Exenatide can reduce glucose independent of islet hormones or gastric emptying

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Exenatide can reduce glucose independent of islet hormones or gastric emptying. Am J Physiol Endocrinol Metab 295: E269–E277, 2008. First published May 20, 2008; doi:10.1152/ajpendo.90222.2008.—Exenatide is a long-acting glucagon-like peptide-1 (GLP-1) mimetic used in the treatment of type 2 diabetes. There is increasing evidence that GLP-1 can influence glycemia not only via pancreatic (insulinotropic and glucagon suppression) and gastric-emptying effects, but also via an independent mechanism mediated by portal vein receptors. The aim of our study was to investigate whether exenatide has an islet- and gastric-independent glycemia-reducing effect, similar to GLP-1. First, we administered mixed meals, with or without exenatide (20 μg sc) to dogs. Second, to determine whether exenatide-induced reduction in glycemia is independent of slower gastric emptying, in the same animals we infused glucose intraportally (to simulate meal test glucose appearance) with exenatide, exendin + the intraportal GLP-1 receptor antagonist exendin-(9-39), or saline. Exenatide markedly decreased postprandial glucose: net 0–135-min area under the curve = +526 ± 315 and −536 ± 197 mg·dl⁻¹·min⁻¹ with saline and exenatide, respectively (P < 0.05). Importantly, the decrease in plasma glucose occurred without a corresponding increase in postprandial insulin but was accompanied by delayed gastric emptying and lower glucagon. Significantly lower glycemia was induced by intraportal glucose infusion with exenatide than with saline (92 ± 1 vs. 97 ± 1 mg/dl, P < 0.001) in the absence of hyperinsulinemia or glucagon suppression. The exenatide-induced lower glycemia was partly reversed by intraportal exendin-(9-39): 95 ± 3 and 92 ± 3 mg/dl with exenatide + antagonist and exenatide, respectively (P > 0.01). Our results suggest that, similar to GLP-1, exenatide lowers glycemia via a novel mechanism independent of islet hormones and slowing of gastric emptying. We hypothesize that receptors in the portal vein, via a neural mechanism, increase glucose clearance independent of islet hormones.

Exendin-4 is a natural 39-amino acid peptide present in the salivary gland of the Gila monster lizard (Heloderma suspectum). The peptide has powerful effects on glucose homeostasis in mammals (15). The effects of exendin-4 are attributable to ~50% amino acid sequence identity with the natural hormone glucagon-like peptide-1 (GLP-1), which is released by intestinal L cells in response to meal ingestion (14). GLP-1 has a multitude of peripheral and central actions that contribute to glucose tolerance acutely and chronically. The systemic physiological roles for GLP-1 are effects on pancreatic islets (stimulation of insulin secretion and suppression of glucagon release), stimulation of β-cell proliferation, and inhibition of apoptosis and on the digestive tract (inhibition of gastric emptying and intestinal motility) (for review see Refs. 8 and 18). However, recent evidence suggests a role for GLP-1 in glucose homeostasis beyond the above-mentioned effects. Reports from several laboratories, including our own, suggest that GLP-1 might contribute to increased postprandial glucose disposition via increased muscle glucose utilization and/or inhibition of hepatic glucose output (19, 27). We have hypothesized that the latter effects are mediated via a central mechanism involving receptors in the portohepatic area.

Exenatide, the synthetic form of exendin-4, is used in the treatment of diabetes. Exenatide has been shown to decrease fasting and postprandial glucose in healthy humans and patients with type 2 diabetes in acute and chronic settings (11, 23, 24), as well as patients with type 1 diabetes (10). The effects on postprandial glycemia have been attributed to several mechanisms, including glucagon suppression, delay of gastric emptying, and insulinotropic effects (23, 26, 30). In addition, it is possible that, similar to GLP-1, exendin-4 may have an effect on glucose homeostasis, independent of changes in pancreatic hormones and gastrointestinal motility. In contrast to GLP-1, which has a very short half-life (1.5–2 min) (7), exendin-4 has a longer half-life: 26 min in intravenous administration (11) and up to 4 h after subcutaneous administration (24). Thus, although a potential GLP-1 effect mediated by portal receptors might be evident only with portal increases of GLP-1 (e.g., after increased endogenous release or intraportal administration), because of its longer half-life, exendin-4 might exert an islet-independent effect on glucose homeostasis, even in systemic administration, as it is used in diabetes therapy. This effect could provide an additional benefit for treatment of patients with type 1 or type 2 diabetes.

