Effects of 1-mo bolus subcutaneous administration of exendin-4 in type 2 diabetes

Josephine M. Egan,1 Graydon S. Meneilly,2 and Dariush Elahi3
1Diabetes Section, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224; 2Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada V6T-2B5; and 3Geriatric Research Laboratory, Massachusetts General Hospital, Harvard Medical School, Boston Massachusetts 02114

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Egan, Josephine M., Graydon S. Meneilly, and Dariush Elahi. Effects of 1-mo bolus subcutaneous administration of exendin-4 in type 2 diabetes. Am J Physiol Endocrinol Metab 284: E1072–E1079, 2003. First published December 10, 2002; 10.1152/ajpendo.00315.2002.—A gut insulinotropic peptide, glucagon-like peptide-1 (GLP-1), when given continuously subcutaneously, has been shown to be an effective agent to treat type 2 diabetes. Because of inactivation by dipeptidyl peptidase IV (DPP IV), it has a very short half-life (90–120 s), hence the need for continuous administration. Exendin-4 is an agonist of the GLP-1 receptor. It is not a substrate for DPP IV, and we previously demonstrated that intravenous administration has potent insulinotropic properties in type 2 diabetic volunteers. We evaluated the efficacy of bolus subcutaneous exendin-4 in insulin-naïve type 2 diabetic volunteers. Ten patients aged 44–72 yr with mean fasting glucose levels of 11.4 ± 0.9 mmol/l were enrolled, and daily or twice-daily bolus subcutaneous exendin-4 was self-administered for 1 mo. Glycosylated hemoglobin, multiple daily capillary blood glucose, β-cell sensitivity to glucose, and peripheral tissue sensitivity to insulin were assessed before and after treatment. The greatest decline in capillary blood glucose was seen before bed, with a drop from 15.5 to 9.2 mmol/l (P < 0.0001). Glycosylated hemoglobin improved significantly with treatment, from 9.1 to 8.3% (P = 0.009). β-Cell sensitivity to glucose was improved, as assessed by C-peptide levels during a hyperglycemic clamp. No significant adverse effects were noted or reported. Our data suggest that, even with this short duration of therapy, exendin-4 treatment had a significant effect on glucose homeostasis.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is an enteric hormone that is released into the portal vein following a meal. GLP-1 also plays a role in the central control of feeding behavior and has other extrapancreatic effects: it inhibits gastric emptying, has cardiac effects, and has insulinomimetic effects on glucose uptake and disposal (2). It acts to regulate islet hormone secretion, insulin biosynthesis, islet cell proliferation, and gastrointestinal motility through a specific G protein-coupled receptor (3). Its exogenous administration to nondiabetic and type 2 diabetic subjects results in lowering blood glucose (6, 15). However, its therapeutic potential is limited in that its rapid degradation by dipeptidyl peptidase IV (DPP IV) within a few minutes of administration represents a significant impediment to the chronic use of the native peptide (12). One way to circumvent this is to give GLP-1 subcutaneously continuously via a pump so that the active and degraded GLP-1 reaches a steady state (29). Another way is to use an agonist of the GLP-1 receptor that is not a substrate for DPP IV. Exendin-4, a peptide and GLP-1 receptor agonist produced in the salivary glands of the Gila monster (Heloderma suspectum), exhibits increased GLP-1 receptor binding, has a longer biological half-life, and is a more potent insulinotropic agent than GLP-1 (4, 5, 14). Because the amino acid sequence of exendin-4 does not contain a position 2 alanine recognized by DPP IV, this partially explains its longer half-life. Long-term experiments that we (8, 24) performed in diabetic mice and rats showed that twice-daily exendin-4 injections lowered mean blood glucose after glucose tolerance testing, lowered hemoglobin A1c, and decreased body weight and, in Zucker rats, decreased total body fat. The enhanced potency and duration of action of exendin-4 in multiple mammalian species suggest that human studies with exendin-4 are warranted. Having demonstrated its prolonged insulinotropic effects and glucose lowering capacity in an acute experiment of both nondiabetic and diabetic volunteers (5), we initiated a long-term study using exendin-4 administered by subcutaneous injections. We began with one daily subcutaneous injection of exendin-4, but it became clear after four volunteers that this was not maintaining 24-h coverage, so we then gave the exendin-4 twice daily. We also used a sequential clamp consisting of three parts to examine the dynamics of exendin-4 treatment on glucose uptake and insulin secretion: a 1-h hyperglycemic clamp followed by 1 h of recovery followed by a 2-h hyperinsulinemic euglycemic clamp and a 0.5-h recovery. Thus we were able to assess β-cell sensitivity to glucose and peripheral tissue sensitivity to insulin before and after

