Peptides that Regulate Food Intake
Glucagon-like peptide 1-(7–36) amide acts at lateral and medial hypothalamic sites to suppress feeding in rats

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Glucagon-like peptide 1-(7–36) amide (GLP-1) potently inhibits feeding behavior after injection into the lateral hypothalamus (LH), dorsomedial hypothalamus (DMH), and ventromedial hypothalamus (VMH). Within the hypothalamus, GLP-1-containing nerve fibers and terminals have been shown to be located in the paraventricular nucleus of the hypothalamus (PVN), the ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), and lateral hypothalamus (LH), well known as integrative areas for feeding regulation (12, 15, 23). In addition, GLP-1 is present in the amygdala (15), a brain region providing neuronal input to the hypothalamus and participating in the regulation of ingestive behavior (3, 9, 21, 42). In all these areas, the presence of GLP-1 receptors has recently been demonstrated (11). GLP-1 might therefore play a regulatory role in the central control of feeding behavior. Previously, GLP-1 has been shown to potently inhibit rat feeding behavior after injection into the lateral cerebral ventricle with a minimal effective dose of 1 μg (41, 48), while after application into the third ventricle GLP-1 appears to be less effective only at a threefold higher dose (7, 53). This is somewhat surprising because important regulatory areas (e.g., PVN or ventromedial nucleus of the hypothalamus) are located

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in the immediate vicinity of the third ventricle and application of GLP-1 into the PVN has been shown to inhibit food intake (25, 26).

While feeding effects observed after ventricular injection of GLP-1 may be attributed to sites of action in the vicinity of the ventricular lumen (e.g., PVN, VMH, DMH), specific conclusions, however, regarding at which brain sites GLP-1 exerts its feeding-suppressive effects cannot be drawn. To further address this issue, microinjection experiments are required by which GLP-1 can be administered directly into brain tissue. Up to now, microinjection data are only available for the PVN (25, 26). Thus it is presently not known whether hypothalamic regions other than the PVN also mediate GLP-1-induced feeding suppression.

Besides the periventricularly located medial hypothalamic nuclei, particularly the LH deserves special attention. The LH has previously been shown to be a physiologically relevant brain area mediating neuronal activity correlated with feeding termination and satiety (38). Thus CCK, another important regulatory neuropeptide, effectively suppresses feeding behavior in LH sites, both exogenously injected (34) and endogenously released (36). Moreover, CCK terminals in the LH do in fact release CCK both after vagal stimulation (38) and after a physiological satiety stimulus, such as a gastric meal load (39, 40). Finally, we have previously demonstrated relevant LH-mediated feeding effects induced by other neuropeptides, such as galanin (35) or neuropeptide Y (37).

Moreover, the amygdala has been demonstrated to participate in the central control of pancreatic exocrine secretion (28) and gastric acid secretion (18) and thus may represent an important part of the so-called brain-gut axis, regulating gastrointestinal function (including ingestion of food) by central integrative processes. On the basis of lesion studies, this extrahypothalamic brain region has previously been implicated in the central control of feeding behavior (9). Because both GLP-1 terminals and GLP-1 receptors are also localized in the amygdala, it was accordingly of interest to additionally look at the amygdala as a potential site of action.

Finally, the intracerebroventricular and the local (PVN) application of a specific GLP-1 receptor antagonist stimulates food intake, suggesting that endogenously released GLP-1 is also of importance (27, 50).

Accordingly, the present study was designed first to reexamine the effect of third ventricular compared with lateral ventricular application of GLP-1, second to determine if hypothalamic loci other than the PVN or the extrahypothalamic amygdala represent brain areas at which GLP-1 contributes to the regulation of food intake, and third to determine the physiological significance of locally released GLP-1 by hypothalamic microinjections of the specific GLP-1 receptor antagonist exendin-(9-39) amide.

