Insulinotropic Actions of Exendin-4 and Glucagon-Like Peptide-1 In Vivo and In Vitro

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This study compares in vitro effects of exendin-4 and glucagon-like peptide (GLP)-1 on basal and glucose-stimulated insulin release from isolated rat islets and in vivo insulinotropic actions of exendin-4 and GLP-1 after an intravenous glucose challenge in rats. In static incubation of isolated islets, changing ambient glucose concentration from 3 mmol/L to 10 mmol/L stimulated insulin secretion 9.8 ± 1.3-fold. The addition of exendin-4 or GLP-1 (1 nmol/L to 1 μmol/L) increased glucose-stimulated insulin secretion up to 5.8 ± 1.6-fold and 3.3 ± 1.0-fold, respectively, over basal rates (defined as no hormones added, 3 mmol/L glucose) and 19.6 ± 2.3-fold and 15.0 ± 3.1-fold at 10 mmol/L glucose. In dynamically perfused isolated islets exposed to 7.5 mmol/L glucose, insulin secretion increased 6.4 ± 1.5-fold, and exendin-4 (20 nmol/L) or GLP-1 (20 nmol/L) increased this similarly by up to 13.5 ± 2.8 and 12.7 ± 3.9-fold,respectively. Anesthetized rats administered 5.7 mmol/kg intravenous glucose increased plasma insulin concentration 3.0-fold. Infusion of exendin-4 or GLP-1 increased this to a maximum of 7.6-fold and 5.3-fold, respectively. As with isolated islet studies, in vivo dose responses and concentration responses with exendin-4 and GLP-1 were bell-shaped. When insulinotropic effects were mapped onto the steady-state plasma concentrations associated with these infusion rates, both peptides exhibited bell-shaped concentration responses with peak insulinotropic effects occurring with plasma peptide concentrations of approximately 1 nmol/L in this model. In summary, exendin-4 and GLP-1 exhibited similar insulinotropic potencies (median effective dose [ED_{50}]) when assessed on a concentration basis in in vitro and in vivo models, while exendin-4 exhibited greater efficacy (maximum response).

EXENDIN-4 IS A recently characterized 39 amino acid peptide isolated from the salivary secretions of the Gila Monster Lizard, *Heloderma suspectum*.[1] Exendin-4 shares about 53% sequence homology with the mammalian gut hormone, glucagon-like peptide-1 (GLP-1).[2] In vitro, exendin-4 is reported to bind to and activate mammalian GLP-1 receptor preparations[3-5] of the type found on pancreatic cells.[6] Initial clinical interest in GLP-1 was based on its insulinotropic effect, the ability to amplify glucose-stimulated insulin secretion without increasing insulin secretion at low plasma glucose concentrations. This glucose dependence was thought to promise a potential safety advantage over agents that increase insulin secretion via glucose-independent mechanisms. But the clinical utility and development of GLP-1 has been frustrated, at least in part, by its short half-life in man and the need for continuous or frequent administration.[7]

Recent clinical and pharmaceutical interest has been shown in exendin-4. Exendin-4 has antidiabetic actions, has a much longer duration of action than GLP-1,[8,9] and up to 3,000-fold greater potency for glucose-lowering in vivo.[9] Exendin-4 has been shown to exhibit glucose-dependent insulinotropic effects in vitro,[3] as has GLP-1,[10] and has been shown to be insulinotropic in human studies.[11] The insulinotropic effects of exendin-4 have not been directly characterized in animals or compared with GLP-1 in vivo. As part of the exploration of the remarkable glucose-lowering potency of exendin-4 (in contrast to GLP-1), the present study compared the actions of both exendin-4 and GLP-1 on insulin release from isolated rat pancreatic islets in 2 in vitro systems, in a static culture incubation system and in a microphysiometer-based perifusion system. We quantify and compare in anesthetized rats the dose-dependent effects of exendin-4 and GLP-1 on insulin secretion stimulated by an intravenous glucose challenge. Using recently developed sensitive and specific 2-site assays for full-length exendin-4 and GLP-1, we define in vivo concentration responses for the insulinotropic effects observed in this animal model. The data support the conclusion that the insulinotropic potency of exendin-4 may not entirely reside in its interaction at β-cell receptors, but in its pharmacokinetic properties, shown elsewhere to differ markedly from those of GLP-1. Preliminary data from this study have been published in abstract form.[12,13]