Address for reprint requests and other correspondence: D. Elahi, Massachusetts General Hospital, Geriatric Research Laboratory, GRB SB 0015, 55 Fruit St., Boston, MA 02114 (E-mail: delahi@partners.org).

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of therapy with exendin-4. Volunteers also kept a diary of capillary blood glucose (CBG), which was monitored at least eight times per day for 1 mo. We show, in type 2 diabetic volunteers, that exendin-4 lowers blood glucose and that HbA1C is improved.

**METHODS**

Selection of volunteers. Nineteen sequential hyperglycemic euglycemic clamps were performed in nine insulin-naïve type 2 diabetic volunteers before and 1 mo after therapy with synthetic, full-length exendin-4 (AC2993: Amylin Pharmaceuticals, San Diego, CA). The volunteers' clinical characteristics are presented in Table 1. Normal electrocardiogram, serum electrolytes, liver and renal function, a fasting glucose of >6.4 mmol/l, and hematocrits of ≥38 in males and 36 in females were requirements for entrance into the study. Volunteers' exclusion criteria included any significant history (physical or laboratory evidence) of hepatic, renal, pulmonary, endocrine (other than type 2 diabetes mellitus), or gastrointestinal disease or any evidence of autonomic insufficiency. Therapy with insulin, steroids (sex or adrenal), diuretics, amphetamines, diphenhydantoin, or enteroactive agents or any medications that might influence carbohydrate metabolism (other than hypoglycemic agents) were also exclusion criteria. Oral hypoglycemic agents were stopped for a 7-day washout period before the initial clamp study. All methods and procedures were approved by the Johns Hopkins Bayview Medical Center/Gerontology Research Center Institutional Review Board (99–02–99–01), along with an investigator-initiated investigational new drug from the Food and Drug Administration. All volunteers provided written informed consent in accordance with the Helsinki II declaration.

Hyperglycemic and hyperinsulinemic euglycemic clamps. All volunteers were weight and activity stable before the initial clamp. Volunteers consumed ≥200 g of carbohydrates for 3 days before the clamp. This clamp procedure has been previously reported in detail (19). The clamp consisted of three steps: 1) a hyperglycemic clamp (5.4 mmol/l above basal) for 1 h and 2) 1 h of glycemic recovery to basal glucose immediately followed by 3) a hyperinsulinemic euglycemic clamp for 2 h with a 0.5-h recovery. The 10-min falling of priming of insulin was followed by a continuous infusion of insulin (480 pmol·m·min⁻¹, Humulin; Eli Lilly, Indianapolis, IN). In type 2 diabetic volunteers, during the recovery period (step 2) the plasma glucose level did not drop to "normal" levels. Therefore, during the hyperinsulinemic euglycemic clamp period (step 3), we did not start the glucose infusion until the plasma glucose level approached 5.3 mmol/l. This level was chosen to clamp the plasma glucose of all of our diabetic volunteers, since it was the mean level encountered in our normal volunteers. The use of sequential clamp for the assessment of glucose uptake during the hyperinsulinemic euglycemic clamp has been validated for diabetic volunteers at this dose of insulin by performing hyperinsulinemic euglycemic clamps without the preceding hyperglycemic clamp (19).