The aim of the present study was to investigate whether exenatide, administered subcutaneously, can produce a reduction in postprandial glycemia beyond that mediated by islet hormones and gastrointestinal motility. If such an independent effect can be identified, it could provide a new pathway for therapy.

METHODS

Animals

Experiments were performed on mature mongrel dogs (27.7 ± 0.8 kg body wt, n = 8) in the conscious relaxed state. The animals were housed under controlled kennel conditions in the University of Southern California Medical School Vivarium and fed a standard diet (27% protein, 32% fat, and 41% carbohydrate; Labdiet, PMI Nutrition, Los Angeles, California).
EXENATIDE’S ISLET-INDEPENDENT EFFECT ON GLYCEMIA

Richmond, IN) once per day. Dogs were used for experiments only if judged to be in good health as determined by body temperature, hematocrit, regularity of food intake, and direct observation. The National Institutes of Health (NIH) Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) was followed, and all surgical and experimental procedures were approved by the University of Southern California Institutional Animal Care and Use Committee.

Surgical Procedures

At least 1 wk before the first experiment, chronic catheters (Tygon, Norton Plastics, Akron, OH) were implanted under general anesthesia in the portal vein, 4 cm upstream from the porta hepatis, and in the jugular vein, with the tip advanced into the right atrium for sampling of right atrial mixed venous blood.

Experimental Design

All experiments were performed in the morning after 12–16 h of fasting. In each of eight animals, we performed the following experiments: mixed-meal tests with or without subcutaneous exenatide (protocols 1a and 1b), intraportal glucose infusions to simulate portal glucose absorption, with or without exendin (protocols 2a and 2c), and (in 5 of the animals) intraportal glucose + exenatide along with intraportal infusion of the exenatide antagonist exendin-(9-39) (protocol 2b).

For all experiments, at approximately 7 AM the animals were brought to the laboratory and placed in a Pavlov sling. After a period of rest, sampling was started at −45 min and continued until 240 min.

**Protocol 1a and 1b: mixed meal with or without exenatide.** After basal sampling, at −15 min, an injection of exenatide (20 μg of Byetta; Amylin, San Diego, CA) or saline was administered subcutaneously. An additional sample was taken at −1 min. At time 0, a mixed meal (471 kcal; 9 ml/kg mixture of equal parts of sour cream and 1 M sucrose solution containing 640 mg of acetaminophen) was administered by oral gavage, and sampling was carried out for 240 min. Although the exenatide dose was at the upper limit of the dose used in humans, it was well tolerated; i.e., there were no signs of discomfort or vomiting.

**Protocol 2a: intraportal glucose infusion with exenatide injection.** After basal sampling, at −15 min, a subcutaneous injection of 20 μg of exenatide was administered, and another sample was obtained at −1 min. At time 0, an intraportal infusion of glucose (20% hydrated dextrose; B Braun, Irvine, CA) was started and continued for 240 min. The intraportal glucose infusion rate was variable: it was designed to result in glycemia matching the systemic glucose seen during the meal test in protocol 1. This glucose infusion rate was determined in a previous study in which an identical meal test was administered and exenatide and saline, respectively, and peaked much later (195 vs. 75 min) in the exenatide group. The exenatide-induced reduction in postprandial glycemia was not due to an insulinoergic effect of the peptide (Fig. 1, B). As expected, insulin concentration increased in response to the meal test in saline and exenatide groups (P < 0.05). However, insulin levels were 83% lower in the exenatide group than in the saline group (P < 0.001) and exenatide (P < 0.05). There was a significant difference between the two experiments [P < 0.001 (drug-time interaction); Fig. 1A]. In the first half of the postprandial period (0–135 min), we measured moderate hyperglycemia in the saline control, while glucose remained below basal levels in the exenatide experiment (net prandial 0- to 135-min AUC = −536 ± 197 and +526 ± 315 mg/dl·min−1 with and without exendin, respectively, P < 0.05). In the saline control group, glucose increased immediately after the meal. However, with exenatide administration, glucose appearance in blood was significantly delayed. Postprandial glucose level was lower (96 ± 4 and 105 ± 5 mg/dl with exenatide and saline, respectively) and peaked much later (195 vs. 75 min) in the exenatide group. The exenatide-induced reduction in postprandial glycemia was not due to an insulinotropic effect of the peptide (Fig. 1B)
trol experiment (Table 1) and, thus, indicating an exenatide-induced decrease in insulin secretion.