MATERIALS AND METHODS

In Vitro Experiments

Islet isolation. Male Lewis rats (Harlan Sprague Dawley, Indianapolis, IN) weighing between 150 and 200 g, were housed at 23 ± 1°C in a 12:12 hour light:dark cycle and fed and watered ad libitum. Islets of Langerhans were isolated from whole pancreas using a method of collagenase digestion originally described by Lacy and Kostianovsky[14] and modified by Lakey et al[15]. Briefly, the pancreas was dissected free of fat, cleaned, and placed into 5 mL collagenase-P (1.8 mg/mL; Boehringer Mannheim, Indianapolis, IN) plus DNase (0.1 mg/mL), minced, and incubated at 37°C for 20 minutes. Digested pancreas was then shaken vigorously, washed in HEPES buffer, and islets were separated on a Ficoll gradient. Selected islets were then hand picked and cultured in RPMI/fetal calf serum (10%) for 3 to 4 days at 37°C until experimentation.

Static incubation. After washing in RPMI media for 1 hour and then incubation in Hanks' Balanced Salt Solution (HBSS) buffer containing 3 mmol/L glucose for 1 hour, triplicate groups of 15 islets were then incubated at 37°C in fresh HBSS buffer for a further hour of treatment, and media was then collected and frozen at -70°C until assay for insulin. Separate treatments consisted of addition of the following to buffer medium: D-glucose 3 mmol/L (control), 7.5 mmol/L or 10 mmol/L, glucose 10 mmol/L + somatostatin 100 nmol/L, glucose 3 mmol/L + L-arginine 10 mmol/L exendin-4 or GLP-1 (100 nmol/L), glucose 10 mmol/L exendin-4 or GLP-1 (1, 10, 100 nmol/L, or 1 μmol/L).

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Microphysiometer perifusion. Groups of 50 islets were entrapped with agarose in a Cytosensor Microphysiometer perifusion chamber (Molecular Devices, Sunnyvale, CA) and were continuously perfused with HBSS buffer (phosphate concentration modified to 1 mmol/L to reduce buffering capacity of medium) containing 3.0 mmol/L glucose. After a 45-minute equilibration period, islets were exposed to a 15-minute treatment with exendin-4 or GLP-1 (20 nmol/L) in the presence of 3 or 7.5 mmol/L glucose. Perifusate was collected in 5-minute fractions (100 μL/min), and secreted insulin was measured by radioimmunoassay.

In Vivo Experiments

Animals. Male Harlan Sprague-Dawley fed rats, weighing 380 to 420 g, were housed at 23 ± 1°C in a 12:12 hour light:dark cycle, with ad libitum access to food (Diet LM-485, Teklad, Madison WI) and water. Anesthesia was induced with 5% halothane and maintained at 2% during surgery and 1% to 1.5% thereafter. Rats were tracheotomized and cannulated via the right saphenous vein for infusions and injections and via the right femoral artery for sampling analytes. Heparinized saline (2 U/mL) was infused via the arterial line at 4.5 mL/h beginning at t = -1 hour. At least 60 minutes after surgery, beginning at t = -30 minutes, saline only (1 mL/h, n = 8), exendin-4, or GLP-1 were infused intravenously in the same volume at 3, 30, 300, and 3,000 pmol/kg/min (n =4 to 8; see legend to Fig 4). Thirty minutes after beginning peptide or saline, D-glucose (5.7 mmol/kg) was injected intravenously at a rate of 0.5 mL/min over 2 to 3 minutes. Colonic temperature was measured and controlled using a thermistor probe/controller (YSI, Yellow Springs, OH). Samples (150 μL) for glucose, lactate, and insulin were taken at -30, -15, 0, 5, 10, 15, 20, 30, 40, 50, 60, and 90 minutes after dextrose. Plasma glucose and lactate were determined by immobilized oxidase chemistries on a YSI 2300 Stat Plus (YSI). Samples for insulin were determined by radioimmunoassay (Linco Research, St Charles, MO). All animal experiments complied with the "guiding principles in the care and use of animals".