Two hours before the start of the clamp (−120 min), a priming dose of [3-3H]glucose (8.7 kBq/kg) was administered, followed by a continuous infusion of [3-3H]glucose (87 Bq·kg⁻¹·min⁻¹) for the duration of the experiment (to 270 min). The priming dose was adjusted for each volunteer by the ratio of the fasting level to 5.3 mmol/l. Steady-state glucose specific activity was achieved by 90 min. To confirm this and to assess fasting glucose and hormone/substrate levels, four arterialized blood samples were obtained at 10-min intervals starting at −30 min. With the start of the clamp at time 0, blood samples were obtained every 2 min from 0 to 10 min and every 5 and 10 min thereafter for the determinations of plasma glucose and hormone levels. During step 3, the 20% glucose solution was spiked with tritiated glucose ("hot Glc") to maintain a stable plasma glucose specific activity (10). The plasma glucose levels were well maintained and stable during the hyperglycemic portion as well as during the euglycemic portion when a level of 5.3 mmol/l was achieved. Fluctuations did not exceed ±0.265 mmol/l in any volunteer during the euglycemic portion. The actual concentration of the 20% glucose solution measured was 10.2 mmol/l, which was 92% of its stated concentration.

Body composition. Fat mass, lean tissue mass, and bone mineral content were determined by dual-energy X-ray absorptiometry (Model Prodigy, LUNAR Radiation, Madison, WI) on the day of each clamp.

Treatment with exendin-4. Insulin-naïve patients were recruited from the Baltimore area. They had to be willing to stay in the area for the duration of the study, and they had to commit to CBG monitoring and recording no less than eight times every day: before and 1 h after breakfast, lunch, and dinner, 1 h after subcutaneous injection of exendin-4, and before retiring to bed. They were also asked to monitor and record CBG any time they perceived symptoms that they

Table 1. Subject characteristics

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ID, subject no.; BMI, body mass index; A, African American; C, Caucasian; Pre and Post, before and after treatment; Meds, medication(s) taken by subject.

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calculated total glucose Ra and the exogenous glucose infusion rate for the appropriate time interval. Glucose turnover rates were calculated at 10-min intervals from −40 to 270 min. The trapezoidal rule was used to calculate the integrated responses of the first-phase insulin release (0–10 min) and over 30-min intervals. The integrated responses were divided by the time interval, which resulted in mean concentration or rates. All data were analyzed using Statistical Analysis System version 8.1 (Cary, NC). Standard methods were used to compute means, standard errors of the mean, and Pearson correlation coefficients. Mixed-model analysis for repeated-measures design was used to analyze hormone and metabolite responses. Differences between clamps were evaluated using a paired t-test. All statistical tests were two-tailed. Except where otherwise stated, data are means ± SE, and P values of <0.05 were regarded as statistically significant.

RESULTS

Clinical characteristics. The clinical characteristics before and after treatment are presented in Table 1. Specifically, exendin-4 did not alter body mass index or percent body fat. The fasting metabolic characteristics obtained on the morning of the clamp, before and after treatment, are presented in Table 2. After treatment, there was a significant decrease in Hb A1c levels from 9.08 ± 0.42 to 8.29 ± 0.46% (P = 0.009).

CBG. Daily glucose excursion throughout the day during treatment with exendin-4 is shown in Fig. 1. The mean levels before breakfast and before initiation of therapy were 13.9 mmol/l and dropped to the 8.3–11.1 mmol/l range while subjects were on daily therapy and were always below 11.1 mmol/l after twice-daily therapy was initiated. The decrease in fasting CBG during the 1-mo therapy before breakfast and bed is significant compared with the levels 10 days before therapy (P < 0.05). The highest levels during the day were seen 1 h after breakfast in the average range of 13.9–18.1 mmol/l while on daily therapy, which dropped to the 11.1–13.9 mmol/l range after twice-daily therapy was started. The decrease in CBG during the 1-mo therapy with exendin-4 at 1 h after breakfast is significant (P < 0.0001) as determined by repeated measures in a mixed model. These levels dropped further to the −11.1 mmol/l range before lunch and increased by ~2.8 mmol/l 1 h after lunch. The levels 1 h

### Table 2. Subject metabolic characteristics

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P-Glc, plasma glucose; NEFA, nonesterified fatty acids.
after dinner were similar to the levels seen 1 h after lunch. The lowest average levels were observed before bed in the range of 5.6–11.1 mmol/l. With >2,000 CBG measurements, only nine incidents of blood glucose ≥3.6 mmol/l were recorded. The lowest value was 3.3 mmol/l.

