GLP-1(7-36)-amide and Exendin-4 Stimulate the HPA Axis in Rodents and Humans


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Glucagon-like peptide-1 (GLP-1) is a potent insulinotropic peptide expressed in the gut and brain, which is secreted in response to food intake. The levels of GLP-1 within the brain have been related to the activity of the hypothalamic-pituitary-adrenal (HPA) axis, and hence, this peptide might mediate some responses to stress. Nevertheless, there is little information regarding the effects of circulating GLP-1 on the neuroendocrine control of HPA activity. Here, we have studied the response of corticosterone to the peripheral administration of GLP-1 (7-36)-amide and related peptides (exendin (Ex-3, Ex-4, and Ex-4(3-39)) in rats, mice, and humans. GLP-1 increases circulating corticosterone levels in a time-dependent manner, both in conscious and anesthetized rats, and it has also increased adrenocortical levels. Moreover, GLP-1 augmented cortisol levels in healthy subjects and diabetes mellitus (DM)-1 patients. The effects of GLP-1/Ex-4 on the HPA axis are very consistent after distinct means of administration (intracerebroventricular, iv, and ip), irrespective of the metabolic state of the animals (fasting or fed ad libitum), and they were reproduced by different peptides in this family, independent of glycemic changes and their insulinotropic properties. Indeed, these effects were also observed in diabetic subjects (DM-1 patients) and in the DM-1 streptozotocin-rat or DM-2 muscle IGF-1 receptor-lens-arginine transgenic mouse animal models. The mechanisms whereby circulating GLP-1 activates the HPA axis remain to be elucidated, although an increase in ACTH after Ex-4 and GLP-1 administration implicates the central nervous system or a direct effect on the pituitary. Together, these findings suggest that GLP-1 may play an important role in regulating the HPA axis. (Endocrinology 151: 2629–2640, 2010)

Glucagon-like peptide-1 (GLP-1) is produced by post-translational processing of pro-pro-glucagon in L cells of the ileum and in different areas of the brain. Specifically, GLP-1 immunoreactive nerve fibers densely innervate hypothalamic regions in the brain such as the paraventricular, dorsomedial, and arcuate nuclei (1–4). GLP-1 (7–36)-amide is the main active form of the peptide, and it is secreted in response to food intake. GLP-1 stimulates insulin secretion, improves functional β-cell mass, and it reduces glucagon release (1–3). Central administration of GLP-1 exhibits feeding behavior (5) and activates monoamine outflow, particularly through the sympathetic branch (6). The GLP-1 receptor (GLP-1r) is widely expressed in the hypothalamus (7), and accordingly, GLP-1 was shown to increase GnRH levels in a hypothalamic cell line in vitro (8), as well as stimulating TSH release from dispersed rat pituitary cells (9).
It has been reported that central administration of GLP-1 activates the hypothalamic-pituitary-adrenal (HPA) axis, increasing the circulating levels of ACTH (10), arginine vasopressin (11, 12), and corticosterone (10, 11). Intracerebroventricular (icv) injection of GLP-1 induces c-fos expression in CRH neurons of the parvicellular region, and this effect is suppressed by the administration of the GLP-1r antagonist, exendin (Ex)-9(39) (11), suggesting that it might be mediated by GLP-1r. The effects of GLP-1 on the HPA axis are of particular interest because Ex-4, a potent GLP-1r agonist (13), is currently used to treat diabetic patients (14). However, recent data showed that some effects of Ex-4 may not be explained by its binding and activation of the GLP-1r alone (15–18). Indeed, we recently provided evidence that Ex-4 but not GLP-1 decreases ghrelin levels in fasted rats (19).

Although GLP-1 may play a role in the central regulation of the HPA axis (10, 20), the effects of peripheral administration of GLP-1 or its analog Ex-4 have been poorly studied to date. Here, we have assessed the effects of GLP-1 and Ex-4 on glucocorticoid and ACTH levels when administered peripherally (ip or iv route), considering that the route of administration might influence the response. Because manipulative stress may modify HPA responses, we studied the effects of GLP-1/Ex-4 in both conscious and freely moving animals and in anesthetized rats that do not suffer experimental stress due to conscious manipulation. Furthermore, regarding the relevance of GLP-1/Ex-4 in metabolic control, and given that the feeding regime may modulate adaptive responses of the HPA axis, we studied the effects of GLP-1/Ex-4 in animals fed ad libitum and in fasting animals that served as a model of metabolic stress, both in controls and in two animal models of experimental diabetes. Finally, to assess the relevance of GLP-1 analogs in the context of human metabolic diseases, we studied the effects of GLP-1 in healthy controls and diabetic subjects.

In all circumstances, these insulinotropic agents (GLP-1/Ex-4) were seen to effectively increase circulating glucocorticoid levels, and the GLP-1/Ex-4-induced glucocorticoid responses in rats were comparable with those elicited by ACTH as the reference secretagogue acting on the HPA axis. Together, these results suggest that GLP-1 might play a relevant role in regulating the HPA axis.