MATERIALS AND METHODS

Experiments were performed in male Wistar rats (body wt 280–320 g; Thomae, Biberach, Germany) that were individually housed in a room with artificial 12:12-h dark-light cycle (lights on from 7:00 AM to 7:00 PM). The rats were maintained on hard rat chow pellets (Altromin, Lage, Germany) and tap water, both available ad libitum. Fasted animals were food deprived for 24 h with free access to tap water, while satiated animals were freely feeding with access to food and water at all times. All experiments were started at 8:00 AM. In addition, our article follows the newest guiding principles of research as recently published by the American Physiological Society (1).

Feeding Bioassay

Groups of animals were trained in three (2 preoperative and 1 postoperative) sessions to feed in single compartments of a divided Plexiglas cage (66 × 27 × 20 cm). Before placement in the observation cage, the rats were handled as if they were to receive injections, to get them acquainted with the injection procedure. Subsequently, the animals had access to a preweighed amount of rat chow pellets and tap water in graduated drinking tubes. This food was the same as that to which the animals had normal daily access. The net amounts of food and water consumed after availability of food were measured in 20-min intervals. This allowed one to assess both the overall cumulative food intake and the rate of feeding (i.e., food consumption per 20 min). The feeding duration was assessed by recording for each 1-min interval whether the animals were feeding or not. This measurement was then expressed as percentage of time spent while feeding. This provides information whether the animals stop feeding totally for a certain time period or whether feeding behavior is associated with a rather steady reduction of the amount of food consumed without substantial changes in feeding activity.

Surgery

Between the second and third training session, rats underwent stereotaxic implantation of 24-gauge stainless steel, thin-wall guide cannulas under ketamine anesthesia (50 mg ip per rat; Ketamin-50-Curamed, Curamed Pharma, Karlsruhe, Germany). Cannulas were unilaterally placed either 0.5 mm above the right lateral ventricle or 1.5 mm above the third cerebral ventricle or the projected brain tissue injection sites, respectively. Stereotaxic coordinates according to de Groot’s system A as taken from the atlas by Pellegrino et al. (32) were as follows (anterior-posterior/lateral/horizontal-vertical; in mm): 5.4/1.0/3.0 (lateral ventricle), 6.0/0.5/–2.0 (3rd ventricle), 6.0/2.2/–2.5 (LH), 6.0/0.75/–3.0 (VMH), 5.6/0.5/–1.8 (DMH), or 5.0/3.5/–3.2 (amygdala). The guide cannulas were inserted through a burr hole in the skull and fixed with skull screws and cranioplast cement (Fastcure, Kerr Laboratory Products Division, Romulus, MI). Each guide was occluded with a 30-gauge stainless steel styllet.

Drugs and Injections

After at least 1 wk of recovery and after a postoperative (3rd) training session, groups of 24-h-fasted rats received GLP-1 [GLP-1-(7–36)amide; mol wt = 3,298 g/mol; Peninsula Laboratories, Belmont, CA] either as lateral ventricular (n = 7) or brain tissue injections (n = 44). For lateral ventricular injections, GLP-1 at either 10, 1, or 0.1 μg, dissolved in 10 μl saline, or 10 μl saline (control) was injected, respectively. For third ventricular injections, GLP-1 at 1 μg, dissolved in 0.5 μl saline, or 0.5 μl saline (control) was injected, respectively. For brain tissue
injections, either GLP-1 at 1 or 0.3 µg, respectively, dissolved in 0.5 µl saline, or 0.5 µl saline (control) was injected.

The local action of the GLP-1 receptor antagonist exendin-(9–39) amide (mol wt = 3,368 g/mol; Peninsula Laboratories) was examined after its injection into the LH. Exendin-(9–39) amide was either tested in 24-h food-deprived rats (i.e., fasted) or in freely feeding animals (i.e., satiated). Fasted rats (n = 8) received LH injections of either exendin-(9–39) amide at 2.5 or 1 µg or 0.5 µl saline, respectively. A first group of satiated rats (n = 8) received either a single LH injection of 1 µg exendin-(9–39) amide or 0.5 µl saline, respectively. A second group of satiated rats (n = 6) received three LH injections of 2.5 µg exendin-(9–39) amide every 20 min (i.e., a total of 7.5 µg) or three injections of 0.5 µl saline, respectively. In all experiments, each animal served as its own control, receiving all injections according to a randomized crossover design. GLP-1 or exendin-(9–39) amide was freshly dissolved in saline on each experimental day.

