Peripheral Versus Central Effects of Glucagon-Like Peptide-1 Receptor Agonists on Satiety and Body Weight Loss in Zucker Obese Rats

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The present study explores the potential utility of peripheral versus central administration of glucagon-like peptide-1 (GLP-1) receptor agonists in the regulation of feeding behavior in Wistar and Zucker obese rats. Acute central (intracerebroventricular [ICV]) and peripheral (subcutaneous [SC]) administration of both GLP-1 (7-36) amide and exendin-4 resulted in a reduction in food intake for at least 4 hours, exendin-4 being much more potent than GLP-1 (7-36) amide, especially after peripheral administration. Both Zucker obese rats (fa/fa) and their lean littermates (Fa/−) responded to acute central and peripheral administration of exendin-4. Moreover, in situ hybridization revealed specific labeling for the mRNA for GLP-1 receptors in several brain areas of both the obese and lean rats. The presence of this receptor was also detected by affinity cross-linking assays. Long-term SC administration of exendin-4 (1 single injection per day, 1 hour prior to the onset of the dark phase of the cycle) decreased daily food intake and practically blocked weight gain in obese rats. In contrast to previous studies, these findings show that peripheral (SC) administration of both GLP-1 receptor agonists also induces satiety and weight loss in rats, and suggest the potential usefulness of exendin-4 as a therapeutic tool for the treatment of diabetes and/or obesity.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) (7-36) amide, one of the multiple products of C-terminal processing of the mammalian proglucagon molecule,[1] exerts several biological effects on peripheral tissues and the central nervous system (CNS). This peptide stimulates insulin secretion in a glucose-dependent manner[2] and inhibits gastric acid secretion[3,4] and gastric emptying.[4] In addition, GLP-1 (7-36) amide increases the arterial blood pressure and heart rate in rats,[5,6] stimulates mucus secretion and pulmonary smooth muscle relaxation,[7] and increases surfactant secretion by rat type II pneumocytes.[8]

The proglucagon gene is also expressed in the brain, with the mRNA transcript[9] being identical to that produced in the endocrine pancreas and intestine, and gives rise to GLPs in the brain.[10,11] The synthesis of GLP-1 and the presence of a high GLP-1 receptor density in neurons of the same brain areas[12-16] suggest that GLP-1 has a local, as well as a more remote, signaling role in the CNS. GLP-1 receptor cDNAs from human[15] and rat[17] brain have been cloned and sequenced, and the deduced amino acid sequences are the same as those found in pancreatic islets. GLP-1 (7-36) amide released either from brain neurons or from intestinal L-cells that enters the CNS through the subfornical organ and the area postrema[18] or through active transport by the choroid plexus—which has a high density of GLP-1 receptors[17]—could be responsible for the previously described central effects of GLP-1 (7-36) amide on the selective release of neurotransmitters,[19,20] appetite,[21-23] fluid homeostasis,[22,23] and thermoregulation.[24] It is noteworthy that in the brain, GLP-1 (7-36) amide serves as a signal to reduce food intake. The colocalization of GLP-1 receptors, glucokinase, and GLUT-2 in hypothalamic neurons involved in feeding behavior[17,22] might play a role in glucose sensing prior to the onset of a state of fullness. Thus, increased glycemia after meals may be recognized by these hypothalamic neurons because of the high-K_m glucose transporter activity of GLUT-2 and the high-K_m glucose phosphorylation of glucokinase. Furthermore, metabolites arising during glucose oxidation in these neurons may be involved in the transduction of signals required to produce a state of satiety. Additionally, hypothalamic receptors may control energy expenditure by mediating the appetite-suppressing or -activating functions of central and peripheral substances such as leptin, glucocorticoids, insulin, galanin, or neuropeptide Y. In addition, GLP-1 (7-36) amide has been shown to exert an antidiabetogenic effect and could therefore be useful in the treatment[25,26] of diabetic patients.

Exendin-4 is a peptide isolated from *Heloderma suspectum* venom that shares 53% structural homology with GLP-1 (7-36) amide. This GLP-1 receptor agonist competes with GLP-1 (7-36) amide for the same binding sites and produces the same second-messenger and biological effects.[27] Exendin-4 has a more prolonged effect than GLP-1 (7-36) amide, suggesting that it could be used as a prototype for the development of clinically useful peptide agonists to treat diabetes mellitus and/or obesity. Although the action of exendin-4 lasts longer than that of GLP-1 (7-36) amide, probably due to its potential dipeptidyl peptidase IV (DPP-IV) resistance, the half-life of the peptide is unknown.

Obesity is the most prevalent nutritional disorder in the Western world. It is associated with hyperlipidemia, hypertension, coronary heart disease, and type 2 diabetes mellitus, with the latter association being very common. Since the effects of exendin-4 are similar to those of GLP-1 (7-36) amide but last longer, the present study was designed to determine the effects of central and peripheral administration of GLP-1 receptor agonists on feeding behavior in Wistar and Zucker obese rats.

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The Zucker rat was used because it is a useful animal model for studying obesity and insulin resistance.

An experimental model was designed in which the effects of GLP-1 (7-36) amide and exendin-4 on food intake and cumulative body weight (BW) were compared after both peripheral and central administration of these peptides. In contrast to the findings of previous studies, peripheral (subcutaneous [SC]) administration of both GLP-1 receptor agonists significantly modified the feeding behavior, with the effect of exendin-4 being more potent than that of GLP-1 (7-36) amide. The interest of these observations and the potential therapeutic use of these peptides are discussed.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 330 to 410 g and Zucker obese (fa/fa) animals and lean controls (Fa/−) aged 12 to 16 weeks (Panlab, Barcelona, Spain) and weighing 380 to 450 g and 294 to 349 g, respectively, at the start of the experiments were used in the studies. The rats were housed individually and maintained in a temperature- and light-controlled environment on a 12-hour light/dark cycle (lights on at 8 AM) with free access to food and water. Animals were allowed at least 2 weeks for acclimatization to the animal room. All procedures were performed according to European Community (EC) ethical regulations for animal research.