Materials and Methods

Animals

Adult male Sprague Dawley rats (300–350 g) were obtained from the faculty of the University of Santiago de Compostela (Santiago de Compostela, Spain). Adult male (8–10 wk of age) muscle IGF-1 receptor-homologous arginine (MKR) transgenic mice (22.94 ± 3.03 g) and their wild-type littermates (26.63 ± 5.26 g) used as controls were provided by Ana Fernández (Instituto Cajal, Consejo Superior de Investigaciones Científicas, Madrid). MKR transgenic mice express a dominant-negative truncated IGF-I receptor, MKR-IGF-I receptor, specifically targeted to the skeletal muscle. Expression of MKR-IGF-I receptor results in the production of dominant negative hybrid mutant receptors that abrogate the normal function of the endogenous IGF-I and insulin receptors, thereby provoking insulin resistance at a relative early age. These mice exhibit hyperinsulinemia and hyperglycaemia, as well as impaired glucose tolerance, providing an excellent model of type 2 diabetes mellitus (DM) not linked to obesity (21).

The animals were maintained with free access to tap water and standard chow (A01; Panlab, Barcelona, Spain) under a 12-h light, 12-h dark cycle (lights on from 0900 to 2100 h) and at a controlled room temperature (20–22°C). Rats and mice were accommodated to the facilities for several days before carrying out the experiments, and they were handled daily to avoid experimental stress. All experimental procedures were carried out in accordance with European Union guidelines regarding the use of animals for experimental purposes (Council Directive CEE 86/609).

Drugs and peptides

Ex-4, Ex-3, Ex-4 (3-39), and GLP-1 (7-36)-amide were obtained from Bachem (Bubendorf, Switzerland), whereas sodium pentobarbital and streptozotocin (STZ) were purchased from Sigma-Aldrich (Alcobendas, Spain). Insulin was supplied by Novo Nordisk (Bagvaard, Denmark) and Synacthen (ACTH-1-24) by Novartis Pharma (Basel, Switzerland).

Experimental procedures in rodents

Freely moving study for peripheral administration to conscious rats

Rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and a polyethylene cannula (PE-50, Becton Dickinson and Company, Sparks, MD) with a silicone tip (4.5 cm in length, inside diameter, 0.635 mm and outside diameter, 1.194; Degania Silicone Ltd, Degania Bet, Israel) was inserted into the right jugular vein and then exteriorized by a tunneling the interscapular area of the animal's back. Rats were allowed to recover for 7 d in individual cages with free access to food and water and were manipulated every day in a simulated experiment to minimize experimental stress. On the day of the experiment, the guide cannula was extended by inserting another 30-cm-long polyethylene cannula (PE-50, Clav Adams), and the animals were left undisturbed for 30 min. A heparin bolus (0.3 ml, 1000 U/ml) was then administered, a basal blood sample (0.3 ml) was taken, and the drugs [Ex-4 or GLP-1 (7-36)-amide] or the vehicle alone (0.9% NaCl) were administered. Three tests were carried out on different groups of animals (n = 6–7/group), rats receiving: iv GLP-1 (7-36)-amide (4 or 20 µg/kg) or saline (experiment 1, Fig. 1, A and B); iv Ex-4 (5 µg/kg) or saline (experiment 2, Fig. 1, C and D) and iv GLP-1 (7-36)-amide (20 µg/kg) or saline (experiment 3, Fig. 1F). All treatments were administered between 1000 and 1200 h. The blood samples were drawn 3, 5, 15, 30, 60, and 120 min after administration and collected in Eppendorf tubes containing EDTA (0.03 M, 10 µl/tube), replacing the volume (300 µl) with saline solution (0.9%). The blood was centrifuged for 7 min at 3500 rpm and 4°C, and the plasma was stored at −20°C until the hormones were measured.

Unless otherwise stated, the drug administration, sampling, and blood processing was performed similarly in all the experiments.

Intravenous administration of glucose to conscious rats

Sixty conscious rats were used per experiment, i.e., 10 rats per group, and 6–7 rats per group, therefore, 33 and 36 rats, respectively, for the two experimental groups. The volume of rats was of around 20 µl and glucose solution in a dose of 0.155 g glucose/kg iv.

We used the following glucose concentrations: 0–6, 6–8.5, 8.5–11, 11–12.5, and 12.5–15 g glucose/kg iv.
Intraperitoneal administration

The time course of the effects of Ex-4 on corticosterone was assessed in trunk blood collected at 0, 5, 15, 30, 60, 120, 240, or 480 min after ip administering Ex-4 (5 µg/kg) or the vehicle alone (0.9% NaCl) to rats fasted for 48 h (n = 6/group; experiment 7; Figs. 4, A and B). Two different studies were carried out to assess the short-term (5–60 min) and long-term effect (hours).

To study the effect of fasting on the corticosterone responses to Ex-4, rats were divided into five groups (n = 12/group) that had free access to food until the day of the study or that were fasted for different times (12, 24, 48, and 72 h) before receiving Ex-4 (experiment 8, Fig. 4C). On the day of the study, Ex-4 (5 µg/kg) or saline was administered ip, and blood samples were collected 2 h later as above.