To conserve blood volume in the present in vivo experiments, plasma concentrations of exendin-4 and GLP-1 corresponding to the peptide infusion rates used here were calculated from parallel continuous infusion pharmacokinetic studies in a similar preparation, reported separately.[16,17] Specificity of the respective immunoassays was validated to show that for the GLP-1 enzyme-linked immunosorbent assay (ELISA), cross-reactivity to active GLP-1 (7-36) amide and GLP-1 (7-36) was 100%, and for GLP-1 (1-36) amide, 1-37, 9-36 amide, 9-37, GLP-2, and glucagon was undetectable. For the exendin-4 immunoradiometric assay (IRMA), the assay detects full-length exendin-4, but not GLP-1 or tested metabolites of exendin-4 or GLP-1. Steady-state plasma GLP-1 and exendin-4 concentrations were calculated from the dose-concentration relationships shown (see Fig 4e. Concentration-response relationships were therefore derived for both peptides (see Fig 4f) to account for potential differences in plasma kinetics.

Chemicals and Reagents

Exendin-4 (1-39), GLP-1 (7-36)-amide, and somatostatin-28 were obtained from Bachem (Torrance, CA). Tissue culture media were obtained from BRL (Gaithersburg, MD). Radioimmunoassay kits for rat insulin (sensitivity, 0.1 ng/mL; interassay variability, 7.4%; intraassay variability, 4.8%) were obtained from Linco Research. All other chemicals were obtained from Sigma (St Louis, MO).

Data Analysis

All results are shown as mean ± SEM. Data were analyzed for statistical changes by 1-way analysis of variance (ANOVA), followed by Dunnett's Multiple Comparison Test (Prism, Graphpad Software, San Diego, CA). Data from islet perifusion experiments were analyzed by unpaired Student's *t* test. All changes stated within the text are significant at $P < .05$. Integrated insulin responses (in vivo experiments) were calculated as the trapezoidal area under the response:time curve for the 60 minutes after glucose injection (AUC) and analyzed using Student's *t* test. Dose responses and concentration responses reported in the text were analyzed using Prism 3.0 (GraphPad Software) with response minima set as values obtained with no peptide added (in vitro) and during saline infusion (in vivo), and maxima set as the maximal response observed with each peptide (in vitro and in vivo). Bell-shaped response curves shown in the figures were constructed as the sums of stimulatory and inhibitory sigmoids. For dynamic islet perifusions, curves fitting the data are single component exponentials.

RESULTS

Static Islet Incubation

Culturing islets in 7.5 and 10 mmol/L glucose resulted, respectively, in increases in rate of insulin secretion of 6.9 ± 1.5-fold and 9.8 ± 1.3-fold over those observed in the presence of 3 mmol/L glucose (designated basal) (Fig 1). Addition of somatostatin (100 nmol/L) to islets cultured in 10 mmol/L glucose reduced the 9.8-fold stimulation of insulin secretion by approximately 50%. Addition of arginine (10 mmol/L) to glucose (3 mmol/L) resulted in an insulin release 5.5 ± 1.3-fold greater than basal. Glucose-dependence, somatostatin-inhibition, and arginine-stimulation of insulin secretion confirmed that the islet preparation used in the present study was responding similarly to those reported elsewhere.[18-22]

Addition of either exendin-4 (100 nmol/L) or GLP-1 (100 nmol/L) to buffer containing arginine (10 mmol/L) + glucose

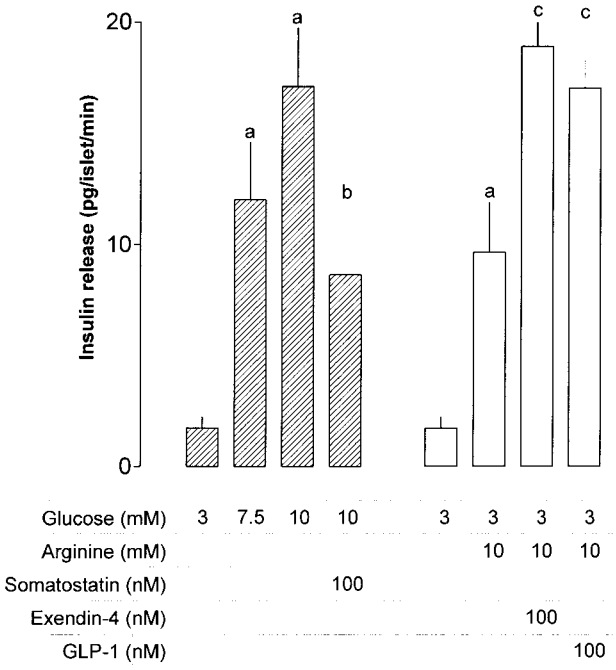

Fig 1. Characterization of isolated rat islets in static culture. Results are shown as mean values ± SEM and indicate glucose stimulation and somatostatin inhibition of insulin secretion (n = 4 to 16). Insulin secretion is further enhanced by addition of arginine, and exendin-4 or GLP-1 (open bars). [a]*P* < .05 *v* glucose 3 mmol/L, [b]*P* < .05 *v* glucose 10 mmol/L, [c]*P* < .05 *v* arginine 10 mmol/L.