Plasma Chemistry and Hypothalamic Monoamine Content

Blood samples were obtained at 12 noon in overnight-fasted animals after SC administration of exendin-4 (250 ng/100 g BW). Plasma glucose, triglyceride, and creatinine levels were measured using a Hitachi (Tokyo, Japan) 737 automatic analyzer. Plasma insulin[28] and corticosterone[29] levels were measured by specific radioimmunoassays. Rat insulin standards were used for the insulin assay. Hypothalamic monoamine content was measured by high-pressure liquid chromatography (HPLC) with electrochemical detection (ED), using previously described methods.[29]

Surgical Procedures

For ICV administration of the different peptides, stainless steel guide cannulas aimed at the lateral ventricle were implanted in the rats. The animals were anesthetized with equithesyn and placed in a David Kopf stereotaxic instrument (Tujunge, CA) with the incisor bar set at 5 mm above the interaural line. A 7-mm guide cannula (23-gauge) was secured to the skull using 2 stainless steel screws and dental cement, and was closed with 30-gauge obturators. The implantation coordinates were 0.6 mm posterior to the bregma, ± 2.0 mm lateral, and 3.2 mm below the surface of the skull. These coordinates placed the cannula 1 mm above the ventricle. After a 7-day postsurgical recovery period, cannula patency was confirmed by the gravity flow of isotonic saline through an 8-mm long 30-gauge injector inserted within the guide to 1 mm beyond its tip. This procedure allowed the animals to become familiar with the injection technique.

Behavioral Testing

Animals were housed individually. Before testing, they were allowed to become accustomed to the handling procedure. To this end, 48 hours prior to testing, the bedding material was removed from the cage and a small can containing food pellets was placed inside the cage for 2 hours. Then, the animals were left undisturbed (undeprived animals) or were food-deprived, but not water-deprived, for 24 hours (deprived groups). Immediately after injection of the peptides, the animals were returned to their home cage, and the bedding material was removed and a small can containing a measured amount of standard food pellets (usually 30 to 40 g) was placed inside the cage. Food pellets and food spillage were weighed at 30, 60, 120, and 240 minutes after starting the test. The amount of food eaten was recorded by trained observers blind to the experimental conditions.

Peptide Treatments

Synthetic human GLP-1 (7-36) amide was purchased from Peninsula Laboratories (St. Helens Merseyside, UK). The GLP-1 receptor agonist, exendin-4, was synthesized as previously described.[27] For peripheral SC administration, the peptides were dissolved in 0.9% saline/0.25% bovine serum albumin and injected in a final vol of 0.1 mL/100 g BW. For ICV administration, the obturator was removed from the guide cannula, and an 8-mm injector (30-gauge stainless steel tubing) connected to 70 cm of calibrated polyethylene-10 tubing was lowered into the ventricle. The tubing was then raised above the rat's head until flow began. A dose of 5 μL peptide solution or vehicle was infused over a 30- to 60-second period. The injector was left in the guide cannula for another 30 seconds and then removed, and the stylet was immediately replaced. The ICV cannula placement was evaluated at the end of each experiment by injection of a blue dye; only rats with proper ICV placement were included in the data analysis.

Zucker obese or lean control rats were also treated SC with exendin-4, using the procedure described in the figure legends. BW and food intake were recorded daily.

In Situ Hybridization Histochemistry

In situ hybridization was performed as previously described.[30] Antisense or sense ^{33}P-labeled cRNA probes were generated with SP6 or T7 polymerase in pGLPR-1 generously donated by Dr B. Thorens (Lausanne, Switzerland), using a standard transcription reaction containing 10 μm ^{33}P-UTP. This resulted in probes with a specific activity of approximately 1.8×10^9 dpm/μg. The probes were hydrolyzed in bicarbonate buffer to an average length of 150 bases. The sense cRNA probe was used as a specificity control and showed no detectable labeling under identical conditions. The slides were dipped in LM-1 photographic emulsion and exposed for 3 weeks, and then were developed and coverslipped.

Cross-Linking of ^{125}I-GLP-1 (7-36) Amide to the Brain Particulate Fractions

For cross-linking experiments, ^{125}I-GLP-1 (7-36) amide was bound to brain homogenates as described previously.[14] The cross-linking reaction was initiated by the addition of 0.5 mmol/L disuccinimidyl suberate dissolved in dimethyl sulfoxide and was performed for 15 minutes at 4°C. The reaction was quenched with 10 mmol/L Tris hydrochloride plus 1 mmol/L EDTA, pH 7.4. The membranes were recovered by centrifugation and subsequently solubilized and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Statistics

The data were assessed by 1-way ANOVA or multifactorial ANOVAs, as required. Following a significant F value, post hoc analyses (Student-Newman-Keuls) were performed to assess specific group comparisons. Calculations were made using the Biomedical Data Processing (BMDP Statistical Software Inc, Los Angeles, CA) statistical package.