To compare the potency of the effects of different Ex on corticosterone levels, Ex-3 (5 µg/kg), Ex-4 (5 µg/kg), Ex-4 (3-39) (5 µg/kg), or the vehicle alone (0.9% NaCl) were injected ip to 48-h-fasted rats (n = 4–6/group; experiment 9, Tables 1 and 2). Blood samples were collected at 15, 30, or 60 min after the injection, and the studies were performed in two different days to test the effect of Ex-4 (3-39) and to compare the effects of Ex-4 and Ex-3.

Intracerebroventricular administration

A permanent polyethylene cannula (PE-50, Clay Adams) was implanted stereotaxically into the lateral ventricle of the rats under sodium pentobarbital anesthesia (50 mg/kg, ip). The drugs, Ex-4, GLP-1 (7-36)-amide, and ACTH(1-24) or the vehicle alone (0.9% NaCl) were administered, and blood samples (300 µl) were taken at different times (basal, 5, 10, 15, 30, 45, and 60 min) after administration. The following treatments were administered to different groups of rats (n = 6–8, after 48-h fasting): GLP-1 (7-36)-amide (1 or 20 µg/kg) or saline (experiment 4, Fig. 2, A and B). Ex-4 at a dose of 0.5, 1, 5, or 20 µg/kg or vehicle alone (experiment 5, Fig. 2C).

We compared the potency of Ex-4 with that of ACTH (experiment 6, Fig. 3), administering overnight fasted rats (n = 6–8/group) with an equimolar dose (1.2 nmol/kg) of Ex-4 (5 µg/kg). ACTH(1-24) (3.5 µg/kg), GLP-1 (7-36)-amide (4 µg/kg), or the vehicle alone.

Intravenous administration to anaesthetized rats

Silicone tips (inside diameter, 0.635 mm and outside diameter, 1.194 mm; Degama Silicone Ltd., Israel) were inserted into the right jugular vein of the rats under sodium pentobarbital anesthesia (50 mg/kg, ip). The drugs, Ex-4, GLP-1 (7-36)-amide, and ACTH(1-24) or the vehicle alone (0.9% NaCl) were administered, and blood samples (300 µl) were taken at different times (basal, 5, 10, 15, 30, 45, and 60 min) after administration. The following treatments were administered to different groups of rats (n = 6–8, after 48-h fasting): GLP-1 (7-36)-amide (1 or 20 µg/kg) or saline (experiment 4, Fig. 2, A and B), and Ex-4 at a dose of 0.5, 1, 5, or 20 µg/kg or vehicle alone (experiment 5, Fig. 2C).

We compared the potency of Ex-4 with that of ACTH (experiment 6, Fig. 3), administering overnight fasted rats (n = 6–8/group) with an equimolar dose (1.2 nmol/kg) of Ex-4 (5 µg/kg). ACTH(1-24) (3.5 µg/kg), GLP-1 (7-36)-amide (4 µg/kg), or the vehicle alone.
FIG. 2. Effect of the iv administration of GLP-1 (7-36)amide and Ex-4 on HPA activity in anesthetized rats. Corticosterone (A) and aldosterone (B) responses after GLP-1 (7-36)amide (1 and 20 μg/kg, iv) administration in rats fasted for 48 h. C, Time course of Ex-4 dose-response effects on corticosterone release in 48-h-fasted rats. D, Ex-4 (5 μg/kg, iv) enhances ACTH release. Data are represented as the mean ± s.e.m. of 6-8 rats per group. **P < 0.01; ***P < 0.001 vs. saline.

Studied in a type 1 diabetes model

Diabetes was induced in rats by administering a single iv injection of STZ (70 mg/kg) in a citrate buffer solution (0.1 M, pH 4.5), whereas control rats received the vehicle alone. Body weight and urine glucose were monitored daily, and only STZ-rats with positive glycosuria were included in the study. STZ-rats received insulin (1U/100g, sc) twice daily for 6 d, whereas the control animals received an equal volume of saline (0.9%). The day of the experiment, glucose levels were determined to ensure the STZ-rats were in a state of hyperglycaemia (mean ± s.e.m. of all experiments = 402 ± 20.2 (STZ) vs. 96 ± 4.2 (controls)).

Control and diabetic animals (n = 6/group) were cannulated and studied under pentobarbital anaesthesia as described above (experiment 12, Fig. 6A). Ex-4 (5 μg/kg) or saline was administered iv, and blood samples were drawn at different times: 5, 10, 15, 30, 45, and 60 min. In another study (experiment 13, Fig. 6B), groups (n = 4–6/group) of control or diabetic rats were maintained ad libitum or fasted for 72 h. Ex-4 (5 μg/kg) or 0.9% NaCl was injected iv, trunk blood samples were collected 2 h later, and the serum was processed as described above.

