmatic vagal deafferentation (SDA), the most selective surgical procedure to lesion abdominal vagal afferents (142), failed to block the satiating effect of GLP-1 infused into the HPV (161). Together these data suggest that intravenously infused GLP-1 acts centrally to inhibit eating. The present demonstration that a meal elicited an increase in HPV, but not systemic, levels of GLP-1 provides further, indirect support for this interpretation. That is, infusion of 1 nmol/kg GLP-1 into the HPV increased VC GLP-1 three min after the start of the infusion to a level that was much higher than any increase in circulating active GLP-1 observed in the HPV during a meal. Thus, even a comparatively low dose of GLP-1 infused into the HPV produces systemic increases in circulating GLP-1 that appear large enough to elicit a central effect. By the same token, however, the lack of a systemic increase in circulating endogenous GLP-1 during a real meal argues against the physiological relevance of the eating-inhibitory effect of intravenously infused GLP-1 under our conditions. Further studies should critically examine this point. The meal-induced increases in plasma glucose and Insulin levels were consistent with previous findings obtained during chow meals in rats under similar conditions (56;57). The 3 g chow meal increased HPV and VC glucose and insulin, and for glucose the increase in the HPV was greater than in the VC. For insulin this difference did not reach statistical significance for single time points, but the AUC of HPV insulin was significantly greater than that of VC insulin. Assuming that active GLP-1 has an endocrine incretin effect (86) under our conditions, the data indicate that it arose in or before the liver, that the short and transient increase of active GLP-1 in the HPV was sufficient for it, and that elevated
circulating GLP-1 is not necessary to maintain the later phase of insulin secretion in response to a meal.

GLP-1 alone acutely increased systemic insulin levels slightly, but significantly. Interestingly, however, the AUC for insulin after the GLP-1 infusion was only a fraction of the AUC for insulin in response to the meal, indicating that even HPV infusion of a GLP-1 dose that substantially increased systemic levels of GLP-1 and reliably reduced food intake under similar conditions (17;19;31) does not mimic the meal-related stimulation of insulin secretion. In contrast to insulin, glucose levels were slightly, but significantly, decreased at 5 and 10 min after GLP-1 administration compared to Veh treatment and baseline. These findings are consistent with previous reports indicating that at basal glucose levels GLP-1 administered into the HPV has only a weak effect on circulating insulin (10). Overall, our data support the hypothesis that GLP-1 synergizes with glucose to increase insulin release (10;140) and that, because of this synergism, GLP-1 by itself does not induce hypoglycemia (53;126).

In summary, we show for the first time that the plasma concentration of active GLP-1 increases in the HPV but not the VC during a normal chow meal in the rat and that HPV infusion of GLP-1 at a dose previously sufficient to inhibit eating increased VC GLP-1 much more than the chow meal increased HPV GLP-1. Together with the observed changes in plasma insulin and glucose concentrations, these data suggest that GLP-1 has a physiologically relevant insulinotropic (=incretin) effect of GLP-1, presumably due to a vagally mediated synergistic effect of GLP-1 and glucose. The rapid meal-contingent increase in HPV GLP-1 is also consistent with a physiologically relevant hepatic or pre-hepatic satiating effect of endogenous intestinal GLP-1. Previous findings suggest that this effect is related to a paracrine action of endogenous GLP-1 on intestinal vagal afferents rather than an endocrine effect of GLP-1, but further studies are necessary to clarify this issue and to determine whether GLP-1 also synergizes with glucose to inhibit eating or has just a permissive effect on satiation.
8 General Discussion

The incretin effect of intestinal GLP-1 is well established, and the site of GLP-1's action as well as the physiological relevance of this metabolic effect are well documented (10;10;49;59;98;103;169;191). In contrast, the physiological relevance of intestinal GLP-1 in the control of food intake (32;96;199), and possible sites of actions in this context (1;78;79;93;96), remain elusive.

The present thesis further investigated these issues using spontaneously eating rats as a translational model. For a thorough discussion of the major open questions in relation to the role of endogenous GLP-1 in the control of eating, it is necessary to know 1) the plasma concentrations of GLP-1 during and after a real, mixed-nutrient meal 2) the effect of GLP-1 administrations that mimic the release of endogenous GLP-1 on eating and on plasma levels of GLP-1, glucose and insulin in the systemic circulation, and 3) the effects of GLP-1 receptor antagonism during spontaneous meals on eating. Despite several years of research into the eating-inhibitory action of GLP-1, surprisingly few such data have been reported for rats. We conducted three series of experiments in an attempt to close this gap.