(3 mmol/L) further augmented insulin secretion (by 10.9-fold and 8.6-fold over basal), confirming the presence of an insulinotropic effect of these peptides in this preparation.

To explore the in vitro concentration responses for enhancement of insulin release by exendin-4 and GLP-1, these peptides were added at final concentrations of 1, 10, 100, or 1000 nmol/L to media containing 3 mmol/L (low) or 10 mmol/L (high) glucose (Fig 2). At both glucose concentrations, exendin-4 produced a concentration-dependent response for augmenting insulin secretion, and in each case, this was bell-shaped (Fig 2).

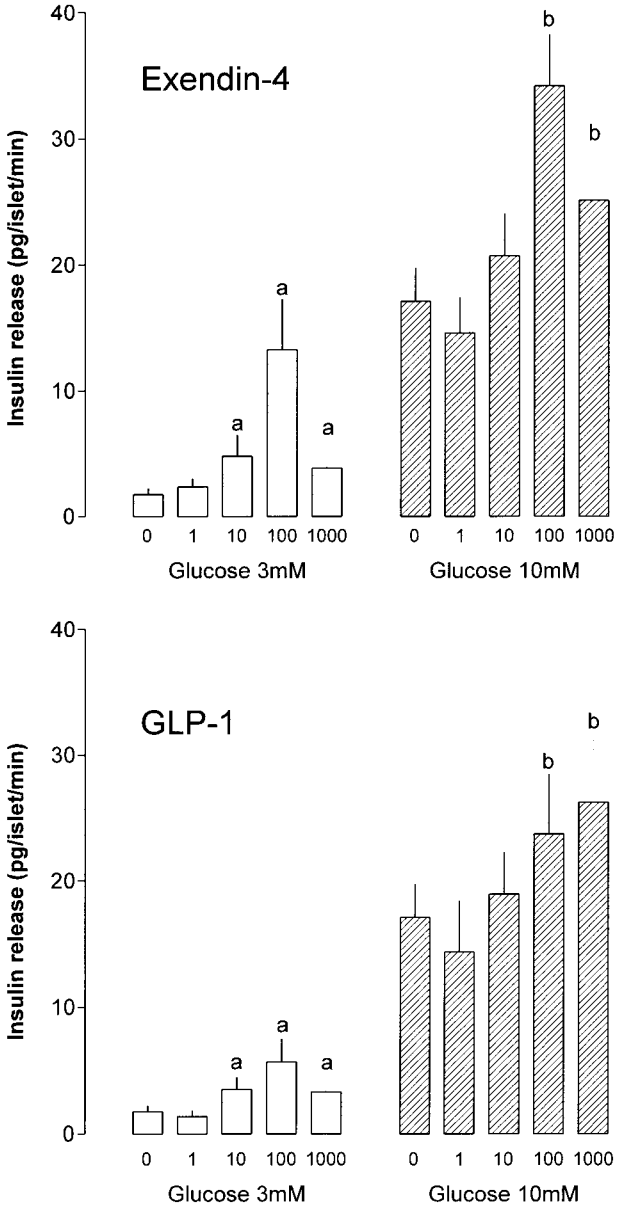

Fig 2. Insulinotropic actions of exendin-4 (1, 10, 100, and 1,000 nmol/L) and GLP-1 (7-36)-amide (1, 10, 100, and 1,000 nmol/L) in isolated rat islets in static culture in the presence of 3 or 10 mmol/L glucose. Results are shown as mean values ± SEM (n = 5 to 16). [a]*P* < .05 *v* glucose 3 mmol/L alone, [b]*P* < .05 *v* glucose 10 mmol/L alone.

Insulin secretion patterns in parallel incubations with GLP-1 instead of exendin-4 were similar, except that maximal secretory rates were greater in islets incubated with exendin-4 (by 76% and 44% at 100 nmol/L peptide concentrations at low and high glucose concentrations, respectively, $P < .05$). At low glucose concentrations, GLP-1 (1, 10, 100, and 1,000 nmol/L) increased plasma insulin in a bell-shaped fashion. At high glucose concentrations, dose-dependent increases in insulin were observed, which were not bell-shaped (Fig 2).