Microinjections were performed during 30 s with a gear-driven microliter syringe connected to a stainless steel injector that protruded the tip of the lateral ventricular guides by 1.5 mm and the tip of the third ventricular or brain tissue guides by 1.5 mm. After the injections, the injector was left in place for a further 30 s after which it was removed and the obturator replaced. Immediately after injection, the animals were placed in the observation cage where food pellets and water were available. Feeding behavior was then recorded as described above. After termination of the experiments, a volume of india ink identical to the respective experiment was injected through the injection cannulas, and the brains were subsequently removed. Brains in which lateral ventricular injections had been performed were examined by knife cuts to verify the presence of india ink in the ventricular lumen. Brains in which third ventricular or brain tissue injections had been carried out were blocked, and the blocks containing the cannula tracts were embedded in paraffin, cut at 10 µm, and stained with Luxol fast blue. The deepest site containing india ink as identified by histology was taken as the site of injection. Furthermore, the spread of india ink was examined, and an overlap to other sites could be excluded. Histological identification of all microinjection sites revealed that 16 injection sites lay in the LH, 11 in the VMH, 6 in the DMH, and 11 in the amygdala. Groups of injections into the same brain locus were analyzed statistically as outlined below.

**Statistics**

Food intake (g) and percentage of time spent while feeding are expressed as means ± SE. Results obtained after GLP-1 or exendin-(9–39) amide injections were compared with those after saline injection by ANOVA followed by Student’s t-test for post hoc test for calculation of statistically significant differences between individual treatments (4). P values of <0.05 were considered significant.

**RESULTS**

**Effect of Intracerebroventricular Injections of GLP-1 on Food Intake in 24-h-Fasted Rats**

Dose response after lateral ventricular injection. After injection of GLP-1 at the highest dose of 10 µg, food consumption during the first 20 min after injection was significantly reduced by 50% (0.7 vs. 1.4 g; F1,34 = 7.94; P < 0.01; Fig. 1B). This initial effect was maintained during the ensuing 20-min intervals (20–40 min: 0.4 vs. 1.3 g; 40–60 min: 0.4 vs. 1.3 g), which resulted finally in a 60% reduction after 60 min (1.5 vs. 3.7 g; F1,34 = 13.75; P < 0.01). Overall food intake after 240 min was still significantly reduced by 30% (6.1 vs. 8.7 g; F1,34 = 4.31; P < 0.05; Fig. 1B).

The inhibitory effect of GLP-1 was clearly dose dependent (food intake, 0–20 min: F3,68 = 5.77; P < 0.01). The data obtained after injection of 10, 1, or 0.1 µg are summarized in Fig. 1. Food intake (0–20 min) was significantly reduced by 36% at 1 µg (F1,34 = 7.28; P < 0.05), while no significant suppression of food consumption was observed at 0.1 µg (1.2 vs. 1.4 g). Thus 1 µg was the minimal effective dose of GLP-1 to suppress feeding in 24-h-fasted rats.

The GLP-1-induced suppression of food intake was paralleled by a significant reduction in time spent while feeding (Fig. 1A).

**Injections of 1 µg GLP-1 into the lateral and third cerebral ventricles.** In saline-injected 24-h-fasted rats (control experiments), food consumption was identical after both lateral and third ventricular injection. Thus, in control experiments, the amount of food consumed 20 min after saline injection was 1.4 g (both lateral and third ventricular), after 60 min 3.7 g (lateral ventricular) or 3.6 g (third ventricular), and after 120 min 6.0 g (lateral ventricular) or 5.6 g (third ventricular), respectively. Accordingly, in Fig. 2, only data obtained after third ventricular saline injection are presented as control values.
was no significant difference in the feeding-suppressive effect of GLP-1 between third and lateral ventricular application (Fig. 2).