Studies using a type 2 diabetes model

Uninjected MKR and wild-type mice (n = 3–5/group) were decapitated at different times of the day, and trunk blood was sampled to measure the plasma glucose and corticosterone (experiment 14, Fig. 6, C and D). MKR and wild-type mice (n = 5/group, experiment 15, Fig. 6E) were fasted for 24 h, and then Ex-4 (5 μg/kg), iv, was administered. Trunk blood samples were collected 2 h later, and the serum was processed as mentioned previously.

Experimental procedures in human subjects

The methods and procedures were approved by the Research Ethics Committee at the Montecello Hospital, and all the volunteers that participated in the study gave their written informed consent. Six healthy, Caucasian, male volunteers with no personal or immediate family history of diabetes were enrolled onto the study. These subjects were 34.71 ± 2.1 yr old (mean ± s.e. range 27–41) with a body mass index of 24.30 ± 0.77 kg/m² (range 21.32–27.72). In another group, six male DM-1 patients with a weak or nonexistent endogenous insulin response to meals were also enrolled. These subjects were 27.77 ± 1.76 yr old (range 22–33) with a body mass index of 25.14 ± 1.08 kg/m² (range 22.12–28.51). The healthy subjects and the type 1 diabetic volunteers received an iv bolus of GLP-1 (7-36)amide (1 μg/kg, all subjects) and saline (all healthy and four diabetic) after overnight fasting in two different tests that were separated by at least 1 wk in each individual subject. Blood samples were drawn 15, 30, and 60 min, and immediately before the treatment was administered (time zero), and then 5, 15, 30, 45, 60, 90, and 120 min after GLP-1

FIG. 3. Comparative effects on corticosterone (A) and aldosterone (B) plasma levels after an equimolar dose (12 nmol/kg, iv) of Ex-4 (5 μg/kg), GLP-1 (4 μg/kg), and ACTH (1-24) (3.5 μg/kg) in anesthetized rats. The data are represented as the mean ± s.e.m. [n = 7–8 rats per group. *P < 0.05; **P < 0.01 vs. saline; ***P < 0.001 vs. saline; ###P < 0.001 vs. ACTH (1-24)-treated group.]

Hormones and cytokines

Table 1

<table>
<thead>
<tr>
<th>Hormone</th>
<th>GLP-1 (7-36)amide</th>
<th>Ex-4</th>
<th>GLP-1 (7-36)amide + Ex-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone</td>
<td>112.4 ± 8.6</td>
<td>106.8 ± 7.2</td>
<td>108.2 ± 8.1</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>14.6 ± 1.2</td>
<td>13.8 ± 1.1</td>
<td>14.2 ± 1.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.5 ± 0.2</td>
<td>4.3 ± 0.1</td>
<td>4.4 ± 0.2</td>
</tr>
</tbody>
</table>

Table footnotes:

1. Data are expressed as the mean ± s.e.m. of 6-8 rats per group.
2. *P < 0.05; **P < 0.01 vs. saline; ***P < 0.001 vs. saline; ###P < 0.001 vs. ACTH (1-24)-treated group.
For 6 d, 24 h, and 48 h the subjects of the study were exposed to 24 h of hyperglycemia. For this experiment,
all groups (n = 6) were fed ad libitum (kcal/kg) or saline for the 24 h, and then all rats
were fasted for 12 h, at which point the study
was completed. The data were analyzed using the Kruskal-Wallis test followed by post hoc Tukey's test to compare three or more groups. Results were considered significant when P < 0.05. The results of the corticosterone variations in MIRK mice were analyzed with the Time Series Analysis Software (Expert Soft Technology, Inc., Richelieu, France).

Results
Peripheral administration of GLP-1/Ex-4 to conscious, freely moving rats
In the first 30 min after the iv administration of GLP-1 (7-36)-amide (20 and 4 µg/kg), freely moving conscious rats exhibited a significant increase in plasma corticosterone (P < 0.05). ACTH levels also increased significantly after 5 min (Fig. 1, A and B). Interestingly, Ex-4 (5 µg/kg, iv) elicited a more potent and lasting activation of the HPA axis, and plasma ACTH remained significantly higher for 60 min after the administration of Ex-4, whereas the rise in corticosterone levels remained significantly higher for 2 h after administration when compared with the control rats (Fig. 1, C and D). When GLP-1 (7-36)-amide (20 µg/kg) was administered ip, a significant increase in corticosterone levels was observed after 60 min (P < 0.05, Fig. 1E), although it was not accompanied by significant changes in ACTH (Fig. 1F).