Hyperglycemic and hyperinsulinemic euglycemic clamps. The data for the first nine patients enrolled in the study (Table 1) are presented for the clamp figures. One additional patient (n = 10) completed 4 wk of exendin-4, but during the last week of therapy she developed an upper respiratory tract infection (unrelated to exendin-4 therapy) for which she was actively being medicated with antibiotic and nasal decongestant at the time when her second clamp study was due. Consequently, her second clamp was not performed, as we thought that the data would be compromised by the new medication. The plasma glucose levels and the glucose infusion rates for the sequential hyperglycemic euglycemic clamp before and after 1 mo of therapy with exendin-4 are presented in Fig. 2. Fasting plasma glucose was 11.4 ± 0.9 and 11.1 ± 0.7 mmol/l before and after treatment, respectively. The fasting plasma glucose levels were rapidly raised by 5.4 mmol/l, and a square wave of hyperglycemia was established from 0 to 60 min (16.4 ± 0.7 and 16.5 ± 0.7 mmol/l). With the termination of the glucose infusion at 60 min, plasma glucose began to fall and reached a level of 13.6 ± 0.7 and 13.3 ± 0.8 mmol/l at 120 min in the pre- and posttreatment clamps, respectively. At 120 min, a square wave of hyperinsulinemia was then created for 2 h. During this period, plasma glucose was allowed to fall to 5.3 mmol/l and was then maintained at this level until 270 min. The glucose infusion required to maintain hyperglycemia and euglycemia during these clamps is shown in Fig. 2. The glucose infusion rates were similar before and after therapy.

Insulin and C-peptide levels during the fasting period and during the two clamp procedures are shown in Fig. 3. Before treatment, fasting insulin levels were 111 ± 24 pmol/l, and the corresponding level after treatment was 108 ± 22 pmol/l. During the hyperglycemic clamp, first-phase insulin response (0–10 min) was not observed before or after exendin-4 therapy. There was a modest second-phase insulin response in both clamps, reaching a level of 158 ± 44 and 156 ± 29 pmol/l, respectively, in the two clamps at 60 min. Whereas plasma glucose levels fell only modestly with the termination of the glucose infusion at 60 min, plasma insulin levels remained elevated in both clamps, and the 120-min levels were 160 ± 38 and 159 ± 38 pmol/l, respectively, in the two clamps at 60 min. Whereas plasma glucose levels fell only modestly with the termination of the glucose infusion at 60 min, plasma insulin levels remained elevated in both clamps, and the 120-min levels were 160 ± 38 and 159 ± 38 pmol/l, respectively. At 120 min, a square wave of hyperinsulinemia was created, and the 120- to 240-min insulin levels averaged 1,092 ± 62 and 1,072 ± 65 pmol/l in the first and second clamps, respectively. With the termination of the insulin infusion at 240 min, plasma insulin levels fell rapidly, and the 270-min levels were 125 ± 16 and 117 ± 16 pmol/l in the two clamps. [P = not significant (NS) for all comparisons between first and second clamp.]

Fasting plasma C-peptide levels were 0.75 ± 0.12 nmol/l before treatment and 0.83 ± 0.08 nmol/l after
treatment. In response to the square wave of hyperglycemia, C-peptide levels increased in both clamps and reached levels of 0.93 ± 0.14 and 1.05 ± 0.10 nmol/l, respectively, at 60 min. The C-peptide level remained elevated at these levels until 120–130 min. Within 10 min after the start of the insulin infusion, C-peptide levels began to fall in both clamps and reached levels of 0.41 ± 0.07 and 0.57 ± 0.09 nmol/l, respectively, at 240 min. The C-peptide levels did not change during the next 30 min when insulin infusion was terminated.

With repeated measures in a mixed model, the change in C-peptide levels during both parts of the clamp is statistically significant (P < 0.001). There is also significant effect of treatment; i.e., posttreatment levels are higher (P < 0.002) but there is no interaction between time and treatment.