During the mixed meal, glucagon did not significantly change from basal in either group (from 46 ± 3 to 66 ± 11 ng/l with saline and from 39 ± 3 to 54 ± 14 ng/l with exenatide, \( P = 0.2 \); Fig. 1C). However, glucagon tended to be lower with than without exenatide (AUC = 3,848 ± 1,927 and 1,581 ± 1,192 ng·l\(^{-1} \cdot \text{min}^{-1} \) with saline and exenatide, respectively, \( P = 0.06 \)). Thus administration of exenatide before a mixed meal resulted in glycemic reduction, without a corresponding increase in insulin secretion and with a smaller rise in glucagon, suggesting that other
mechanisms, such as delayed gastric emptying, accounted for the hypoglycemic effect.

To investigate the role of gastric emptying in exenatide-induced reduction of postprandial glycemia, we measured plasma appearance of acetaminophen. There was a significant difference between saline and exenatide plasma acetaminophen [P < 0.001 (drug-time interaction); Fig. 1D]. During the control meal, acetaminophen concentration increased immediately. In contrast, exenatide induced a delay in the plasma acetaminophen rise. Additionally, maximum acetaminophen concentration was lower with exenatide (1.9 ± 0.6 and 4.8 ± 1.0 μg/ml with exenatide and saline, respectively, P < 0.05). AUC was reduced 53% with exenatide (278 ± 87 and 588 ± 112 μg·ml⁻¹·min⁻¹ with exenatide and saline, respectively, P < 0.05), indicating a significant exenatide-induced delay in gastric emptying (Fig. 1D).

NEFA levels were significantly suppressed during the meal with or without exenatide; however, there was less suppression of NEFA in the exenatide than in the control experiment, possibly due to lower insulin levels (Table 1).

Plasma active GLP-1 levels increased with meal administration, from a basal level of 15 ± 5 pmol/l to a peak of 23 ± 12 pmol/l at 30 min (P < 0.05; Table 1). In contrast, with exenatide, GLP-1 did not significantly increase from basal level (from 11 ± 3 to 12 ± 7 pmol/l, P = 0.7).

**Intraportal Glucose Infusions**

Mixed-meal results showed a major effect of exenatide to decrease postprandial glycemia, with no corresponding increase in insulin secretion but with a tendency to lower glucagon, and delay of gastric emptying. To examine whether the hypoglycemic effect of exenatide is maintained in the absence of gastric motility changes, we performed paired experiments (with or without exenatide) in which glucose was infused intraportally at the same rate in both experiments, simulating identical appearance of glucose from the intestinal bed.

Glycemia decreased with exenatide in the fasting state, before the start of the glucose infusion (from 93 ± 2 to 89 ± 2 mg/dl, P < 0.05; Fig. 2A). Intraportal glucose infusion at identical rates resulted in significantly different glycemia during the saline or exenatide experiments [P < 0.01 (drug-time interaction); Fig. 2A]. In the saline experiment, we measured mild hyperglycemia very similar to the mixed meal: glucose increased from 93 ± 3 to 104 ± 4 mg/dl (P < 0.01 vs. basal) and returned to the basal level at the end of the experiment. Subcutaneous exenatide injection before the glucose infusion considerably reduced systemic glycemia: glucose levels further decreased 10 min after the start of glucose infusion (from 93 ± 2 mg/dl to a nadir of 86 ± 3 mg/dl, P < 0.001 vs. basal). For the duration of the experiment, glycemia was significantly lower with exenatide than with saline (AUC = 992 ± 349 vs. 419 ± 407 mg·dl⁻¹·min⁻¹, P < 0.001; Fig. 2A).