RESULTS

Effect of SC Administration of Either GLP-1 (7-36) Amide or Exendin-4 on Food Intake in Food-Deprived Wistar Rats

Peripheral administration (SC) of GLP-1 (7-36) amide to Wistar rats elicited a small but significant effect on food intake

(Fig 1A), characterized by a decrease in the amount of food eaten after delivery of 500 ng peptide/100 g BW. At lower doses (50 ng/100 g BW), exendin-4 proved to be more potent than GLP-1 (7-36) amide (Fig 1B). Subcutaneous administration of both GLP-1 (7-36) amide and exendin-4 also resulted in a reduction of food intake. SC administration of exendin-4 (250 ng/100 g BW) decreased the content of serotonin and noradrenaline, but not dopamine, in the hypothalamus, indicating a selective alteration of the central neurotransmitter systems involved in the regulation of food intake (Table 1). In Zucker obese and lean control rats, SC administration of exendin-4 also decreased the hypothalamic content of serotonin (Table 2). These effects were not associated with a decrease in glycemia. SC administration of the peptide increased plasma levels of corticosterone (Table 1). In addition, SC administration of exendin-4 to Wistar and Zucker lean and obese rats resulted in a significant ($P < .05$) reduction in water intake (Table 3).

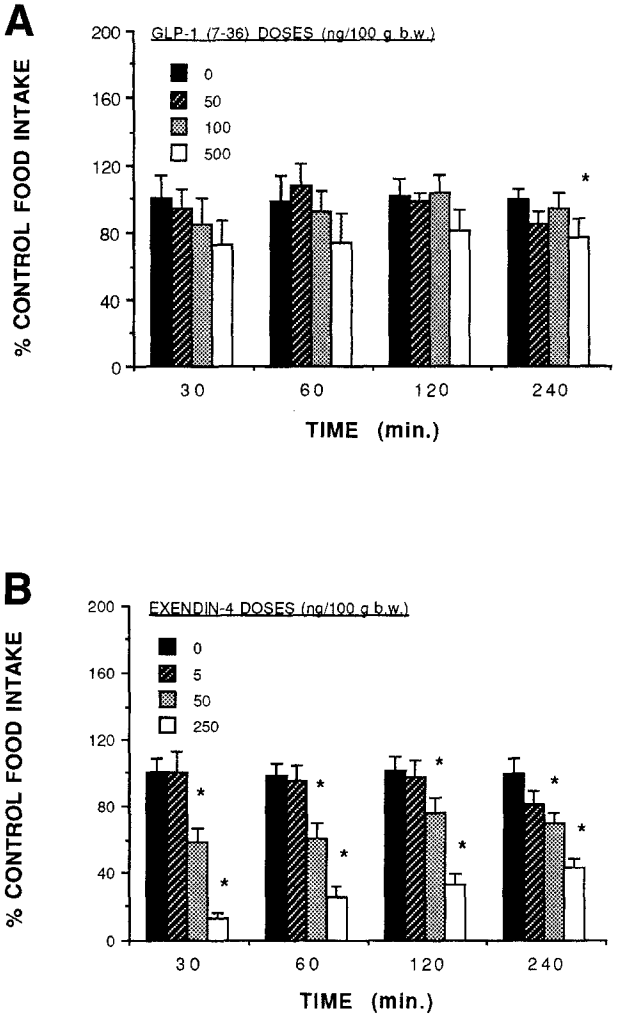

Fig 1. Effects of peripheral (SC) administration of (A) GLP-1 (7-36) amide (0, 50, 100, or 500 ng/100 g BW) or (B) exendin-4 (0, 5, 50, or 250 ng/100 g BW) on food intake in food-deprived Wistar rats. Values are the mean ± SEM percentage of cumulative food intake during 4 consecutive intervals for 10-12 determinations per group after acute administration of the peptide. *$P < .05$, Newman-Keuls, *v* control group. Absolute values for food intake (g) in the control group: 30 min, 3.4 ± 0.5; 60 min, 3.8 ± 0.6; 120 min, 5.9 ± 0.6; 240 min, 8.2 ± 0.6.

Table 1. Effects of SC Administration of Exendin-4 (250 ng/100 g BW) on Plasma Chemistry and Hypothalamic Monoamines in Wistar Rats

Parameter	Control	Exendin-4–Treated
Plasma chemistry		
Glucose (mg · dL^{-1})	121.2 ± 2.7	139.1 ± 6.6
Triglycerides (mg · dL^{-1})	149.8 ± 24.9	105.5 ± 17.0
Creatinine (mg · dL^{-1})	0.49 ± 0.02	0.49 ± 0.01
Insulin (ng · mL^{-1})	3.8 ± 0.4	4.6 ± 0.8
Corticosterone (ng · mL^{-1})	171.5 ± 25.7	310.8 ± 39.8†
Hypothalamic monoamine content (ng · mg^{-1})		
Noradrenaline	4.22 ± 0.32	3.46 ± 0.27*
Dopamine	0.83 ± 0.08	0.70 ± 0.09
DOPAC	0.37 ± 0.05	0.27 ± 0.05
Serotonin	12.10 ± 1.2	9.51 ± 0.72†
5-HIAA	7.93 ± 0.44	6.44 ± 0.59

NOTE. Data are the mean ± SEM of 13 animals per group in samples obtained 60 minutes after acute SC administration of the peptide (exendine-4–treated) or vehicle (control).

Abbreviation: DOPAC, 3,4,dihydroxy-phenylacetic acid.

*$P < .05$, †$P < .01$: vehicle *v* exendin-4 by Newman-Keuls test.