In the first series of experiments the effects of intrameal HPV infusions of GLP-1 on spontaneous eating and the effects of HPV and Intraperitoneal (IP) GLP-1 infusions on eating in rats after subdiaphragmatic vagal deafferentation (SDA) or sham surgery (Sham) were compared. We found that HPV and IP infused GLP-1 primarily reduced meal size, which is consistent with the hypothesis that GLP-1 induces satiation. Furthermore, IP administered GLP-1 depended on intact vagal afferents to reduce meal size, whereas HPV administered GLP-1 did not. This indicates that peripherally administered GLP-1 can inhibit eating through receptors located in at least two different sites, of which one, that is accessible for IP administered GLP-1, requires intact vagal afferents to signal the brain, whereas the other one, that is accessible for GLP-1 from the HPV, does not. Also, GLP-1 inhibited eating with equal potency after HPV and VC infusion. Kim et al. (96) reported that GLP-1 reduced food intake after infusion into the jugular vein even
more potently than after infusion into the HPV. Together these findings suggest that intravenously infused GLP-1 acts directly in the brain to inhibit eating, whereas IP administered GLP-1 may act on vagal afferents terminating in the lamina propria of the intestinal mucosa. A central site of action for exogenous circulating GLP-1 to inhibit eating is also supported by our finding that HPV infused GLP-1 increased GLP-1 levels in the VC, which did not increase during and after a regular chow meal. Infusion of GLP-1 into the HPV increased systemic GLP-1 levels much more than a meal increased HPV GLP-1 concentrations. This indicates that even a comparatively low dose of GLP-1 infused into the HPV leads to systemic GLP-1 levels that are high enough to reach the brain despite the rapid degradation of circulating GLP-1 by DPP-IV (41;42;127). On the other hand, given its dependence on subdiaphragmatic vagal afferents (1;161), IP infused GLP-1 must act in the abdomen to inhibit eating, and the most likely site appears to be GLP-1 receptors on vagal afferents terminating in the lamina propria of the intestinal mucosa. Therefore, IP administration of GLP-1 may mimic a paracrine action of endogenous GLP-1. A similar intestinal paracrine action has been implicated in the satiating effect of endogenous CCK (36;176).

Whereas IP administered GLP-1 inhibits eating by acting through vagal afferents signaling to the NTS, circulating GLP-1 was shown to pass the BBB by active transport (93) and at the AP (145), a CVO in the hindbrain that contains GLP-1 receptors. GLP-1 may also directly activate receptors in the NTS, i.e., in close proximity to the AP. A hindbrain site of action for GLP to inhibit eating is suggested by the finding that GLP-1R blockade in the NTS (78) increased food intake whereas it failed to do so in the Arc (162). Also, the effect of IP administration of the GLP-1R agonist Ex (4-39) on eating, sympathetic and parasympathetic responses was not modified in chronic decerebrate (CD) rats (79), an animal model in which the neural connections between hindbrain and forebrain are disconnected, indicating that hindbrain circuitries are sufficient for these effects. Furthermore, experiments in our laboratory recently showed that the infusion of 1 nmol/kg GLP-1 into the HPV increased the number of cells expressing c-Fos protein, a marker of neuronal activation, in the AP, the NTS and the central area of
General Discussion

the amygdale (CeA), but not in the in the hypothalamic Arc or PVN (Baumgartner et al., in preparation). These findings implicate the hindbrain and the CeA also in the effects of intravenously administered GLP-1.

The finding that HPV GLP-1 infusion alone at basal glucose levels transiently increased systemic insulin levels and slightly reduced systemic glucose levels is in line with previous findings about the incretin effect of GLP-1 (10;49;103;112). However, the insulin release after GLP-1 infusion was only a fraction of the insulin release after a meal, when glucose levels increased. This provides further evidence that GLP-1 synergizes with glucose to release insulin (10).