The lower glycemia measured in the exenatide experiment was not a result of increased insulin secretion, since there was no significant difference in plasma insulin with or without exenatide [P = 0.23 (drug-time interaction); Fig. 2B]. Without exenatide, insulin significantly increased from a basal level of 53 ± 6 pmol/l to a maximum of 99 ± 23 pmol/l (P < 0.01); with exenatide, there was no significant insulin increase: from a basal level of 50 ± 5 pmol/l to a maximum of 71 ± 8 pmol/l (P = 0.09). There was no statistically significant difference between the insulin AUC with saline and exenatide: 1,631 ± 2,161 and 619 ± 1,853 pmol·l⁻¹·min⁻¹, respectively (P = 0.71; Fig. 2B). The insulin results were paralleled by C-peptide measurements, which showed significantly increased insulin secretion in the saline but not the exenatide group (Table 2) and no significant difference between saline and exenatide [P = 0.27 (drug-time interaction)]. Since glycemia was significantly lower during exenatide treatment, no difference in insulin secretion suggests an increased insulin response to glucose in the exenatide-treated group; however, the ratios of insulin to glucose (0.58 ± 0.04 and 0.61 ± 0.06 pmM⁻¹·dl⁻¹ with exenatide and saline, respectively, P = 0.26) and C-peptide to glucose (1.53 ± 0.10 and 1.45 ± 0.10 nmM⁻¹·dl⁻¹ with exenatide and saline, respectively, P = 0.28) were not significantly higher in the exenatide group.

Suppression of glucagon was not involved in the hypoglycemic effect of exenatide: glucagon levels were similar during experiments with or without exenatide (Fig. 2C). Glucagon concentration changed slightly and nonsignificantly from the basal level in both experiments (from 42 ± 3 to 64 ± 10 ng/l with saline and from 49 ± 3 to 72 ± 22 ng/l with exenatide, P = 0.69), and there was no significant glucagon difference between saline and exenatide [P = 0.67 (drug-time interaction); Fig. 2C].

There were no significant differences in NEFA or GLP-1 between matched intraportal glucose infusions with or without exenatide (Table 2). However, in the exenatide experiment, NEFA levels were suppressed earlier (30 and 60 min with

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**Table 1. C-peptide, NEFA, and GLP-1 during meal test with exenatide or saline**

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<tr>
<th></th>
<th>Basal</th>
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Values are means ± SE. NEFA, nonesterified fatty acid; GLP-1, glucagon-like peptide-1 Ex, exenatide (20 μg, 15 min before test meal); Sal, saline. Significantly different from saline: *P < 0.05; †P < 0.01. ‡Significantly different from saline: P < 0.05.
exenatide and saline, respectively), suggesting increased insulin sensitivity.

**Effect of Antagonist Exendin-(9-39)**

To investigate whether the exenatide-induced reduction of glycemia is mediated via a pancreatic-type portal GLP-1 receptor, in a separate experiment, after exenatide injection, we intraportally infused glucose (for 240 min) and exendin-(9-39) (from 0 to 60 min). Exendin-(9-39) partly reversed exenatide’s hypoglycemic effect (Fig. 3A). Peripheral glycemia was significantly higher during antagonist administration than during exenatide experiments (95 ± 3 vs. 92 ± 3 mg/dl, P < 0.01), although it did not reach the levels of the saline control (99 ± 3 mg/dl; Fig. 3A). In contrast, after the antagonist infusion was stopped, glucose decreased to values significantly lower than the saline control (92 ± 3 vs. 96 ± 2 mg/dl, P < 0.05) and was not different from the level attained during the exenatide experiments (92 ± 3 vs. 91 ± 2 mg/dl, P = 0.43; Fig. 3A). The effects of antagonist administration are more evident in the AUC analysis during and after antagonist infusion (Fig. 3B). During antagonist administration, there was a statistically significant increase in the glucose AUC (−15- to 60-min AUC = −142 ± 63 and 51 ± 33 mg·dl⁻¹·min⁻¹ with exenatide and antagonist, respectively, P < 0.05); after antagonist infusion was stopped at 60 min, glucose reverted to levels that were not different from the exenatide experiment (60- to 240-min AUC = −98 ± 92 and −277 ± 362 mg·dl⁻¹·min⁻¹ with antagonist and exenatide, respectively, P = 0.71).