Effect of SC Administration of Either GLP-1 (7-36) Amide or Exendin-4 on Food Intake in Zucker Obese Rats

Exendin-4 injected into the SC adipose tissue may be a good tool for the treatment of diabetes mellitus and/or obesity. In an attempt to gain insight into these issues, the effects of both peptides on feeding behavior were studied in Zucker obese rats. Plasma insulin was significantly higher ($P < .001$) in obese (vena cava, 18.5 ± 1.9 ng/mL; portal vein, 33.3 ± 3.4) versus lean (vena cava, 7.6 ± 2.1 ng/mL; portal vein, 13.7 ± 3.3) Zucker rats. Both control and obese Zucker rats responded to acute ICV administration of both GLP-1 (7-36) amide and exendin-4 (Fig 2 A to D) with a significant reduction in food intake.

These results prompted us to use Zucker obese rats to determine whether SC treatment with a GLP-1 receptor agonist in fact reduces food intake and BW. Both acute and chronic SC exendin-4 treatment reduced food intake in Zucker obese rats (Fig 3A to C). A single dose of exendin-4 per day caused a highly significant reduction in food intake and cumulative weight gain (Fig 3C and D). Additionally, a transient change was observed in the pattern of spontaneous motor activity, similar to that described after central administration of GLP-1 (7-36)-amide: namely a decrease in horizontal locomotor activity (data not shown).

In Situ Hybridization Histochemistry and Cross-Linking of ^{125}I-GLP-1 (7-36) Amide to Brain Membranes of Zucker Rats

The physiological effects of GLP-1 agonists on feeding behavior were associated with the presence of the mRNA and protein of GLP-1 receptors in the brain of Zucker rats (Figs 4 and 5). By in situ hybridization histochemistry, with the antisense probe (Fig 4A, B, and D), labeled cells were found throughout the entire brain, whereas no labeling was detected with the sense probe (Fig 4C). Positive cells were found to be widely distributed throughout the cerebral cortex, thalamus, and hypothalamus, with certain specific areas showing higher

Table 2. Effects of SC Administration of Exendin-4 (250 ng/100 g BW) on Plasma Chemistry and Hypothalamic Monoamines in Zucker Rats

Parameter	Lean Zucker Rats		Obese Zucker Rats	
	Control	Exendin-4–Treated	Control	Exendin-4–Treated
Plasma chemistry				
Glucose (mg · dL^{-1})	—	—	380.70 ± 4.1	348.80 ± 36.7
Triglycerides (mg · dL^{-1})	54.20 ± 3.5	58.70 ± 2.7	250.20 ± 57.3	313.80 ± 37.2
Creatinine (mg · dL^{-1})	0.82 ± 0.14	0.72 ± 0.4	0.81 ± 0.04	0.71 ± 0.06
Insulin (ng · mL^{-1})	7.60 ± 2.1	8.30 ± 1.7	18.50 ± 1.9	21.80 ± 2.1
Hypothalamic monoamine content (ng · mg^{-1})				
Noradrenaline	3.20 ± 0.19	3.50 ± 0.39	3.30 ± 0.32	2.95 ± 0.22
Dopamine	1.02 ± 0.40	0.87 ± 0.04	0.95 ± 0.16	1.24 ± 0.21
DOPAC	0.47 ± 0.22	0.16 ± 0.03	0.22 ± 0.05	0.26 ± 0.11
Serotonin	13.30 ± 1.31	9.70 ± 1.40*	8.73 ± 0.60	6.90 ± 0.45*
5-HIAA	9.30 ± 2.80	7.10 ± 2.13	4.20 ± 0.88	5.41 ± 1.40

NOTE. Data are the mean ± SEM of 4-13 animals per group in samples obtained 60 minutes after acute SC administration of the peptide (exendin-4–treated) or vehicle (control).

*$P < .05$, vehicle *v* exendin-4 by Newman-Keuls test.

concentrations. In the hippocampus, positive cells were found in neurons from the granule cell layer and in cells in the hilus and stratum moleculare. Numerous labeled cells were found dispersed throughout the hypothalamus, especially in the paraventricular nucleus ([PVN]; Fig 4A, B, and D). GLP-1 receptor was identified (Fig 5) as a band of 56 kd by affinity cross-linking, indicating that GLP-1 receptors are synthesized in the brain of Zucker rats. Affinity cross-linking data also revealed a band of 90 kd that disappeared in the presence of excess GLP-1 (7-36) amide. This could be explained by the existence of complexes of the ligand-receptor-α-subunit of G proteins, since other investigators[31] have reported that seven-transmembrane-segment receptors may be solubilized from tissue as high-affinity receptor–G protein complexes.

DISCUSSION

We present experimental evidence herein indicating that GLP-1 receptor agonists injected either SC or ICV induce satiety and BW loss in Zucker rats. It is thought that GLP-1 (7-36) amide is a satiety mediator that acts only through a central mechanism[21-23] since, in previous studies, peripheral administration of this peptide by intraperitoneal injection was not found to affect food intake. However, in the present study, we observed that using another peripheral route, ie, SC injection, both GLP-1 (7-36) amide and exendin-4 administration reduced food intake. Moreover, Flint et al[32] have clearly demonstrated that peripheral administration of GLP-1 to humans induces satiety. GLP-1 (7-36) amide could enter the brain by binding to blood-brain barrier–free organs such as the subfornical organ and the area postrema.[18] Alternatively, it could be transported into the brain through the choroid plexus, which has a high density of GLP-1 receptors.[17] Thus, GLP-1 (7-36) amide released from intestinal L-cells or injected peripherally into the SC tissue may be responsible for part of the central physiological effects of this peptide.