Glucagon and nonesterified fatty acid (NEFA) levels during the fasting period and during the clamp procedures are shown in Fig. 4. Fasting plasma glucagon levels were 24.7 ± 4.6 pmol/l before treatment and 24.4 ± 3.1 pmol/l after treatment. During both clamps, plasma glucagon levels began to fall throughout all phases of the clamp, reaching levels of 20.1 ± 3.4 and 19.5 ± 1.9 pmol/l at 240 min. The fall in plasma glucagon levels is statistically significant (P < 0.05). There was no significant effect of treatment and no interaction between time and treatment. Fasting plasma NEFA levels were 0.60 ± 0.04 mmol/l before treatment and 0.77 ± 0.07 mmol/l after treatment (P < 0.0001). With the start of the hyperglycemic clamp, plasma NEFA began to fall and fell further during the recovery period. During the hyperinsulinemic euglycemic portion of the clamp, NEFA reached a stable level of 0.13 ± 0.01 and 0.24 ± 0.01 mmol/l from 150 to 240 min before and after treatment, respectively (P < 0.001). With repeated measures in a mixed model, the fall in NEFA during the clamp is statistically significant (P < 0.0001). When NEFA levels were expressed as a difference from basal levels and with repeated measures in a mixed model, there is no significant effect of treatment (P = 0.96). However, there is a significant interaction between time and treatment (P = 0.002), indicating a faster rate of suppression of NEFA following treatment.

Glucose turnover rates. Basal HGP before therapy was 18.61 ± 0.89 μmol·kg⁻¹·min⁻¹. HGP dropped to
11.9 ± 4.3 μmol·kg⁻¹·min⁻¹ during the last 30 min of the hyperglycemic portion of the clamp. However, during the euglycemic portion of the clamp, HGP fell further and reached a rate of 6.3 ± 2.1 μmol·kg⁻¹·min⁻¹ during the last 0.5 h of the insulin infusion. Glucose disposal rates during the last 30 min of the hyperglycemic portion of the clamp and last 30 min of the hyperinsulinemic euglycemic portion of the clamp were 14.4 ± 1.3 and 22.8 ± 4.6 μmol·kg⁻¹·min⁻¹. After 1 mo of therapy, basal HGP was 16.5 ± 1.5 μmol·kg⁻¹·min⁻¹. During the last 30 min of the hyperglycemic clamp, HGP dropped to 5.0 ± 1.6 μmol·kg⁻¹·min⁻¹. During the euglycemic portion of the clamp, HGP was further suppressed to 2.4 ± 1.7 μmol·kg⁻¹·min⁻¹. Glucose disposal rates during the last 30 min of the hyperglycemic and hyperglycemic euglycemic portions of the clamp were 9.4 ± 1.2 and 18.2 ± 3.2 μmol·kg⁻¹·min⁻¹. Although both basal and stimulated HGP and glucose disposal rates were lower during all portions of the clamp following therapy, changes were not significant.

Safety. Two patients experienced nausea after the subcutaneous injection, one for three evenings and one for four evenings, during the 1st wk of exendin-4 treatment. Thereafter, even with addition of a second dose or escalation of the previous nighttime dose, there was no more nausea recorded.

DISCUSSION

We demonstrate that exendin-4 administered subcutaneously, even in this limited study, can impact positively on blood glucose levels in poorly controlled, community-dwelling, insulin-naive type 2 diabetic subjects. CBG levels were clearly lower before bedtime (2200–2400) than at any other time of the day, reflecting the fact that exendin-4 had been given at ~2100. The nighttime injection also lowered fasting blood glucose levels, although at the dose we used levels were still in the 11.1 mmol/l range. The nighttime dose did not control postbreakfast blood glucose levels, and this was evident in the first four volunteers for the entire month. Introduction of a second injection in the morning, just before breakfast after the 1st wk of once-daily nighttime treatment in the rest of the volunteers, resulted in lower blood glucose 1 h after eating. However, it can be seen that, even with the introduction of the second morning injection, postlunch and -dinner blood glucose levels were not lowered. This means that twice-

Fig. 3. Plasma insulin (A) and C-peptide (B) levels during hyperglycemic and hyperinsulinemic euglycemic clamps before (○) and after (●) 1 mo of therapy with exendin-4. Data are means ± SE.

Fig. 4. Plasma glucagon (A) and nonesterified fatty acid (B) levels during hyperglycemic and hyperinsulinemic euglycemic clamps before (○) and after (●) 1 mo of therapy with exendin-4. Data are means ± SE.
daily subcutaneous exendin-4, at this dose, does not give full 24-h blood glucose control. Therefore, exendin-4, should it become a treatment for type 2 diabetes, is unlikely to be used in the present mode and/or dose of administration.