Intravenous administration of GLP-1/Ex-4 to anesthetized rats
Circulating levels of corticosterone increased 10 to 30 min after GLP-1 (7-36)-amide (20 µg/kg) was adminis-

TABLE 1. Corticosterone response after the ip administration of Ex-4 (3-39)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corticosterone levels (ng/ml) at 15 min</th>
<th>Corticosterone levels (ng/ml) at 30 min</th>
<th>Corticosterone levels (ng/ml) at 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex-4 (3-39) (5 µg/kg)</td>
<td>418.73 ± 77.27</td>
<td>411.50 ± 79.88</td>
<td>156.84 ± 26.45</td>
</tr>
<tr>
<td>Control (CIN 0.9%)</td>
<td>384.15 ± 85.96</td>
<td>325.46 ± 65.25</td>
<td>174.09 ± 12.80</td>
</tr>
</tbody>
</table>

Ex-4 (3-39) in 48-h-fasted rats. The data are represented as the mean ± SE (n = 4-6 rats per group).
TABLE 2. Corticosterone response after the i.p. administration of Ex-3 and Ex-4

<table>
<thead>
<tr>
<th></th>
<th>Corticosterone levels (ng/ml) at 15 min</th>
<th>Corticosterone levels (ng/ml) at 30 min</th>
<th>Corticosterone levels (ng/ml) at 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex-3 (5 µg/kg)</td>
<td>539.88 ± 68.23a</td>
<td>577.40 ± 26.58a</td>
<td>637.68 ± 39.73a</td>
</tr>
<tr>
<td>Ex-4 (5 µg/kg)</td>
<td>482.75 ± 49.00</td>
<td>583.97 ± 36.64a</td>
<td>685.27 ± 43.60a</td>
</tr>
<tr>
<td>Control (CINa 0.9%)</td>
<td>325.72 ± 27.87</td>
<td>364.09 ± 36.40</td>
<td>174.72 ± 31.96</td>
</tr>
</tbody>
</table>

Ex-3 and Ex-4 in 48-h-fasted rats. The data are represented as the mean ± se (n = 4-6 rats per group).

" P < 0.05.
" P < 0.01 vs. control.
" P < 0.001 vs. saline.

sterized w to anesthetized rats that had fasted for 48 h and likewise, the circulating aldosterone levels also rose significantly 30 min after GLP-1 (7-36)-amide administration (Fig. 2A and B). Ex-4 (5 and 20 µg/kg) also significantly augmented the plasma corticosterone levels in a dose-dependent manner from 10 to 60 min after iv administration to rats fasted for 48 h (Fig. 2C), and this increase was greater than that produced by GLP-1. In addition, effective doses of Ex-4 (5 µg/kg) administered iv to anesthetized rats previously fed ad libitum promoted a marked increase in ACTH from 15 to 60 min (Fig. 2D). Moreover, an identical time course in the response to iv administration of an equimolar dose (1.2 nmol/kg, 3.5 µg/kg) of ACTH1-(1-24) (Synacthen) and Ex-4 on corticosterone levels was evident, reaching similar plasma corticosterone levels. By contrast, no such response was elicited by the same molar dose of GLP-1 (7-36)-amide (Fig. 3A). Ex-4 (5 µg/kg) also enhanced aldosterone secretion (Fig.

**FIG. 5.** Effect of the icv administration of GLP-1 (7-36)-amide and Ex-4 on ACTH and corticosterone release in 24-h-fasted rats. A. Dose-dependent effect of GLP-1 (7-36)-amide and the interaction of the effect of GLP-1 (7-36)-amide with Ex-4 (5 µg) on serum corticosterone levels 1 h after administration. B. Effect of various doses of Ex-4 and the interaction of the effect of Ex-4 with GLP-1 (7-36)-amide (20 µg) on serum corticosterone levels 1 h after administration. Time course of the effect on ACTH (C) and corticosterone (D) plasma levels of icv administration of Ex-4 (2 µg) to freely moving rats. The data are represented as the mean ± se (n = 4-8 rats per group): * P < 0.05; ** P < 0.01 vs. saline; *** P < 0.001 vs. saline; ## P < 0.001 vs. GLP-1-treated group.)
Central administration of GLP-1 and EX-4

The icv administration of GLP-1 (7-36)-amide augmented the levels of corticosterone in a dose-dependent manner, and the response to the highest dose (20 μg/rat) was similar to that produced by mean doses of EX-4 (Fig. 5A). Moreover, the effect of lower doses was significantly enhanced by the administration of an additional dose of EX-4 (5 μg/rat, P < 0.05). EX-4 potently stimulated corticosterone release when icv administered, and at all the doses tested (0.1, 1, 3, and 10 μg/rat), a robust and significant increase in corticosterone concentration was observed 1 h after administration (P < 0.01, Fig. 5B). High levels of corticosterone were reached even with the lowest dose of EX-4 tested (0.1 μg/rat), an effect that was not enhanced by the addition of GLP-1 (7-36)-amide (20 μg/rat). Plasma ACTH and corticosterone levels also increased in response to icv administration of EX-4 (2 μg/rat) to freely moving rats, and both remained significantly high 2 h after administration (Fig. 5, C and D).