Table 3. Effects of SC Administration of Exendin-4 (5, 50, or 250 ng/100 g BW) or Vehicle on Water Intake (mL) Measured 240 Minutes After Peptide Injection in Wistar Rats and Zucker Obese and Lean Control Rats

Group	Vehicle	Dose of Exendin-4 (ng/100 g BW)		
		5	50	250
Wistar	16.5 ± 1.5	15.3 ± 0.8	10.8 ± 1.8*	7.0 ± 1.0*
Lean Zucker	10.2 ± 1.2	8.3 ± 1.2	8.8 ± 1.0	6.3 ± 0.7*
Obese Zucker	11.5 ± 1.4	9.6 ± 0.9	8.5 ± 1.0*	6.6 ± 1.6*

NOTE. Data are the mean ± SEM; n = 10-12.

*$P < .05$, vehicle *v* exendin-4.

Since both GLP-1 (7-36) amide and exendin-4 are able to delay gastric emptying,[4] an intriguing question is whether the observed effects on food intake are due to inhibition of gastric motility or to a direct central nervous action. In this regard, damage to the arcuate nucleus and sensory circumventricular organs by administration of monosodium glutamate to Wistar rats completely abolishes the inhibitory effect of GLP-1 (7-36) amide on feeding behavior.[33] Because these findings indicate that centrally acting GLP-1 (7-36) amide modulates food intake via neurons present in the arcuate nucleus and the circumventricular organs, we believe that the peripheral administration of GLP-1 receptor agonists may also interfere with the central mechanisms involved in feeding behavior. It is noteworthy that the effect of GLP-1 (7-36) amide on gastric emptying seems to be centrally mediated, as it is not found in vagotomized subjects.[34]

We found exendin-4 to be more biologically potent than GLP-1 (7-36) amide. Differences in the actions of these 2 peptides may be due to a higher affinity of exendin-4[27] for common receptors and/or to a longer half-life of this peptide. The lower activity of GLP-1 (7-36) amide may be the result of the effects of at least 2 enzymes: DPP-IV and neutral peptidase NEP24.11. DPP-IV cleaves GLP-1 at the NH_2-terminal end; as a consequence, exendin-4 and GLP-1 analogs are resistant to enzyme breakdown.[35] NEP24.11[36] is a membrane-bound ectopeptidase that is important for the extracellular processing of neuropeptides, with GLP-1 (7-36) amide being a good substrate and exendin-4 being a poor substrate for this enzyme. These data suggest a longer half-life for exendin-4, although this aspect remains to be elucidated. If this is indeed the case, exendin-4 may be a good prototype for the development of clinically useful peptide agonists.