The EC_{50} values for the insulinotropic effect of exendin-4 or GLP-1 are shown in Table 1. EC_{50} values for exendin-4 or GLP-1 enhancement of insulin release were not different when compared in either 3 mmol/L or 10 mmol/L glucose incubations ($P = .39$, $P = .94$, respectively). In most (3 of 4) sets of incubations, stimulation of insulin secretion with the highest (1 μmol/L) concentration of peptide was less than with 100 nmol/L peptide. That is, concentration response curves were bell-shaped.

Microphysiometer Islet Perifusion

Increasing perifusate glucose concentration from 3 mmol/L to 7.5 mmol/L for 15 minutes in the absence of exendin-4 or GLP-1 stimulated insulin secretion up to 6.4 ± 1.5-fold (Fig 3), and insulin secretion remained elevated during exposure to high glucose. In the presence of 3 mmol/L glucose, neither exendin-4 nor GLP-1 (20 to 100 nmol/L) had any effect on secretion of insulin from the perfused islets (data not shown). During 15 minutes elevation of perifusate glucose concentration to 7.5 mmol/L and concurrent exposure to exendin-4 (20 nmol/L) or GLP-1 (20 nmol/L), maximum insulin secretion increased to 13.5 ± 2.8-fold and 12.7 ± 3.9-fold, respectively, significantly greater than the 6.4 ± 1.5-fold observed in the presence of 7.5 mmol/L glucose alone (Fig 3). Insulin secretion returned towards control levels within 5 minutes of cessation of exendin-4, GLP-1 or elevated glucose treatment.

Anesthetized Rats

In saline-treated, exendin-4–treated, and GLP-1–treated rats, plasma glucose concentrations were similar before injection, increased by similar amounts after glucose injection, decayed at similar rates for 60 minutes after the glucose challenge, and resulted in similar 60-minute glucose AUC values with all doses of peptide treatment (Fig 4c). That is, the glycemic stimulus was similar in all treatment groups.

Plasma insulin concentration in saline-treated rats increased 3.0-fold after the glucose challenge (322 ± 28 to a peak of 935 ± 56 pmol/L) (Fig 4a and b). During exendin-4 infusions, the increase in plasma insulin concentration was 2.0-fold (3 pmol/kg/min), 7.6-fold (30 pmol/kg/min), 6.6-fold (300 pmol/kg/min), and 5.1-fold (3,000 pmol/kg/min) (ED_{50} = 5.8 ± 19 pmol/kg/min) (Fig 4a). During infusions of GLP-1, plasma insulin concentration increased 3.1-fold (3 pmol/kg/min), 2.9-fold (30 pmol/kg/min), 5.3-fold (300 pmol/kg/min), and 4.7-fold (3,000 pmol/kg/min) (ED_{50} = 51.3 ± 7.9 pmol/kg/min) (Fig 4b). The 60-minute plasma insulin AUC in saline-treated rats was 31 ± 1 nmol/L/min. At an exendin-4 infusion rate of 30 pmol/kg/min, this was increased 96% (61 ± 6 nmol/L/min; $P < .01$ *v* saline; $P < .05$ *v* GLP-1 infusion) and by 23% in

Table 1. Insulinotropic Actions of Exendin-4 and GLP-1 in Isolated Islets in Static Culture

Treatment	EC_{50} (nmol/L)	Maximum Response (fold increase over basal)*	Maximum Response (secretion) (pg/islet/min)	Dose Response Relationship	No. (range)
Exendin-4					
Glucose 3 mmol/L	7.9 ± 3.1 nmol/L	5.8	13.3 ± 4.1†	Bell-shaped	6-8
Exendin-4					
Glucose 10 mmol/L	11.8 ± 2.0 nmol/L	19.6	34.2 ± 4.1‡	Bell-shaped	5-8
GLP-1					
Glucose 3 mmol/L	4.0 ± 5.0 nmol/L	3.3§	5.7 ± 1.8†§	Bell-shaped	7-8
GLP-1					
Glucose 10 mmol/L	12.0 ± 1.9 nmol/L	15.0§	26.2 ± 5.5‡§	Linear	5-6

NOTE. Results are shown as mean ± SEM.

*Fold-increase calculated as: peptide-treated secretion rate/basal (3 or 10 mmol/L glucose alone) secretion rate.