During a real chow meal we measured an increase in GLP-1 levels in the HPV but not in the VC. This supports previous findings (10;180) indicating that GLP-1 released from the intestine exerts its eating-inhibitory and metabolic actions prior to the liver. Our findings of differential modes of actions of GLP-1’s eating inhibitory effect after IP or HPV administration suggest that, if intestinal GLP-1 is physiological relevant for satiation, the site of action is at the level of the intestine. Presumably, GLP-1 receptors on intestinal vagal afferents terminating in the lamina propria of the mucosa are involved. The physiological relevance of this effect remains open to discussion, however, because in our hands under the same conditions IP administered Ex (9-39) failed to increase food intake, although it blocked the satiating effect of exogenous GLP-1. Also, the lack of a dose response curve after IP and HPV administration does not support a physiological relevance of GLP-1 in satiation because satiation in response to food is a graded response (123). Reports of the effects of GLP-1 antagonism on eating are conflicting: whereas Williams et al. reported an increase of food intake after IP Ex (9-39) (200), others failed to see such an effect after IV administration of (32;96), another GLP-1R antagonist (96), and GLP-1R deficient mice do not display altered body weight or eating behavior (169). Our own findings, however, do not disprove that endogenous GLP-1 has a physiologically relevant role in satiation. As our IP Ex (9-39) infusions started 2-3 min after meal onset, Ex (9-39) may have reached the
critical receptor sites in the lamina propria too late or in too low a concentration to effectively compete with endogenous GLP-1 released from the L-cells in close proximity (161). Also, GLP-1 may well have a permissive effect in satiation or interact with other hormones or metabolites, and both features might account for the failure of Ex (9-39) to stimulate eating under the conditions of our experiment. So far, GLP-1 has been shown to synergize with leptin to inhibit eating (199) and with glucose to release insulin (10), but further interactions are well feasible.

In summary, our findings do not support a physiological role for endogenous intestinal GLP-1 in satiation, but they strongly suggest that GLP-1 receptors on vagal afferents terminating in the lamina propria of the intestinal mucosa may mediate a possible effect of intestinal GLP-1 on eating. Further experiments are necessary to clarify the open issues. To examine whether circulating GLP-1 acts in the hindbrain to inhibit eating it is necessary to test whether the effect of HPV infused GLP-1 on food intake is blocked after GLP-1R antagonism in the hindbrain. Also, the meal-contingent changes in circulating GLP-1 should be measured with high carbohydrate, high fat meals, i.e., in situations which presumably stimulate a greater GLP-1 release that the chow meal used as a stimulus in our experiment. Finally, further studies should pursue the role of receptors on vagal afferents terminating in the lamina propria of the mucosa. Possible experiments in this context include administrations of GLP-1 or Ex (9-39) that specifically target the distal jejunum and the ileum, i.e., the major sites of GLP-1 release.
9 References


10. **Balkan B, Li X** 2000 Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. 1. Am J Physiol Regul Integr Comp Physiol 279:R1449-R1454


12. **Banks WA** 2006 The blood-brain barrier as a regulatory interface in the gut-brain axes. 1. Physiol Behav 89:472-476


14. **Beglinger C, Degen L** 2004 Fat in the intestine as a regulator of appetite--role of CCK. 1. Physiol Behav 83:617-621


References


20. **Berthoud HR** 2006 Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. 3. Obesity (Silver Spring) 14 Suppl 5:197S-200S


 References


References


36. Cox JE 1998 Cholecystokinin satiety involves CCKA receptors perfused by the superior pancreaticoduodenal artery. 1 76. Am J Physiol 274:R1390-R1396


40. Deacon CF 2004 Circulation and degradation of GIP and GLP-1. 34 70. Horm Metab Res 36:761-765

References


44. **Dezaki K, Sone H, Yada T** 2008 Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. 1. Pharmacol Ther 118:239-249

45. **Dockray GJ** 2009 Cholecystokinin and gut-brain signalling. 1. Regul Pept 155:6-10

46. **Duggan JP, Booth DA** 1986 Obesity, overeating, and rapid gastric emptying in rats with ventromedial hypothalamic lesions. 1. Science 231:609-611


50. **Eisen S, Davis JD, Rauhofer E, Smith GP** 2001 Gastric negative feedback produced by volume and nutrient during a meal in rats. 1. Am J Physiol Regul Integr Comp Physiol 281:R1201-R1214
References


58. **Gamse R** 1982 Capsaicin and nociception in the rat and mouse. Possible role of substance P. 1. Naunyn Schmiedebergs Arch Pharmacol 320:205-216
References


62. **Geary N** 2004 Endocrine controls of eating: CCK, leptin, and ghrelin. 5. Physiol Behav 81:719-733