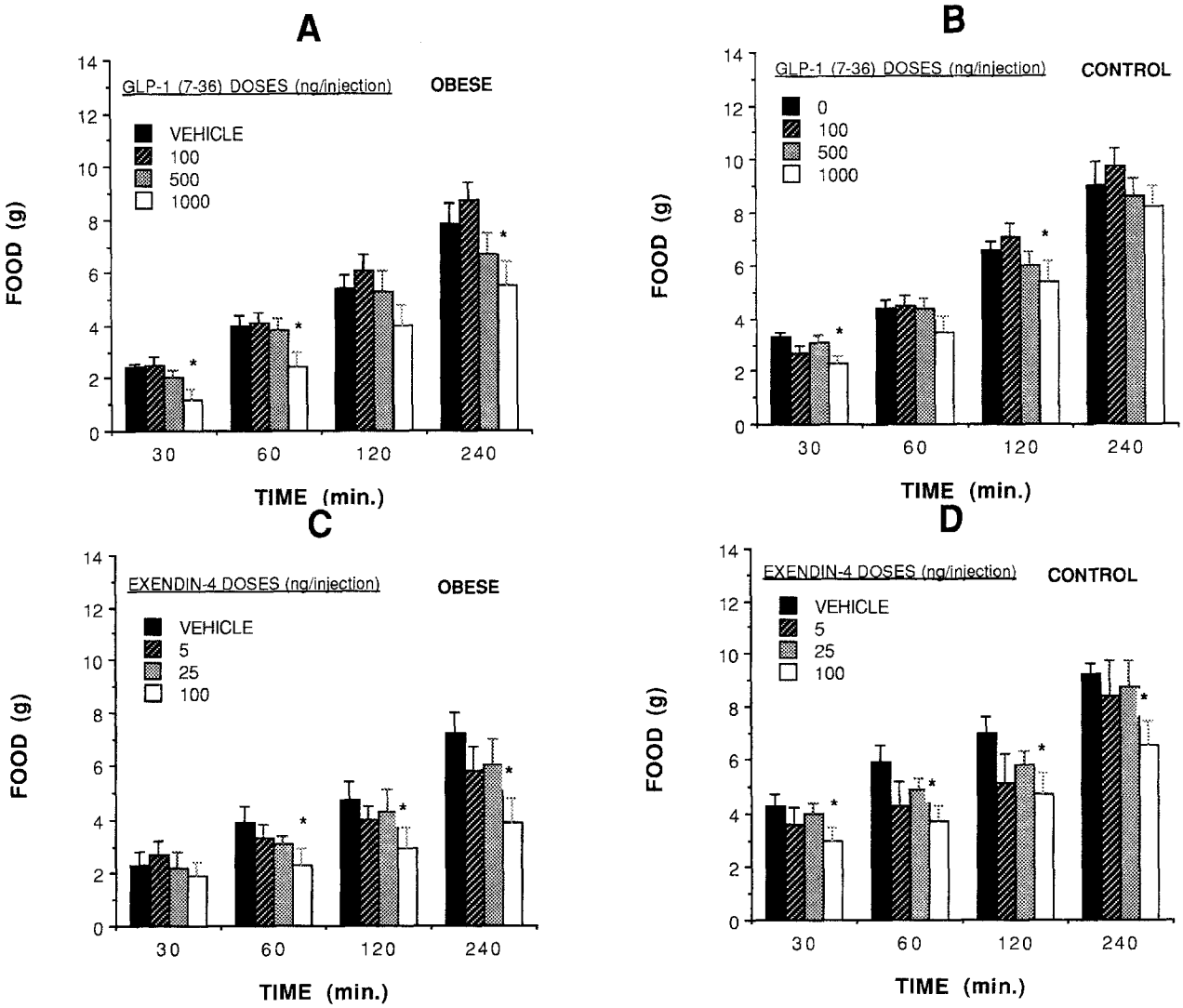

Fig 2. Effect of ICV administration of either GLP-1 (7-36) amide or exendin-4 in Zucker rats. (A) and (B). Effects of acute ICV administration of GLP-1 (7-36) amide (0, 100, 500, and 1,000 ng) on food intake in food-deprived Zucker (A) obese and (B) lean controls. (C) and (D). Effects of central administration of exendin-4 (0, 5, 25, and 100 ng) on food intake in food-deprived Zucker (C) obese and (D) lean controls. Values are the mean ± SEM cumulative food intake during 4 consecutive intervals for 10-12 determinations per group. *$P < .05$, Newman-Keuls, either GLP-1 (7-36) amide or exendin-4 *v* control group.

SC administration of exendin-4 also decreased the hypothalamic content of serotonin and norepinephrine, but not dopamine, indicating a selective alteration of central neurotransmitter systems involved in the regulation of food intake. Activation of serotonin release and either direct or indirect activation of serotonergic receptors (such as 5HT 1b, 5HT 2a, or 5HT 2c) decrease food intake through a variety of mechanisms, including the regulation of meal size and the continuity of feeding.[37] The PVN of the hypothalamus has been proposed to be an important terminal field involved in serotonin-mediated satiety. The present results suggest that activation of GLP-1 receptors in the medial basal hypothalamus (including the lateral hypothalamic area and the PVN) activates serotonin input into the hypothalamus, as reflected by changes in the main metabolite of serotonin, 5-HIAA, in animals exposed to exendin-4. This effect is associated with the onset of decreased food intake in exendin-4–exposed rats. In addition, central administration of GLP-1 (7-36) amide stimulates corticotropin-releasing hormone (CRH) neurons,[38] which may be responsible for the inhibition of feeding behavior and the activation of the hypothalamic pituitary-adrenocortical axis induced by this peptide. In the present study, we found that SC administration of exendin-4 significantly increased plasma corticosterone levels, as reported by other investigators.[38] This could be interpreted as a stress-like response induced by this GLP-1 agonist and could also account for its effects on satiety. In this regard, it is reported that GLP-1 (7-36) amide, in addition to reducing food intake when administered centrally, causes illness in rats.[39,40] Regardless of the fact that these effects only appear with the use of much higher doses of the peptide (3- to 30-fold) than those administered here, a recent report[41] suggests that the amygdala-hypothalamus-pituitary-adrenal (AHPA) axis is strongly activated during food intake processes, as revealed by the enhanced release of CRH in the amygdala and increased plasma cortico-