†Basal secretion in presence of 3 mmol/L glucose = 1.7 ± 0.5 pg/islet/min (n = 16).

‡Basal secretion in presence of 10 mmol/L glucose = 17.1 ± 2.7 pg/islet/min (n = 16).

§$P < .05$ *v* exendin-4.

GLP-1–treated rats (37 ± 1 nmol/L/min, not significant [NS] *v* saline).

ED_{50} for the integrated insulin responses (60-minute AUC) was 2.9 ± 9.7 pmol/kg/min for exendin-4 and 10.6 ± 3.9 pmol/kg/min for GLP-1. As with the static islet incubations, maximal insulinotropic effects of exendin-4 and GLP-1 were observed at submaximal doses, with diminution of response at the highest doses. Maximal effect of exendin-4 was observed at an infusion rate of 30 pmol/kg/min, while maximal effect of GLP-1 was observed at an infusion rate 10-fold higher (see Fig 4d).

To perform in vivo concentration response analyses, the independently determined relationships between exendin-4 or GLP-1 infusion rate and steady-state plasma concentration shown in Fig 4e were used to construct the in vivo concentration responses for the insulinotropic effects of exendin-4 and GLP-1 shown in Fig 4f. By this concentration response analysis, peak insulinotropic effect of exendin-4 and GLP-1 each occurred at a similar steady-state plasma concentration (≈ 1 to 2 nmol/L). That is, insulinotropic potency was similar. The magnitude of the maximal effect was 63% greater with exendin-4 than with GLP-1 ($P < .03$). Infusion of exendin-4 has been shown to produce approximately 10-fold higher plasma concentrations than infusion of equimolar doses of GLP-1, most likely due to resistance of exendin-4 to degradation, leading to slower clearance from blood.[16]

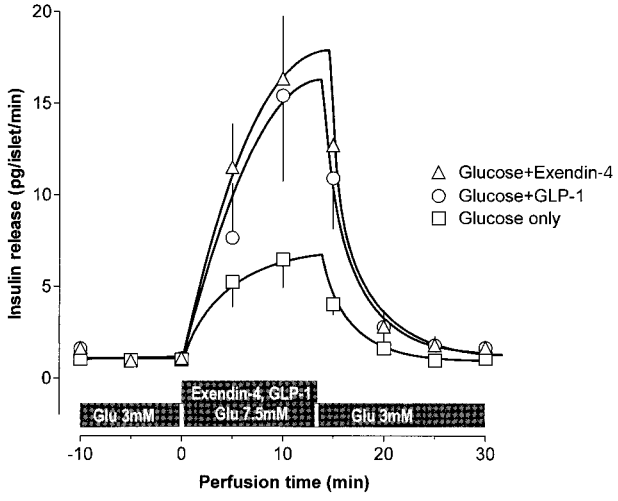

Fig 3. Insulinotropic actions of exendin-4 or GLP-1 (7-36)-amide (20 nmol/L) in isolated, perifused rat islets. Results are shown as mean insulin secretion rate ± SEM (n = 6 to 19). Glu, glucose.

DISCUSSION

In the present study using islets whose functional integrity was well characterized, exendin-4 and GLP-1 augmented glucose-stimulated insulin secretion in a dose-dependent manner. We have further shown that exendin-4 and GLP-1 can potentiate the release of insulin during an intravenous glucose challenge in rats. Maximal insulinotropic effects of exendin-4 were greater than those of GLP-1 in both static islet culture and in vivo, and infusion rates required to reach maximal insulinotropic effect were approximately 10-fold higher for GLP-1 than for exendin-4 during an intravenous glucose challenge. The steady-state plasma concentration of peptide required to achieve this response was similar for each, in a concentration-response analysis, suggesting that slower clearance of exendin-4 from blood may contribute to the greater insulinotropic action versus GLP-1 during equimolar dosing. Consistent with our in vivo findings is a recent report by Greig et al,[23] in which a greater insulinotropic action of exendin-4 was seen versus GLP-1 when examining unstimulated insulin release after equimolar dosing of the 2 peptides to anesthetized rats.