Changes in glycemia during infusion of the antagonist exendin-(9-39) were independent of changes of pancreatic hormones (Fig. 4). There were no differences in plasma insulin concentration (Fig. 4A) or insulin secretion, as indicated by C-peptide concentrations between groups with or without the antagonist (Fig. 4B). Additionally, glucagon levels were similar in the presence and absence of exendin-(9-39) (Fig. 4C).

Active GLP-1 was not significantly increased from the basal level during exenatide treatment with (from 7 ± 3 to 7 ± 3 pmol/l, P = 0.49) or without (from 13 ± 8 to 12 ± 8 pmol/l, P = 0.49) antagonist administration. **Fig. 2.** Glucose (A), insulin (B), and glucagon (C) plasma concentrations (left) and 0- to 240-min AUC (right) during intraportal glucose infusion with exenatide (●, solid bars) or without exenatide (○, open bars). ***P < 0.001.
**DISCUSSION**

Exenatide is widely used in the treatment of type 2 diabetes, because it lowers glycemia and improves glucose tolerance, decreases HbA1c, induces weight loss, and stimulates β-cell proliferation and neogenesis (9). The mechanisms by which exenatide and its natural hormone counterpart GLP-1 exert these effects are not completely understood. Our present results show that exenatide lowers glycemia, in part, via a novel effect that is independent of changes in pancreatic hormones or gastrointestinal motility.

Administration of exenatide before a mixed meal resulted in lower glycemia than the saline control. Interestingly, this lower glycemia was evident without a corresponding increase in insulin secretion. Exendin-4 and GLP-1-induced reduction of postprandial glycemia has been noted in healthy humans (11) and in patients with type 1 (10) and type 2 (23) diabetes. The absence of a corresponding insulin increase in our study, as well as in those cited above, is presumably due to a lower glycemogenic signal. This conjecture is supported by the direct correlation between glucose and insulin levels and by the timing of glycemic increase and insulin increase in the mixed meals with and without exenatide (Fig. 1A). In the absence of a corresponding increase in insulin secretion, the postprandial hypoglycemic effect of exenatide has been explained by glucagon suppression and by delay of gastric emptying (11, 23). In our study, the exenatide-induced glucagon suppression was still evident, thus demonstrating that the slowing of gastric emptying could not totally account for the exenatide-induced reduction of glycemia. There was no difference in glucagon concentration between the two experiments; therefore, other mechanisms, such as increased glucose disappearance independent of changes in pancreatic hormones and gut, account for the phenomenon. Moreover, in the exenatide experiment, NEFA levels were suppressed 30 min earlier than in the saline experiment, suggesting increased insulin sensitivity.

Chronic administration of exenatide or GLP-1 has been shown to improve glycemia, insulin sensitivity, and parameters of glucose tolerance, although, at least in part, the chronic effects on insulin sensitivity can be attributed to reduction of food intake and increased β-cell mass and function (13, 17, 40, 41). Data regarding the acute effect of GLP-1 or exendin-4 on insulin sensitivity are more controversial. A number of investigators reported increases in insulin sensitivity with acute administration of GLP-1 (3, 4, 25, 32), while others failed to show these effects. Our present results suggest that exenatide lowers glycemia independent of changes in pancreatic hormones or gastrointestinal motility.

**Table 2. C-peptide, NEFA, and GLP-1 during intraportal glucose infusion with exenatide or saline**

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Values are means ± SE. Significantly different from basal: *P < 0.05; †P < 0.01

P = 0.23) antagonist. Exendin-(9-39) did not change NEFA levels, which were similar with and without antagonist (data not shown).
find an effect (16, 29, 37, 38). It has been suggested that the delivery route (peripheral vs. the more natural, portal route) of GLP-1 could account for some of the differences in outcome between various studies. Indeed, when GLP-1 was delivered intraportally in studies performed in our laboratory and in others (6, 19, 27), an insulin secretion-independent effect of GLP-1 on glucose disappearance was measured. Since exenatide has a longer half-life than GLP-1 (24), even subcutaneous administration will considerably increase the peptide’s levels in portal circulation and produce an effect, as reported in the present study. We found only one study in humans in which effects of exendin-4 on insulin sensitivity were investigated in an acute setting (38). In this study, infusion of GLP-1 or exendin-4 during a euglycemic-hyperinsulinemic clamp in healthy volunteers produced only a minimal, nonstatistically significant increase in glucose infusion rate and glucose disap-