67. **Goke R, Larsen PJ, Mikkelsen JD, Sheikh SP** 1995 Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. 36. Eur J Neurosci 7:2294-2300


69. **Grill HJ** 2006 Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. 1. Obesity (Silver Spring) 14 Suppl 5:216S-221S
References


78. Hayes MR, Bradley L, Grill HJ 2009 Endogenous hindbrain GLP-1 receptor activation contributes to the control of food intake by mediating gastric satiation signaling. 64. Endocrinology


82. Hillebrand JJ, de Wied D, Adan RA 2002 Neuropeptides, food intake and body weight regulation: a hypothalamic focus. 9 83. Peptides 23:2283-2306


References


96. **Kim DH, D’Alessio DA, Woods SC, Seeley RJ** 2009 The effects of GLP-1 infusion in the hepatic portal region on food intake. 66. Regul Pept


99. **Konner AC, Klockener T, Bruning JC** 2009 Control of energy homeostasis by insulin and leptin: targeting the arcuate nucleus and beyond. 1. Physiol Behav 97:632-638


105. **Langhans W** 1991 Hepatic and intestinal handling of metabolites during feeding in rats. 2. Physiol Behav 49:1203-1209
References


113. Leibowitz SF 1978 Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. 3. Pharmacol Biochem Behav 8:163-175
References

114. Lenard NR, Berthoud HR 2008 Central and peripheral regulation of food intake and physical activity: pathways and genes. 1. Obesity (Silver Spring) 16 Suppl 3:S11-S22


References


130. Mollet A, Gilg S, Riediger T, Lutz TA 2004 Infusion of the amylin antagonist AC 187 into the area postrema increases food intake in rats. 1. Physiol Behav 81:149-155
References


132. **Moran TH, Baldessarini AR, Salorio CF, Lowery T, Schwartz GJ** 1997 Vagal afferent and efferent contributions to the inhibition of food intake by cholecystokinin. 44. Am J Physiol 272:R1245-R1251


138. **Naslund E, Hellstrom PM** 2007 Appetite signaling: from gut peptides and enteric nerves to brain. 1. Physiol Behav 92:256-262

References


144. **Oesch S, Ruegg C, Fischer B, Degen L, Beglinger C** 2006 Effect of gastric distension prior to eating on food intake and feelings of satiety in humans. 3 71. Physiol Behav 87:903-910


References


References


160. Ruttimann EB, Arnold M, Geary N, Langhans W 2009 GLP-1 Antagonism with Exendin (9-39) Fails to Increase Spontaneous Meal Size in Rats. Physiol Behav in Review:


References


168. Scott KA, Moran TH 2007 The GLP-1 agonist exendin-4 reduces food intake in nonhuman primates through changes in meal size. 6. Am J Physiol Regul Integr Comp Physiol 293:R983-R987


References


173. Simerly RB 2008 Hypothalamic substrates of metabolic imprinting. 1. Physiol Behav 94:79-89


References


184. **Talsania T, Anini Y, Siu S, Drucker DJ, Brubaker PL** 2005 Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. 19. Endocrinology 146:3748-3756


References


200. **Williams DL, Baskin DG, Schwartz MW** 2008 Evidence that intestinal GLP-1 plays a physiological role in satiety. 62. Endocrinology

201. **Woods SC** 2009 The control of food intake: behavioral versus molecular perspectives. 2. Cell Metab 9:489-498


Abbreviations

10 Abbreviations

AgRP  Agouti-related protein
Arc  Arcuate nucleus
AP  Area postrema
BBB  Blood-brain-barrier
BW  Body weight
CCK  Cholecystokinin
CD  Decerebrated rats
DPP-IV  Dipeptidyl peptidase-4
DMH  Dorsomedial nucleus
DMV  dorsal motor nucleus of the vagus
Ex-4  Exendin (4-39)
Ex (9-39)  Exendin (9-39)
GLP-1  Glucagon-like peptide-1
GLP-1R  Glucagon-like peptide-1 receptor
HPV  Hepatic portal vein
IP  Intraperitoneal
LHA  Lateral hypothalamic area
α-MSH  Alpha- melanocyte stimulating hormone
NPY  neuropeptide Y
NTS  Nucleus tractus solitarii
POMC  proopiomelanocortin
PVN  Paraventricular nucleus
SDA  Subdiaphragmatic vagal deafferentation
sham  Sham surgery
VC  Vena cava
VMH  Ventromedial nuclei
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