EX-4 in diabetic animal models

EX-4 (5 μg/kg, iv) increased plasma corticosterone levels in anesthetized STZ-diabetic rats, and the time course of this response was similar to that in control rats that received EX-4 (Fig. 6A). Moreover, the corticosterone release in control-fed rats after ip administration of EX-4 (5 μg/kg) was reproduced in fed and fasted STZ-treated rats (Fig. 6B). Despite the high basal corticosterone levels in STZ-diabetic rats after 72 h of fasting (P < 0.001 vs. fed STZ-treated rats), EX-4 (5 μg/kg) was still capable of enhancing corticosterone release (P < 0.05, Fig. 6B).

The blood glucose profile in MKR transgenic mice, a well-recognized type 2 diabetes-like obese-free model, was very high with respect to their wild-type littermates (Fig. 6C). While the time course of corticosterone, release over 24 h displayed a circadian rhythm identical to that of their wild-type littermates, with higher circulating corticosterone levels (Fig. 6D). Indeed, a significant increase in corticosterone levels was observed in both groups of animals (MKR and wild type) 2 h after EX-4 administration (5 μg/kg, ip; Fig. 6E).

Intraperitoneal administration of EXs

When administered ip, EX-4 strongly enhanced corticosterone levels from 15 to 120 min after its injection to conscious rats (Fig. 4, A and B, and Tables 1 and 2). This increase appeared to be independent of metabolic status because it occurred in rats that were fed and or that had fasted for different periods of time (12-72 h, Fig. 4C). EX-3 reproduced the effects of EX-4 on corticosterone release at 15, 30, and 60 min, unlike EX-4 (3-29), a truncated form of EX-4 that lacks the first two amino acids (Tables 1 and 2). Thus, these two amino acids of the N-terminal domain appear to be essential to produce the effects of EX-4.

3B), but in this case, the effect was not as potent as that produced on ACTH-(1-24).

FIG. 6. A, Time course of the response to iv administration of EX-4 (5 μg/kg) in control and STZ-treated rats. B, Two hours after administration, EX-4 (5 μg/kg, ip) raises serum corticosterone levels in STZ-treated rats that were fed ad libitum and those that were fasted for 72 h. Serum glucose (C) and corticosterone (D) profiles over 24 h in MKR and wild-type (WT) mice. E, EX-4 (5 μg/kg, ip) enhances corticosterone release in MKR and wild-type, 2 h after administration. The data are represented as the mean ± S.E. (n = 5-8; animals per group). *P < 0.05; **P < 0.01; ***P < 0.001 vs. saline; ¥P < 0.05; ¥¥P < 0.01; ¥¥¥P < 0.001 vs. vehicle (citrate buffer)-treated rats. §§§P < 0.001 vs. fed STZ-treated rats.
FIG. 7. Cortisol and glucose response to GLP-1 (7-36)-amide (1 μg/kg, iv) injection in healthy volunteers (A and B) and type 1 diabetic patients (C and D) after overnight fasting. The data are represented as the mean ± s.e. (n = 6 subjects; *, P < 0.05 vs. saline; **, P < 0.01 vs. saline; ***, P < 0.001 vs. saline).

GLP-1 (7-36)-amide in humans

Finally, GLP-1 (7-36)-amide (1 μg/kg) produced a potent increase in cortisol release in healthy volunteers when administered iv (Fig. 7A). Cortisol levels rose significantly between 15 and 90 min after GLP-1 injection, peaking at 30 min (P < 0.01 vs. saline-treated control subjects). Moreover, a significant reduction in glucose levels was also observed in GLP-1-treated subjects after 15 min (P < 0.05) due to its insulinotropic effects (Fig. 7B). Accordingly, in type 1 diabetic patients who fail to produce endogenous insulin, GLP-1 (7-36)-amide did not reduce the glucose levels at any time studied (Fig. 7D). Nevertheless, in these DM-1 subjects, GLP-1 administration still produced a significant increase in circulating cortisol levels that also peaked at 30 min, although reaching slightly lower levels than in controls (P < 0.05, Fig. 7C).

Discussion

Peripheral GLP-1 administration

GLP-1 was previously shown to raise corticosterone and ACTH levels after iv administration (10, 11). Here, we show for the first time that peripheral administration (iv) of GLP-1 (7-36)-amide markedly increased circulating levels of adrenal steroids (corticosterone and aldosterone) in a time-dependent manner in conscious, freely moving and anesthetized rats. Likewise, GLP-1 (7-36)-amide administration augmented cortisol secretion in humans. This marked elevation of corticosteroid levels triggered by GLP-1 was preceded by an increase in ACTH levels, suggesting that ACTH mediates the adrenal steroid responses. However, when GLP-1 was administered ip, the activation of pituitary-adrenal activity was clearly weaker, although a significant increase in plasma corticosterone was observed after 60 min in our study. GLP-1 is physiologically secreted into mesenteric circulation, where most of it is degraded by dipeptidyl peptidase (DPP)-IV and captured in the liver. Hence, less than 15% of the GLP-1 secreted enters into general circulation and can therefore produce a biological effect on target organs (2). GLP-1 administered by the ip route will be processed similarly, yielding a much lower effective dose than when administered by the iv pathway.