**Effect of Brain Tissue Injections of GLP-1 on Food Intake in 24-h-Fasted Rats**

**Distribution of microinjection sites.** Microinjections were performed at three hypothalamic loci, i.e., the LH, the VMH, and the DMH, and one extrahypothalamic region, the medial amygdala. Histological verification of the microinjection sites in all experiments revealed that 16 sites lay in the LH, 11 in the VMH, 6 in the DMH, and 11 in the medial amygdala. Figure 3 depicts histological photomicrographs of representative microinjection sites for all brain regions examined, as well as the anatomic localization of these brain regions on three schematic drawings of coronal sections through the rat brain (Fig. 3E).

**Mapping results (intracerebral injections of 0.3 or 1 μg GLP-1).** LH injection of 1 μg GLP-1 resulted in a significant reduction of food intake compared with saline injection by 43% during 0–20 min (1.2 vs. 2.1 g), by 44% during 20–40 min (0.9 vs. 1.6 g), and by 40% during 40–60 min (0.6 vs. 1.0 g), respectively. The overall reduction of food intake was 43% after 60 min ($F_{1,30} = 16.62; P < 0.01$) or 33% after 120 min ($F_{1,30} = 13.61; P < 0.01$), all differences being statistically significant (Fig. 4). Similar to the experiments with intraventricular GLP-1 injection, the percentage of time spent while feeding was significantly reduced parallel to the reduction of the amount of food consumed ($P < 0.05$). The lower dose of 0.3 μg GLP-1 also significantly suppressed feeding behavior at LH injection sites, i.e., by 33% after 20 min (1.4 vs. 2.1 g; $F_{1,30} = 7.26; P < 0.05$), by 28% after 60 min (3.4 vs. 4.7 g), or by 27% after 120 min (5.3 vs. 7.3 g) ($P < 0.05$; Fig. 4).

A significant suppression of food intake was further observed after microinjection of 1 μg GLP-1 into the VMH or DMH. While DMH injection of 1 μg GLP-1 resulted in a significant reduction of food intake at 0–20 min by 48% (1.1 vs. 2.1 g; $F_{1,10} = 8.56; P < 0.05$), which was comparable to the feeding-suppressive effect observed after LH injection, the reduction of the amount of food consumed after 120 min was no longer statistically significant. After VMH injection, GLP-1 significantly reduced food intake by 30% after 20 min ($F_{1,20} = 4.61; P < 0.05$), by 20% after 60 min, and by 16% after 120 min ($P < 0.05$). In contrast to the LH, at both medial hypothalamic loci, the lower dose of 0.3 μg GLP-1 was ineffective (Fig. 4).

No alteration of feeding behavior was observed when GLP-1 was injected into the medial amygdala (Fig. 4).

**Effect of LH Injections of the Specific GLP-1 Receptor Antagonist Exendin-(9–39) Amide on Food Intake**

**Twenty-four-hour fasted rats.** In 24-h-fasted rats, injection of exendin-(9–39) amide into the LH did not significantly alter deprivation-induced feeding compared with control experiments (i.e., saline injection), either at 1 μg (Fig. 5B) or at a higher dose of 2.5 μg.

**Freely feeding rats.** In freely feeding (i.e., satiated) rats, however, LH injection of 1 μg exendin-(9–39) amide resulted in a significant increase in food consumption, but only during the first 20 min (0.6 vs. 0.1 g; $F_{1,14} = 6.86; P < 0.05$) (Fig. 5A). In view of this significant but shortlasting effect, we hypothesized that an increase in the dose of exendin-(9–39) amide might lead to an even stronger feeding stimulation. Because drug solubility, however, was limited to a concentration of 2.5 μg per 0.5 μl (injection volume), we attempted to administer a total dose of 7.5 μg by giving three sequential injections of 2.5 μg each at 20, 40, and 60 min. In these experiments, a clear stimulation of feeding behavior was observed during the entire 60-min observation period (Fig. 6). Thus repeated LH injections of exendin-(9–39) amide enhanced the overall percentage of time spent while feeding fourfold (17 vs. 4%), thereby causing a 300% increase in overall food consumption during the 60-min observation period compared with control experiments (1.3 vs. 0.3 g; $F_{1,10} = 20.93; P < 0.01$) (Fig. 6).