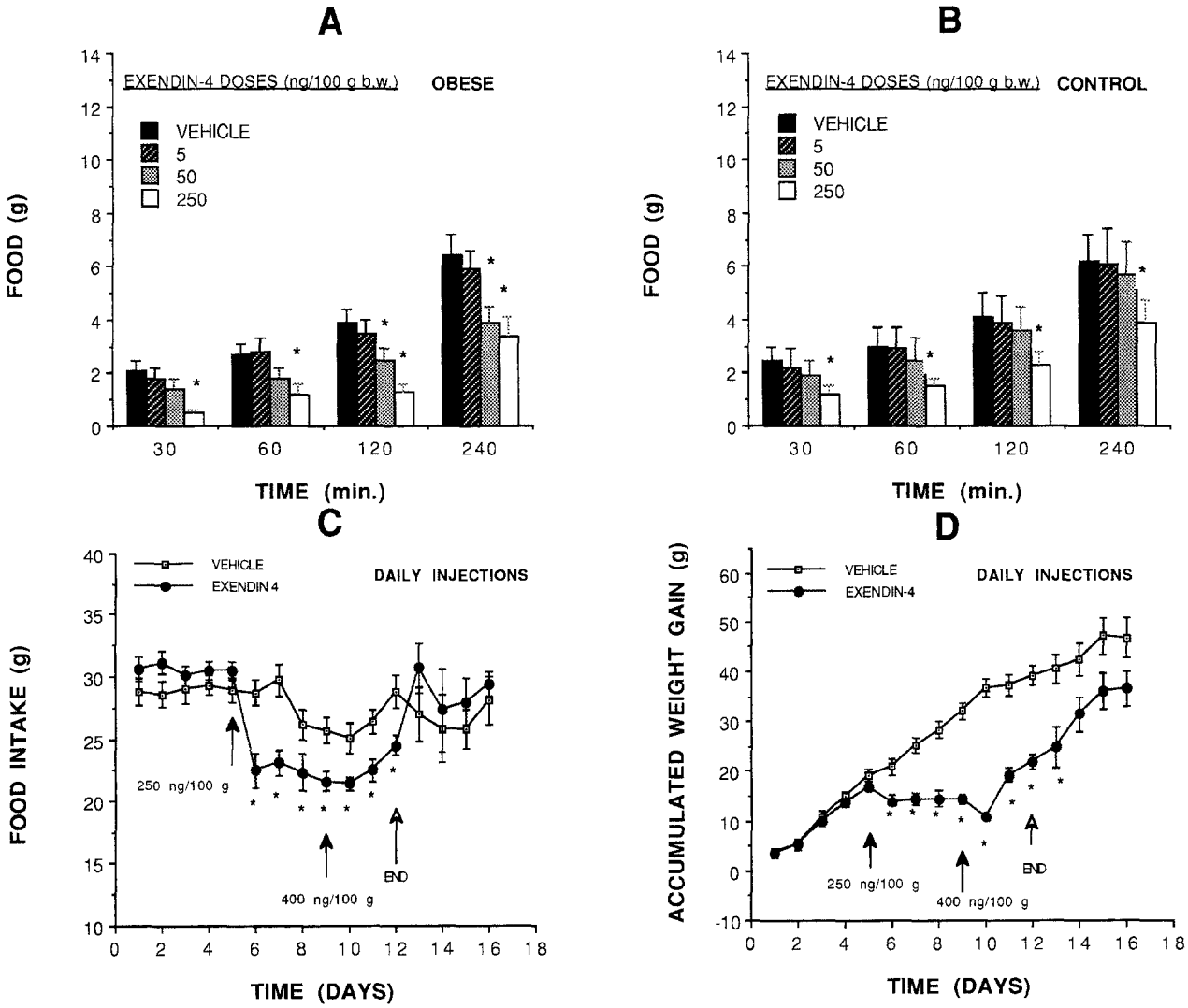

Fig 3. Effects of acute or chronic SC treatment with exendin-4 in Zucker obese rats. (A) and (B) Effects of SC administration of exendin-4 (0, 5, 50, and 250 ng) on food intake in food-deprived Zucker (A) obese and (B) lean controls. (C) and (D) Effects of chronic SC administration of exendin-4 (vehicle days 1-4, 250 ng/100 g BW days 5-8, 400 ng/100 g BW days 9-11, and vehicle again days 12-16) on (C) daily food intake and (D) cumulative weight gain in Zucker obese rats. Values are the mean ± SEM of 7-8 determinations per group. *$P < .05$, Newman-Keuls, exendin-4 *v* control group.

tropin or plasma corticosterone associated with feeding and satiety. These experimental data support the notion that the AHPA axis is a system with a much broader role than previously believed. Rather than evoking fear and anxiety reactions, this system could help to draw attention to events or cues of biological significance (both potential benefits and threatening situations), associated with feeding. These findings open the way to further studies aimed at understanding the satiety induced either by stress or by GLP-1 receptor agonists. Our results also indicate that SC administration of pharmacological doses of GLP-1 (7-36) amide significantly decreases food ingestion, although under physiological circumstances, the smaller amounts of GLP-1 (7-36) amide released from intestinal L-cells cannot modulate food intake. In contrast, exendin-4 has a more potent effect, and when injected into the SC adipose tissue, it may be a good tool for the treatment of diabetes mellitus and/or obesity.

To gain further insight into these issues, the effect of both peptides on feeding behavior was studied in Zucker obese rats. These animals were hyperinsulinemic as compared with their littermates, and both experimental groups responded to acute ICV or SC administration of either GLP-1 (7-36) amide or exendin-4 with a significant reduction in food intake. These physiological effects of GLP-1 agonists on feeding behavior were associated with the presence of the mRNA and protein of GLP-1 receptors in the brain of Zucker rats. Our findings indicate that GLP-1 receptors are synthesized in the brain of Zucker rats, and the localization of the mRNA for GLP-1 receptors is similar to that previously described in Wistar rats.

These results prompted us to use Zucker obese rats to determine whether chronic SC treatment with exendin-4 in fact reduces food intake and BW. Interestingly, a single daily dose of this GLP-1 receptor agonist was enough to produce a highly significant reduction in food intake and BW gain, and this effect