There are different possibilities to explain how circulating GLP-1 could exert effects on the HPA axis. It is
known that GLP-1 can cross the blood-brain barrier by simple diffusion (23), and thus, peripheral GLP-1 could gain access to the paraventricular nucleus and directly activate CRH-producing neurons. Indeed, a neural pathway in the central nervous system that is influenced by peripherally released GLP-1 has already been proposed (2, 6, 24). Accordingly, the hormone may bind to and activate sensory afferent neurons, which in turn may activate neurons of the solitary tract nucleus, whose ascending fibers could activate hypothalamic neurons. Alternatively, the peptide could act directly on the pituitary, where GLP-1r expression has also been detected by in situ hybridization (23), inducing ACTH secretion. Moreover, because GLP-1 crosses the blood-brain barrier by diffusion, it might also theoretically enter into circulation and reach the pituitary after iv administration, directly eliciting ACTH secretion by the corticotroph. It is most unlikely that the peptide could enhance corticosterone and aldosterone release through a direct interaction with the adrenal gland, because no GLP-1r expression has been identified at this site (26) and no stimulatory effect of GLP-1 on glucocorticoid secretion by dispersed rat adrenocortical cells has been detected (27).

Alternatively, central and peripheral administration of GLP-1 (7–36)-amide and Ex-4 increases blood pressure and heart rate, and they activate autonomic regulatory neurons in rats (6, 28, 29). In addition, adrenal splanchic innervations may modulate the diurnal rhythm of corticosterone by increasing adrenal responsiveness to ACTH (30). Thus, although the increase in corticosterone levels elicited by GLP-1 and Ex-4 is preceded by an increase in ACTH levels, it is also possible that activation of the sympathoadrenal system by these peptides may enhance the secretogogue effect of ACTH. This might explain why GLP-1 significantly stimulated corticosterone secretion when it was administered ip, despite reducing the increase in plasma ACTH.

**Ex-4 and the adrenal axis**

As expected in the light of previous data using GLP-1, central icv Ex-4 administration markedly elevates circulating ACTH and corticosterone levels, eliciting an even more potent response than that elicited by GLP-1. Indeed, low doses of Ex-4 (0.1 μg) nearly induced the same corticosterone response as 200-fold higher doses of GLP-1 (20 μg). Furthermore, when administered by the icv route, the influence of GLP-1 on corticosterone release was enhanced by Ex-4.

We also show that peripheral administration of Ex-4 strongly stimulates activity of the adrenal axis. Again, this effect is more potent than that produced by GLP-1, producing higher hormone levels that persist for longer. Unlike GLP-1, Ex-4 is not degraded by DPP-IV, and thus, it has a half-life of about 1–2 h rather than 1–2 min (31, 32). The longer half-life of Ex-4 might explain the stronger effects in vivo and the need for lower doses of this drug to produce a robust increase in adrenal corticoids and ACTH release. Furthermore, the corticosterone responses to iv administration of Ex-4 are as potent as those elicited by equimolar doses of ACTH-(1–24), showing a similar time course. These effects of Ex-4 suggest that its influence in regulating the HPA axis may be as relevant as its insulinotropic activity. Interestingly, the effects of Ex-4 on the activity of the HPA axis appear to be independent of the animal’s metabolic status, and indeed, it acted similarly in rats fed ad libitum and fasted rats. It is remarkable that in fasted rats, where basal corticosterone levels are already higher, GLP-1/Ex-4 still induced marked elevation in corticosterone, indicating that their effects are relevant in the activation of the HPA axis even in conditions of metabolic stress.

**Effects of Ex-4 in diabetic models**

Peripheral administration of Ex-4 (iv and ip) markedly increased corticosterone levels in diabetic STZ-rats, as well as in nondiabetic controls. Elevation of corticosterone levels after chronic Ex-4 administration over 1 wk was observed previously in STZ-diabetic rats (33). Here, Ex-4 could still increase circulating corticosterone levels in fasting STZ-diabetic rats, which could be considered as a very stressful metabolic state, highlighting the strength of Ex-4 as an activator of the HPA axis.

We also assessed the corticosterone responses to Ex-4 in a model of type 2 DM, MKR mice. As far as we know, the activity of the HPA axis of MKR mice has not yet been defined. Here, we show that MKR mice exhibit a circadian rhythm of corticosterone similar to that observed in wild-type littermates, reaching the lowest corticosterone levels during daylight hours and peaking between 1600 and 2000 h, with a mild increase in the mean levels (midline estimating statistic of rhythm, 54.4 ± 5.5 vs. 44.1 ± 4.3, P < 0.01) and no change in amplitude (39.7 ± 7.8 vs. 38.6 ± 6.1, not significant) with respect to the wild type. Conversely, MKR mice had significantly higher glucose levels that did not vary significantly during the course of the day. There was a significant increase in circulating corticosterone levels in response to ip Ex-4 injections in MKR mice, also acting as a strong corticosterone-releasing factor in this model of type 2 diabetes. This mouse model of type 2 DM is not associated with obesity, a state that greatly alters HPA function and that is a common cause of hypercortisolism in humans. Thus, the effects on the HPA axis in this model can be attributed to the actual
alterations in diabetes rather than to other conditioning factors as obesity.

