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Cellular Specificity of Proexendin-4 Processing in Mammalian Cells *in Vitro* and *in Vivo*

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Glucagon-like peptide-1 (GLP-1) is a potent stimulator of glucose-dependent insulin secretion. Exendin-4$^{1–39}$ (Ex-4), isolated from Gila monster venom, is a highly specific GLP-1 receptor agonist that exhibits a prolonged duration of action *in vivo*. Although the processing mechanisms underlying liberation of GLP-1 from its prohormone have been elucidated, those for Ex-4 remain unknown. To examine the requirements for proEx-4 processing in mammalian cells, BHK fibroblasts, InR1-G9 islet A cells, and AtT-20 corticotropes, which express different prohormone convertases (furin, prohormone convertase 2, and prohormone convertase 1, respectively) were transfected with full-length lizard proEx-4, and the processing of proexendin was examined by HPLC and RIA (n = 3). All of the transfected cell lines exhibited Ex-4-like immunoreactivity in the media, and Ex-4-like immunoreactivity was detected in extracts of InR1-G9 and AtT-20 cells. However, only media and extracts from AtT-20 cells (not InR1-G9 and BHK cells) contained a single peak by HPLC corresponding to synthetic Ex-4. To establish whether proEx-4 can be processed to Ex-4 in nonimmortalized mammalian cells *in vivo*, the molecular forms of exendin-4 were examined in mice expressing a metallothionein-proEx-4 transgene (n = 3–6 for both males and females). ProEx4 mRNA transcripts were detected by RT-PCR in a broad range of both endocrine and nonendocrine tissues. Ex-4-like immunoreactivity was detected in pituitary, fat, adrenals, and testes; however HPLC analyses demonstrated that processed Ex-4 was found only in adrenals and testes. These results indicate that lizard proEx-4 is processed to mature bioactive Ex-4 in both rodent endocrine and nonendocrine mammalian cell types *in vitro* and in murine tissues *in vivo*. These findings may be useful for engineering cells that express a lizard pro-Ex4 transgene for the treatment of type 2 diabetes. (*Endocrinology* 143: 3464–3471, 2002)

GLUCAGON-LIKE peptide-1$^{7–36NH_2}$ (GLP-1) is a potent stimulator of glucose-dependent insulin secretion that also inhibits glucagon release and gastric emptying (reviewed in Refs. 1 and 2). GLP-1 may also stimulate β-cell neogenesis in the pancreas (3, 4). Because of its pluripotent effects to reduce both fasting and fed glycemia, GLP-1 is currently under consideration as a therapeutic agent for the treatment of patients with type 2 diabetes (5–7). However, a major impediment to clinical use of GLP-1 is its very short half-life (<2 min) due to cleavage of GLP-1 at Ala^2 by the circulating protease, dipeptidylpeptidase IV (DP IV) (8, 9).

The rapid degradation of GLP-1 suggests that frequent injections or continuous infusion of the peptide would be required to maintain sustained biological activity *in vivo*. Therefore, an alternative approach to the use of the native peptide is the development of long-acting, DP IV-resistant GLP-1 analogs. Several such analogs have been synthesized and shown to exhibit enhanced biological activity *in vivo* due at least in part to enhanced stability and resistance to DP IV-mediated degradation (10–12). A naturally occurring analog of GLP-1 has also been described, the *Heloderma suspectum* peptide, exendin-4$^{1–39}$ (Ex-4) (13). Ex-4 binds and activates the GLP-1 receptor with the same potency as GLP-1 (14). However, Ex-4 contains a Gly at position 2, which confers DP IV resistance to the peptide and thus markedly increases its biological activity *in vivo* (15, 16). Ex-4 has been shown to reduce glycemia in a variety of animal models of diabetes (4, 15–17), and is currently being tested in clinical trials for therapeutic use in patients with type 2 diabetes (18).

Within proglucagon, the sequence of 37-amino acid GLP-1 is flanked by a pair of basic amino acids in the N-terminal sequence, a single basic amino acid at position 6, and a pair of basic amino acids in its C-terminal sequence. Studies of prohormone processing using reconstituted purified enzyme preparations and heterologous cell lines have demonstrated that the endoprotease, prohormone convertase 1 (PC1), is required for liberation of GLP-1 from proglucagon by cleavage at all of these sites (Fig. 1) (19–22). PC1 (also known as PC3) and the related enzyme PC2 are distributed widely throughout neural and endocrine cells and are responsible for the posttranslational processing of a number of different prohormones (23, 24). Although lizard Ex-4 is a GLP-1 receptor agonist, its amino acid sequence shares only 53% identity with that of mammalian GLP-1. Furthermore, cloning of the lizard gene for Ex-4 has shown that it is encoded within a prohormone, proEx-4, that is distinct from proglucagon (Fig. 1) (25). Analysis of the cleavage site required for liberation of Ex-4 from proEx-4 reveals the presence of a classical PC1 and/or PC2 consensus sequence (*e.g.* a pair of basic amino acids), as well as a typical furin cleavage site (*e.g.* Arg–X–Lys/Arg–Arg) (23, 24). Furin is a ubiquitous endoprotease that has been implicated in the processing of many proproteins (23, 24). As information about the factors involved in the processing of proEx-4 may be useful in the development of cell-based or gene transfer strategies for the delivery of bioactive Ex-4 to patients with type 2 diabetes

Abbreviations: DP IV, Dipeptidylpeptidase IV; Ex-4, exendin-4$^{1–39}$; Ex-4-LI, Ex-4-like immunoreactivity; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLP-1, glucagon-like peptide-1; MT, metallothionein; PC, prohormone convertase; TFA, trifluoroacetic acid.