**DISCUSSION**

GLP-1 and a high density of GLP-1 receptors have previously been demonstrated in the brain, providing the morphological basis for a potential regulation of nutrient homeostasis not only by peripheral but also by central mechanisms involving GLP-1-containing neuronal systems (5, 11, 19, 20, 46, 47, 52).

This notion is further supported by mapping studies that have shown GLP-1 to be present in several brain areas known to be involved in the regulation of food intake. Thus GLP-1 is present in neurons connecting the gastrointestinal tract to the regulatory brain cen-
ters for ingestive behavior (23). However, synthesis of GLP-1 is exclusively localized to noncatecholaminergic neurons in the nucleus of the solitary tract, which send off fibers and terminals to hypothalamic nuclei (23). Within the rat hypothalamus, GLP-1-containing nerve fibers and terminals have been demonstrated in the preoptic, supraoptic, paraventricular, dorsomedial, ventromedial, and lateral hypothalamic area (15). Furthermore, the amygdala providing neuronal input to the hypothalamus also contains GLP-1 fibers (21). These data strongly suggest a functional role of GLP-1 in feeding regulation. Moreover, GLP-1 receptors have been demonstrated in all these brain areas mentioned (11).

In addition to this morphological evidence, functional studies have subsequently shown that intracerebroventricularly injected GLP-1 inhibits food intake in rats (22, 41, 48, 51), and this is confirmed by the present dose-response studies over a total experimental period of 4 h with a minimal effective dose of 1 μg. Application of GLP-1 into the third ventricle, however, appeared to be effective only at a clearly higher dose of 3–10 μg (7, 53). The third ventricle is of special interest because important hypothalamic areas for the regulation of feeding behavior are located in its immediate vicinity. The present data demonstrate that in our experimental design, third ventricular application of GLP-1 at a dose of 1 μg is similarly effective in suppressing food intake as it is after lateral ventricular injection.

The data on food consumption are paralleled by the data on time spent while feeding, which show a fairly even reduction of feeding activity during the observation period. These findings are in accord with the notion that the feeding-suppressive effect of GLP-1 is compatible with physiological satiety. This is further

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**Fig. 3.** Photomicrographs of representative microinjection sites identified on histological coronal sections through the rat brain. A: lateral hypothalamus (LH). B: ventromedial hypothalamus (VMH). C: dorsomedial hypothalamus (DMH). D: medial amygdala (AMY). Each arrow indicates tip of cannula through which the microinjections were accomplished. E: schematic drawings present 3 coronal sections at the diencephalic level [6.4 (a), 5.8 (b), or 5.2 mm (c)] according to de Groot's system A as taken from the atlas by Pelligrino et al. (32), illustrating the LH, VMH, DMH, and AMY microinjection target regions. Landmark structures are as follows: 3V, internal capsule (IC), optic tract (OT), fornix (F).

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supported by normal behavioral aspects of the animals (i.e., motor activity, grooming) during the entire observation period. It must be noted, however, that GLP-1 when administered intracerebroventricularly at a relatively high dose (10 μg) appeared to be an effective unconditioned stimulus when tested in a conditioned taste aversion (CTA) paradigm (49, 50). In the PVN, however, microinjections of lower doses of GLP-1 (0.1 or 0.2 μg) were not associated with a CTA response (26). Because in our study no paradigm was used to assess CTA, such effects cannot be ruled out completely.

When looking at studies in mice with disrupted GLP-1 receptor expression, the importance of GLP-1 in the control of food intake does not appear to be essential. These animals show normal eating behavior and weight gain while postprandial insulin secretion is attenuated (43–45). These observations emphasize the overall importance of GLP-1 as an incretin, whereas loss of the satiating effect of GLP-1 can obviously be compensated, presumably by the large number of other

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**Fig. 4.** Effect of hypothalamic or extrahypothalamic microinjections of GLP-1 at 1 or 0.3 μg or 0.5 μl saline (open bars) on cumulative food intake in 24-h-fasted rats, presented for the brain regions examined, i.e., LH (A; n = 16), VMH (B; n = 11), DMH (C; n = 6), and AMY (D; n = 11), as measured 20, 60, or 120 min after injection. *Significant difference vs. saline (P < 0.05).