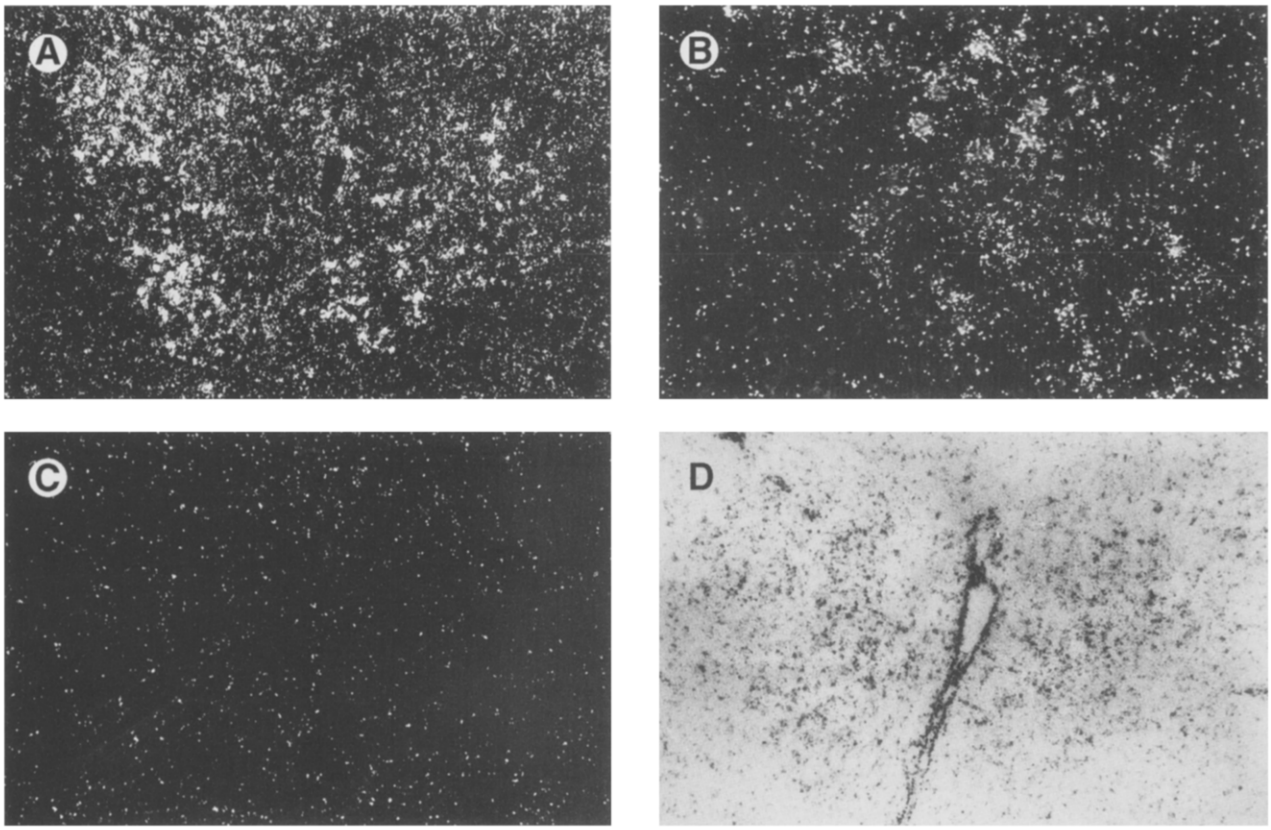

Fig 4. In situ hybridization histochemistry of GLP-1 receptor mRNA. (A) and (B) Dark-field photomicrograph of labeled cells of the PVN. Clusters of white grains represent cells positive for GLP-1 receptor mRNA. (C) Dark-field photomicrograph in the same anatomical area and magnification as B for in situ hybridization using a sense control riboprobe. No specific labeling is seen. (D) Light-field photomicrograph in the third ventricle and PVN. Black grains represent cells positive for GLP-1 receptor mRNA.

was even more dramatic at the lower dose. Donahey et al[42] have reported that intraventricular administration of GLP-1 (7-36) amide reduces short-term, but not long-term, food intake and BW in lean and obese Zucker rats. We have recently observed (F.R. Fonseca, M. Navarro, and E. Blázquez, unpublished observations, February 1997) that continuous ICV administration of exendin-4 over several days induces a desensitization of the satiety-induced response to this peptide, resulting in a normalization of daily weight gain. However, discontinuous SC treatment with low-dose exendin-4 did not result in desensitization. Furthermore, at low doses, GLP-1 (7-36) amide does not cause illness.[39,40]

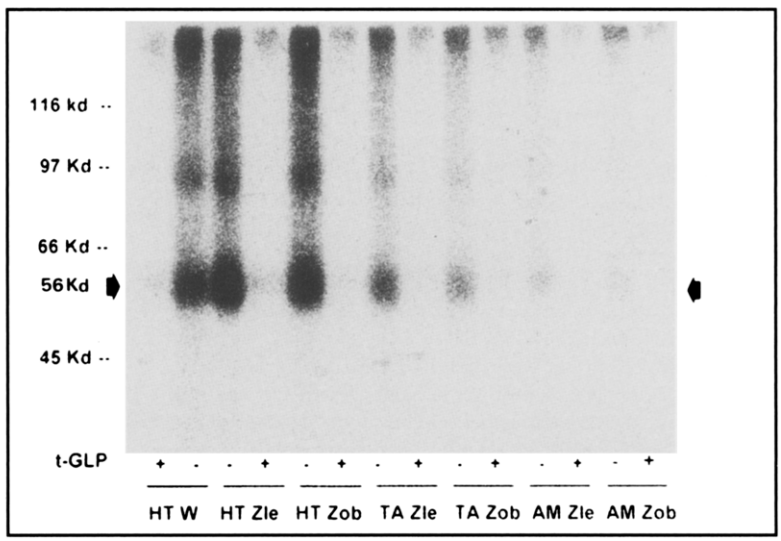

Fig 5. Cross-linking of ^{125}I-GLP-1 (7-36) amide to brain membranes. Cross-linking reveals the presence of GLP-1 receptor protein in several brain areas (hypothalamus, HT; thalamic region, TA; and amygdala, AM) of Wistar (W) and Zucker rats (obese, Zob; lean, Zle).

These observations highlight the possible usefulness of exendin-4, or its structural analogs yet to be developed, as a tool for treating obesity and/or diabetes. GLP-1 (7-36) amide regulates blood glucose through stimulation of glucose-dependent insulin secretion,[2] inhibition of glucagon secretion,[43] and inhibition of gastric emptying,[4] which facilitate the decrease of blood glucose in both type 1 and type 2 diabetic patients.[25,26] Although diabetic patients treated with GLP-1 (7-36) amide require less exogenous insulin and have reduced postprandial hyperglycemia, their preprandial glycemia is increased, probably due to the short duration of this peptide.[44] In light of these results, different *N*-terminally substituted GLP-1 (7-36) amide analogs resistant to DPP-IV have recently been developed.[45] These analogs have prolonged metabolic stability in vivo and improved biological activity, which may be of great interest in diabetes therapy. Likewise, exendin-4 is resistant to DPP-IV cleavage in addition to being a poor substrate for the ectopeptidase NEP24.11. This may explain the results reported here, and suggests a potential use of exendin-4 or structural analogs in the treatment of type 2 diabetes and/or obesity.

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