The islet preparations used in the present study appeared to behave similarly to islet preparations described by others,[18-21] in that they exhibited increased insulin release in response to physiologic changes in glucose or arginine, increased glucagon secretion in response to arginine (data not shown), and suppressed insulin secretion in response to somatostatin. Consistent with our in vitro observations in the present study, several groups have reported GLP-1 to directly stimulate secretion of basal (ambient glucose)[24] and glucose-stimulated insulin release from isolated islets.[24-28]

Collectively, these findings indicate that behavior of our isolated islet preparation was similar to that typically described by other investigators. It was therefore initially surprising to us

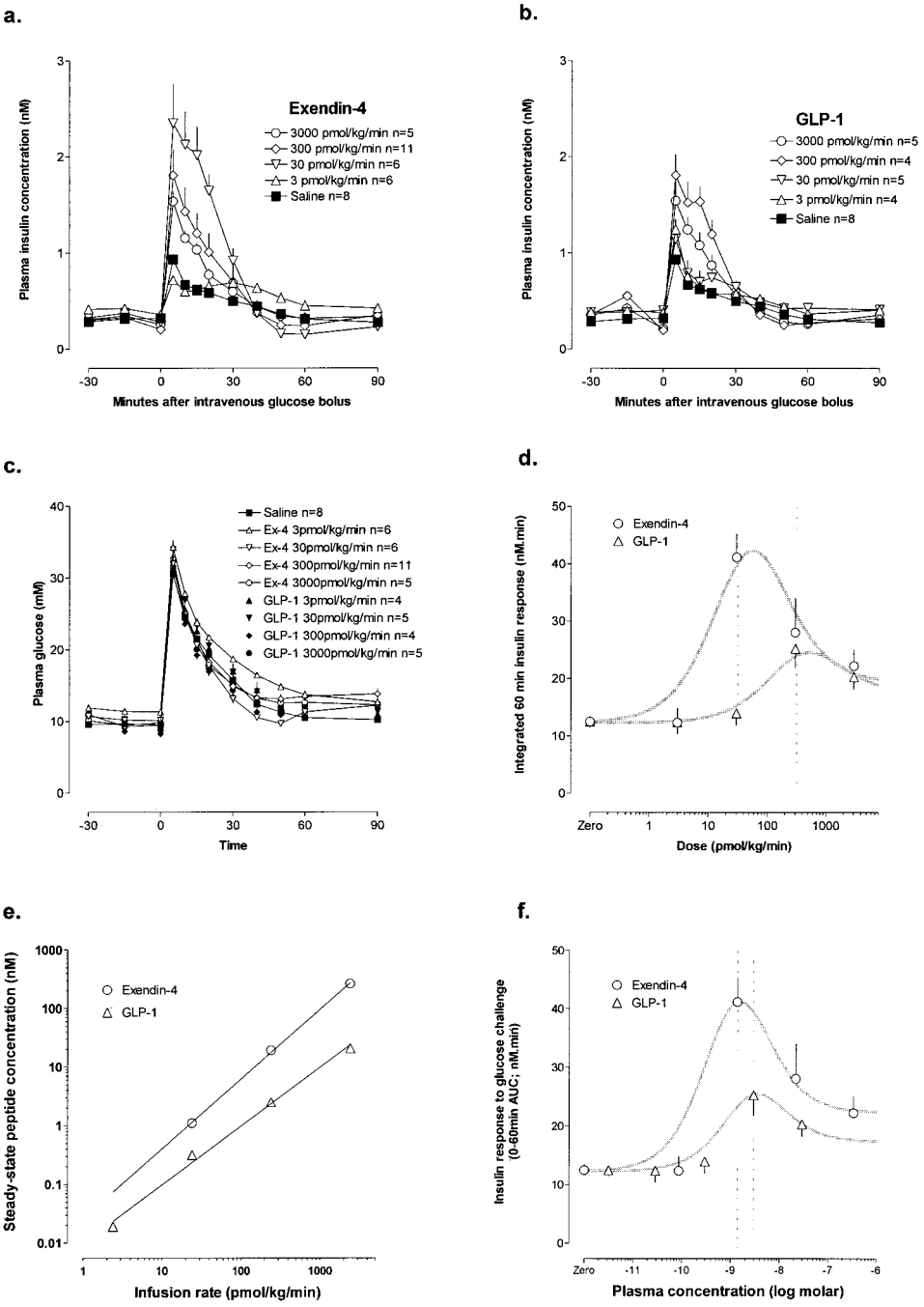

Fig 4. Plasma concentrations in fasted, anesthetized rats (n = 4 to 11). Saline, exendin-4 (a) or GLP-1 (b) (3, 30, 300, or 3,000 pmol/kg/min) were infused for 2 hours from t = -30 minutes, and plasma insulin concentrations assessed at the time points shown. Glucose (5.7 mmol/kg intravenous) was administered at t = 0 minute. (c) Plasma glucose concentrations shown as mean ± SEM of animals treated with saline, exendin-4, or GLP-1 (d) peptide dose *v* integrated 60-minute plasma insulin concentrations. (e) Plasma concentration *v* peptide dose in anesthetized rats (n = 4); regression slopes were not significantly different (*P* = .112); exendin-4 slope = 1.188 ± 0.028, GLP-1 slope = 0.999 ± 0.054. (f) Calculated peptide concentration *v* integrated 60-minute insulin concentrations in anesthetized rats (n = 4 to 8).

that in static isolated islet cultures, and also for the intravenous glucose challenges, bell-shaped concentration responses were observed for both exendin-4 and GLP-1. The consistency of the phenomenon in vitro and in vivo, its presence with both exendin-4 and GLP-1, and the otherwise regular behavior of our islets supports a conclusion that the phenomenon of a bell-shaped dose/concentration response for these insulinotropic peptides is not artefactual. In fact, a bell-shaped dose response for insulin stimulation by GLP-1 is apparent in diabetic humans[29] and in the Rin m5F model of β cells,[30] although the investigators did not comment on this observation. We presently identify no mechanisms underlying these high-dose phenomena.

Others report that exendin-4 and GLP-1 exhibit similar in vitro affinity to cloned GLP-1 receptors[3,4] of the type that are reported to be present on cells within pancreatic islets.[31] These receptors have been proposed from GLP-1 receptor knockout studies[32] and receptor blockade studies[33] to be involved in mediating GLP-1's incretin/insulinotropic effect. Approximately equal potencies of exendin-4 and GLP-1 in activating