**Fig. 5.** Effect of LH microinjections of the specific GLP-1 receptor antagonist exendin-(9–39) amide at a dose of 1 μg (solid bars) or 0.5 μl saline (control; open bars) on food intake of freely feeding (i.e., satiated) rats (A; n = 8) or 24-h-fasted rats (B; n = 8). *Significant difference vs. saline (P < 0.05).

**Fig. 6.** Effect of 3 repeated LH injections of the specific GLP-1 receptor antagonist exendin-(9–39) amide at a dose of 2.5 μg per each injection (●) or 0.5 μl saline (control, ○). As indicated by arrows and by the labeling, injections (Inj) were given at 0, 20, and 40 min in freely feeding (i.e., satiated) rats; (n = 6), each experiment starting at 8.00 AM. *Significant difference vs. saline (P < 0.05).
neurotransmitters involved in these regulatory processes.

Nevertheless, GLP-1 may play an important role in the acute regulatory process of feeding termination. Several lines of evidence suggest that GLP-1 most likely may act as a phasic rather than as a tonic regulatory factor. Thus 1) continuous intracerebroventricular application of GLP-1 has no effect on food intake or body weight compared with acute administration (7), 2) the GLP-1 agonist exendin-4 has been expressed in transgenic mice, resulting in prolonged GLP-1 receptor signaling without effect on food intake (2), and 3) permanent loss of GLP-1 receptor, as mentioned above, does not alter food intake and body weight (43–45). Effects similar to the latter have also been observed for stimulators of feeding behavior, e.g., prolonged vs. acute administration of melanin concentrating hormone has no effect on body weight, and similarly neur peptide Y-deficient mice show normal weight gain (13, 33).

Extensively performed morphological mapping studies within the hypothalamus have found GLP-1 to be localized in fibers and terminals of the ventromedial, the dorsomedial, and the paraventricular nuclei and also the lateral hypothalamic area, all of these regions being well known to contain neuronal mechanisms affecting ingestive behavior (30). The densest innervation by GLP-1-immunoreactive nerve fibers was found in the dorsomedial and paraventricular nuclei (23).

A functional role of GLP-1 within the hypothalamus has thus far been demonstrated for the PVN by McMahon and Wellman (25, 26) and by Choi and Anderson (6). The present data extend these previous findings by demonstrating that GLP-1 also affects ingestive behavior when given into other sites of the hypothalamus. In the VMH and DMH, the lowest effective dose was 1 μg, which is still somewhat higher than the previously reported dose of 0.1 and 0.2 μg in the PVN (25, 26). This might suggest that among these three medial nuclei the PVN is the most important site of action, at least with regard to GLP-1 effects. It is conceivable, though, that GLP-1 injected into the DMH or VMH could affect neurons in the PVN via diffusion. Supportive evidence against this possibility comes from the fact that india ink injected into the VMH or DMH does not reach other injection sites. However, it must be kept in mind that the spread of india ink and peptides may not entirely be comparable. On the other hand, peptide diffusion at least in other sites of the brain is not a major problem even at a very short distance as shown for vaspressin and its effect on thermoregulation (17).

In view of the effectiveness of identical doses of GLP-1 when given into the third ventricle or the various periventricular nuclei, it is somewhat surprising that parenchymally injected GLP-1 did not act at much lower doses. This could in part be due to the fact that after third ventricular injection, GLP-1 has access to all three periventricular nuclei (PVN, VMH, DMH), thus inducing synergistic effects, whereas direct tissue injection of GLP-1 may predominantly affect only distinct neurons in one single brain area.