Fig. 4. Insulin (A), C-peptide (B), and glucagon (C) AUC during exenatide (solid bars) or exenatide + antagonist (gray bars) infusion from −15 to 60 min in the presence of antagonist (left) or from 60 to 240 min without antagonist (right).
The present study (75 pmol) generally. Even though the dose of exendin-(9-39) used in the effects of GLP-1 when exendin-(9-39) is administered peripheral to glucose in obese diabetic (ob/ob) might not have been

Another argument supporting an exenatide-induced increase in glucose disappearance independent of pancreatic hormones is the lowering of glycemia in the fasted state, an effect found in our present study and previously reported by several investigators (11, 40). Edwards et al. (11) found that exendin-4 reduced fasting glycemia in healthy volunteers, without changes in insulin or glucagon, whereas Young et al. (40) showed that acute administration of exenatide decreased fasting glucose in obese diabetic (ob/ob and db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys. Interestingly, the effect appeared to be glucose dependent, with the greatest effect obtained at the highest fasting glycemia. Similarly, in the present study, we measured lower glycemia, with a trend toward significance in the fasted state; after the glucose load, the difference in glycemia became more pronounced and statistically significant.

To investigate the mechanism of exenatide-induced increase in glucose disappearance, we intraportally infused a known antagonist of the GLP-1 receptor, exendin-(9-39). The antagonist infusion partly reversed the effect of exenatide on glycemia: the glucose AUC during antagonist administration significantly increased compared with that during exenatide administration; however, it did not completely revert to saline control values, indicating only a partial blocking effect at a dose of 75 pmol·kg⁻¹·min⁻¹. Selection of an appropriate dose of exendin-9 was affected by the lack of information regarding dosage and effects of exenatide and exendin-(9-39) in dogs, so human data were used. In humans studies, a ratio of GLP-1 to exendin-(9-39) of 1:30, strongly suggesting that local action in the portohepatic area might be involved in the effect. Alternatively, the partial blocking could be explained by the fact that exenatide has other peripheral effects, independent of the effects in the portal vein. In a previous study (19), we presented data supporting a portal mechanism of GLP-1 action, by showing that intraportal glucose and GLP-1, but not peripheral glucose and GLP-1, produce an increase in glucose disappearance, a result that is concordant with data from other investigators (2, 21, 27). The portohepatic area is richly innervated and is sensitive to GLP-1 (1, 28). Recent data suggest that circulating GLP-1 can bind to nerve receptors in the portal vein and initiate signals that are carried in vagal afferent nerves (22); administration of a GLP-1 antagonist locally to block receptor binding impaired glucose tolerance (36). Further studies are necessary to elucidate the precise location of the receptor that mediates exenatide’s effect on glycemia and the exact mechanism of this effect. GLP-1 has been shown to have insulin-independent effects on suppressing hepatic glucose production and increasing liver glucose uptake in several studies (6, 31); in other studies, GLP-1 has been shown to primarily increase peripheral glucose utilization (25, 27, 32, 34).

An interesting aspect of GLP-1 physiology revealed by the present study is the potential impact of exenatide on endogenous GLP-1 secretion. Exenatide caused an 88% reduction of postprandial plasma active GLP-1 concentration. This result correlates with findings from other studies (11) in which administration of exedin-4 in healthy humans reduced endogenous GLP-1 secretion by a similar extent. Since during the meal test we measured a considerable slowing of gastric emptying after exenatide injection, it is possible that the delivery of nutrients to GLP-1-secreting L cells was delayed, thus explaining the lower GLP-1 levels. An alternative explanation that has been advanced for this effect is that the presence of exenin-4 results in downregulation of endogenous GLP-1 production (11).

In conclusion, our study presents, for the first time, clear evidence that exenatide, in subcutaneous administration similar to the one used in the treatment of type 2 diabetes, reduces peripheral glycemia via a mechanism independent of a delay of gastric emptying or corresponding changes in pancreatic hormone secretion. In addition, the present study suggests that this effect may be mediated by pancreatic-type GLP-1 receptors, perhaps located in the portohepatic area. This finding could provide an additional tool in the use of exenatide for the treatment of type 1 and type 2 diabetes.

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EXENATIDE’S ISLET-INDEPENDENT EFFECT ON GLYCEMIA