GLP-1 receptors on β cells in those studies, and in the present studies, similarities in potency in vitro in islets, and for concentration responses in vivo, would on the surface appear to indicate that insulin secretion was simply being determined by the pharmacology of β cells. But, it must also be noted how in vitro and in vivo responses differ. In the present experiments, insulinotropic effects were observed in vitro in islets for both exendin-4 and GLP-1 when glucose concentration was as low as 3 mmol/L. In contrast, these peptides do not stimulate insulin secretion in vivo when plasma glucose is as low as 3 mmol/L. In nondiabetic, fasted humans administered exendin-4[34] or GLP-1,[10,35], for example, insulin secretion is suppressed as the decline in plasma glucose approaches 4 mmol/L. The fact that this does not happen with islets in static culture suggests that any apparent "glucose-dependence" of the insulinotropic effect is a property that is not entirely intrinsic to islets. We may need to look at other glucose-sensitive structures, such as the brain, to fully appreciate nutrient-stimulated secretion from the endocrine pancreas.[36] The approximately 100-fold greater potency of concentration responses for exendin-4 and GLP-1 in vivo versus that observed in vitro in the present study is another example of a disparity that points to secretory control residing at least partly outside the islet. Alternatively, changes in islet responsiveness, once they are removed from their in vivo setting, may contribute to the observed differences in insulin responsiveness.

Of interest, in all groups of animals receiving an intravenous glucose tolerance test (IVGTT) (saline, exendin-4, and GLP-1), the glycemic stimulus and glucose decay profile was similar, despite higher circulating insulin levels in the exendin-4– and GLP-1–treated rats. This may be explained by studies showing that in the rat (unlike in humans), glucose disposal is more glucose-dependent than insulin-dependent, in which plasma glucose simply falls down its concentration gradient.[37-39] Hence, the decay of glucose observed in the present study may be unaffected by 2- to 3-fold higher levels of circulating insulin in the GLP-1– and exendin-4–treated rats.

In conclusion, we have shown in in vitro and in vivo models that both exendin-4 and GLP-1 stimulated secretion of insulin in a dose-dependent manner. In vivo, exendin-4 was more potent than GLP-1 on a dose basis, but similar in potency when analyzed on a basis of plasma concentration. Exendin-4 demonstrated a greater maximal effect than GLP-1. This study provides the first parallel comparison of these 2 peptides with respect to insulinotropic actions, in which in vivo concentration response analyses permitted efficacy comparisons independent of pharmacokinetic differences, which may exist between these peptides. Disparities between secretory behavior in vitro and in vivo may point to additional extrapancreatic influences on control of insulin secretion, in which exendin-4 and GLP-1 may participate.

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