Feeding suppression was also observed after GLP-1 injection into the LH. This is quite interesting, because the LH has previously been shown to play a relevant role in the gut-brain satiety loop. Thus ingestion of food and subsequent activation of gastrointestinal function (e.g., gastric distension) causes correlated neuronal CCK release in the LH (39, 40) via afferent vagal fibers (38). Moreover, endogenous CCK, locally released from LH neurons by meal intake, significantly contributes to suppression of ongoing feeding behavior (36). These findings clearly demonstrate the importance of LH neurons for termination of food intake and satiety. In conjunction with previous observations that stimulatory neuropeptides such as galanin (35) and neuropeptide Y (37) also act at the LH to influence feeding behavior, the present findings on GLP-1-induced feeding suppression in the LH underscore the relevance of this particular hypothalamic area as a projection site for peripheral satiety signals.

Besides hypothalamic regions, GLP-1 has also been shown to be present in the amygdala (15), which does not only provide afferent neuronal input to the hypothalamus via the amygdalofugal pathway (21) but also may play a role in the regulation of ingestive behavior (3, 9, 42). At the extrahypothalamic region of the amygdala, however, GLP-1 was not able to alter feeding behavior. These findings are consistent with our previous observation that CCK did not affect feeding at loci in the medial amygdala (34).

Having shown that exogenous GLP-1 exerts strong feeding-suppressive effects via the LH, it is clearly of great interest to determine whether under physiological conditions (i.e., free feeding) endogenous GLP-1 also affects food intake. This issue can be addressed by blockade of endogenous GLP-1 receptors by specific antagonists. Previously, it has been shown that intracerebroventricular application of the specific GLP-1 receptor antagonist exendin (9–39)-amide stimulates food intake, which emphasizes the relevance of endogenously released GLP-1 in the regulation of feeding termination (27, 50, 51). Our results indicate that in the LH, endogenously released GLP-1 significantly contributes to the regulation of feeding termination, similarly to what has recently been shown for the PVN (50).

Exendin-(9–39) amide-mediated GLP-1 receptor blockade resulted in an increased food intake only in freely feeding rats. This can be explained by the fact that after an overnight feeding period the rats were largely satiated, as shown by the low food intake at the start of the experiment, and accordingly, feeding behavior was probably suppressed by endogenously released GLP-1. In contrast, in fasted rats, driven to feed, the GLP-1 antagonist was ineffective. This may be attributed to the relatively short duration of action of exendin-(9–39) amide, which lasts only ~20 min (as can be derived from the experiments in freely feeding rats), and in this early postprandial period (i.e., first 20 min) satiety signals are presumably still of minor reg-
ultralim port. Our data obtained with LH injections of exendin-(9–39) amide are in good agreement with the findings by Turton et al. (51) that intracerebroventricularly injected exendin-(9–39) amide did not affect food intake in fasted rats, but more than doubled food intake in rats fed ad libitum (51). These findings support the hypothesis that endogenous GLP-1 indeed contributes to feeding termination and that the LH is an important site of action.

While a single injection of exendin-(9–39) amide is able to increase food intake of freely feeding rats during the first 20 min after injection, no prolonged effectivity can be observed during the next 20 min or even later. This suggests that the duration of action of LH-injected exendin-(9–39) amide is very short. We therefore administered the GLP-1 receptor antagonist exendin-(9–39) amide every 20 min and also chose to go up with the dose to the maximally possible concentration of 2.5 μg/0.5 μl (which represents the upper limit of drug solubility). With this experimental design, we were able to record a longlasting increase in feeding activity with a threefold higher amount of food consumed compared with control animals. Interestingly, exendin-(9–39) amide was effective when injected repeatedly, which suggests that the rapid decrease in the exendin-(9–39) amide effect is not due to neuronal insensitivity but rather a problem of degradation. The present data are in agreement with the notion that, in satiated rats, food intake is restricted in part by endogenous GLP-1 in the LH and extend a previous report demonstrating increased food intake and weight gain by repeated daily third ventricular injections of exendin-(9–39) amide (27).

In summary, our data demonstrate that GLP-1 acts at multiple hypothalamic sites to suppress feeding in rats. Moreover, endogenous GLP-1 contributes to feeding termination and satiety in the LH. These findings therefore strongly support a regulatory role for GLP-1 in the hypothalamic control of feeding and satiety.

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