We were restricted from altering the upper limit of the total 24-h dose by the conditions of the protocol. Despite the imperfect blood glucose control, exendin-4 lowered mean blood glucose levels, and there was a decrease in \( \text{Hb A}_{1\text{C}} \), even though the study was only of 1-mo duration.

We did not obtain blood for assessment of various metabolic factors during the 1-mo treatment. However, we can undoubtedly attribute the control of the plasma glucose levels mainly to the well-established insulino-tropic effect of exendin-4 (1, 4, 5, 8, 18, 23, 24, 27, 28). Another mechanism, which also may have contributed to the improvement in glucose homeostasis during this treatment, is reduction in gastric emptying, which would retard postprandial absorption of carbohydrates and which is a well-known feature of GLP-1 and exendin-4 (4, 16).

The effect on prebedtime CBG lasted for the duration of the treatment, indicating that there was probably no desensitization of the GLP-1 receptor to exendin-4 in the islets over time.

In the clamps performed after 1 mo of therapy with exendin-4, the last dose of the peptide was administered \( \geq 12 \) h before the start of the hyperglycemic portion of the clamp. Therefore, it is not surprising that neither first-phase nor second-phase insulin responses were augmented during the hyperglycemic portion of the clamp. C-peptide levels, on the other hand, were significantly augmented by exendin-4 treatment. Therefore, it appears that insulin secretion is indeed augmented following exendin-4 treatment but that insulin extraction from the portal vein from the first pass through the liver must also be augmented.

An assay for exendin-4 in not commercially available at this time. However, the pharmacokinetic actions of exendin-4 have been reported with a two-site immunoradiometric assay that measures the full-length molecule (17). When exendin is administered intravenously or subcutaneously, the half-life is reported to be 18–41 min or 90–216 min, respectively. Bioavailability of subcutaneously administered exendin-4 was estimated to be 65–75%, and plasma clearance rate of intravenously administered exendin-4 was 4–8 ml/min. For GLP-1, the corresponding estimates of intravenously and subcutaneously administered peptide are 0.8–4.7 and 0.6–13.5 min, respectively. Estimates of bioavailability and clearance rates are 36–67% and 35–38 ml/min.

This 1-mo treatment with exendin-4 resulted in a significant increase in NEFA. We have no explanation for this, and further studies are underway to elucidate the mechanism involved. However, during the clamp, NEFA levels were suppressed following treatment in the same pattern as before treatment. This is undoubtedly due to their exquisite sensitivity to hyperinsulinemia. Furthermore, NEFA were suppressed at a significantly faster rate following treatment, indicating improved adipose tissue sensitivity.

We did not instruct our patients to modify their daily habits in any way. None reported changes in appetite and none had any change in body weight. GLP-1 has been reported to decrease food intake with short-term administration (7, 9, 25, 26) but not with longer administration. Two patients complained of nausea the 1st wk of treatment. Nothing else of note was seen.

To our knowledge, there are relatively few studies that have used exendin-4 in vivo (1, 8, 18, 23, 24, 27, 28), and most studies have utilized animal models. The duration of treatment with exendin-4 in those studies ranged from acute studies to 13 wk. There are two reports of acute intravenous administration of exendin-4 in humans (4, 5). Infusion of exendin-4 has been shown to reduce postprandial glucose profile in normal volunteers (4). We (5) have previously reported the acute effects of exendin-4 administered intravenously in normal and diabetic volunteers during a hyperglycemic clamp. A very potent insulino-tropic effect of exendin-4 with a long biological half-life was demonstrated. The exendin-4 dose used in the present study was calculated from our own experience with this agent and with reported effects observed in acute studies by our colleagues (13). Studies using different doses and modes of administration as well as longer durations of therapy are needed.

In summary, subcutaneously administered exendin-4 lowers blood glucose, but the manner of administration and the dosing need optimizing. There was no indication of desensitization to its effects. We are working on a continuous subcutaneous delivery system that would deliver exendin-4 from a transdermal-type formulation.

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