GLP-1 increases cortisol levels in healthy subjects and DM-1 patients

The most relevant fact shown here for the first time is that the elevation of circulating adrenal corticoid levels after acute exposure to GLP-1 is not restricted to rodents, as it also occurs in both healthy humans and type 1 diabetes patients to whom relatively low doses of GLP-1 were administered (1 µg/kg). When given as an iv bolus, GLP-1 produced a rapid and time-dependent increase in cortisol that remained high for 90 min despite the short half-life of the peptide. As expected, GLP-1 produces a transient reduction in glycemia in healthy volunteers, which might induce a compensatory response in the adrenal axis that justifies the rise in cortisol. However, GLP-1 also promoted a significant increase in circulating cortisol levels in DM-1 patients that cannot be attributed to a transitory reduction in glycemia, nor can it be linked to the insulinotrophic properties of GLP-1 because these patients lack endogenous insulin production. Why the elevation in glucocorticoid levels induced by GLP-1 does not increase glucos levels afterwards is not clear. One possible explanation is that GLP-1 induced several regulatory effects, besides of incretin mechanism, and those tend to minimize glucos excursions. These might include a reduction in the levels of other counter regulatory hormones, such as glucagon and possibly GH (34), and a reduction in gastric motility that attenuates carbohydrate absorption. In addition, the subjects in this study were fasted overnight, and during the experimental fasting, glucocorticoids can prevent hypoglycemia but not necessary induce hyperglycemia (35).

The GLP-1-induced rise in cortisol in humans was observed at doses lower than those used in the rat (1 us. 4 µg/kg), and thus, we infer that the relevance of circulating GLP-1 in HPA regulation could be even greater in humans than in rodents.

A previous report did not find an increase in plasma cortisol after iv infusion of GLP-1 (7-36)-amide (34). However, this was a hyperinsulinemic and hypoglycemic clamp study that was not designed to test the serum cortisol responses to GLP-1 but rather, to explore the insulinotropic and glucagon suppressive actions of continuous GLP-1 infusion in hypoglycemic conditions. Therefore, the clamped glycemia and the high insulin levels in this study could mask the effects of GLP-1 on cortisol levels. We present here the first evidence of acute effects of GLP-1 after iv bolus administration in normal control and diabetic subjects under unmanipulated metabolic conditions, and with GLP-1 doses in the range of several other secre-

tagogues and activating factors active in the endocrine system: GHRH [1 µg/kg (36)], Grehlin [1 µg/kg (37)], CRH [100 µg in bolus (38)], and TRH [10 µg/kg (39)].

However, whether changes in the physiologic range of GLP-1 levels in the body fluids may modulate the activity of the HPA axis in humans needs to be further clarified. At present, and given that endogenous secreted GLP-1 is extracted efficiently by the liver and quickly degraded by DPP-IV (2), its relevance in the direct control of the HPA axis must be studied further. However, indirect influences through afferent neural regulatory signals mediated by the autonomous nervous system appear to be likely.

We also provide evidence that Ex-4 is a potent activator of the HPA axis in diabetic and healthy animals, in accordance with the association of continuous Ex-4 administration with enhanced cortisol secretion in nondiabetic humans (40). Therefore, further studies will be needed to determine the effects of prolonged Ex-4 administration on the regulation of the HPA axis in humans.

In summary, we show here that acute peripheral administration of GLP-1 (7-36)-amide strongly stimulates the activity of the HPA axis in healthy and diabetic subjects and in rodents. The stimulatory effect on adrenal corticoid release can be extended to other members of the GLP-1 family. Particularly, the effect of the exogenous GLP-1 agonist, Ex-4, is of great interest because it is currently used to treat type 2 diabetic patients. Here, we report that peripheral or central administration of Ex-4 markedly increases circulating corticosterone levels in diabetic and nondiabetic rodents. This effect is more potent than that produced by GLP-1, and it is of a similar magnitude and timing to that of equimolar doses of ACTH in the rat. The capacity of GLP-1/Ex-4 to increase the activity of the HPA axis appears to be of great relevance, because it is produced in rodents (mice and rats) and humans when administered by different pathways (iv, iv, and ip), independent of the metabolic state (ad libitum-fed or fasting animals), as well as in both healthy and diabetic subjects (DM-1 patients and animal models of DM-1 and DM-2). The stimulation of pituitary-adrenal activity produced by these insulinotropic peptides was irrespective of the changes in glycemia and their insulinotropic properties. The mechanism whereby circulating GLP-1 activates the HPA axis remains unclear, although the increase in ACTH after Ex-4 and GLP-1 administration implicates a central mechanism or a direct effect on the pituitary-inducing ACTH release by corticophor cells.

Acknowledgments

We thank Dr. Ana Fernández for kindly supplying the MKR mice.
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References

and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. J Clin Endocrinol Metab 87:1239–1246

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