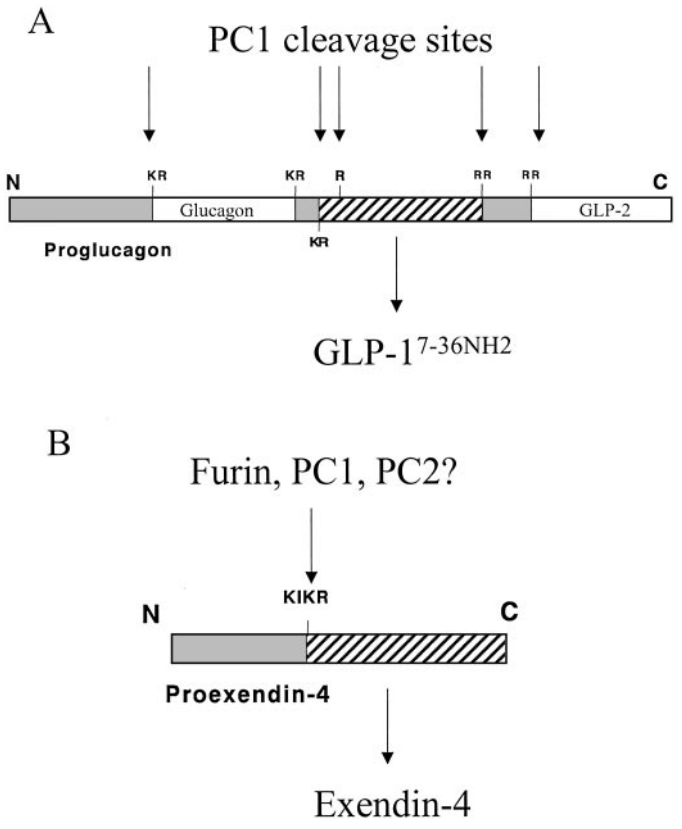

FIG. 1. Schematics of proglucagon (A) and proEx-4 (B) and their known or potential posttranslational processing sites. Proglucagon is cleaved by PC1 at selected pairs of basic amino acids as well as at a single basic amino acid to liberate several biologically active peptides, including GLP-1. Note that GLP-1 normally also undergoes amidation, probably through the actions of peptidylglycine-α-amidating monooxygenase (47). The cleavage site for Ex-4 contains the consensus sequences for cleavage by furin, PC1, and/or PC2.

(26), we have investigated the mechanisms underlying the biosynthesis of Ex-4 using endocrine and nonendocrine cell lines as well as transgenic mice that express a metallothionein (MT) promoter-lizard proEx-4 transgene (27).

Materials and Methods

Cell lines

Baby hamster kidney (BHK) fibroblasts, mouse AtT-20 corticotropes, and hamster InR1-G9 islet A cells were maintained in culture as previously described (19, 20). Cells were transfected using the calcium phosphate method (28) with the full-length lizard proEx-4 cDNA (25) cloned in both the sense and antisense (control) directions into the *Eco*RI site of pcDNA3.1 (Invitrogen, Carlsbad, CA), and transfected cells were selected using 500-1000 μg/ml G418 (Sigma, St. Louis, MO). To minimize the potential for clonal variability, pools of several dozen G418-resistant clones were mixed, propagated, and used for the processing studies. Confluent plates of cells were washed twice and then incubated for 24 h in medium containing 200 μg/ml G418. Medium was centrifuged to remove floating cells, cells were washed twice, and medium and cell peptides were extracted as described below.

Transgenic mice

The creation of MT-proEx-4 transgenic mice expressing the full-length lizard proEx-4 cDNA under the control of 1.9 kb of the mouse MT-1 promoter has been described previously (25). Transgene expression was induced by the addition of 25 mM $ZnSO_4$ to the drinking water for 3 d. The following tissues were collected from adult male and female transgenic mice and age-matched controls: 1) brain: cerebral cortex, cerebellum, hypothalamus, and pituitary; 2) peripheral tissues: muscle and fat; 3) internal organs: gonads, kidneys, liver, heart, lung, adrenals, and spleen; and 4) gastrointestinal tract: stomach, duodenum, jejunum, ileum, colon, and pancreas. Tissue peptides and RNA were extracted as described below. All procedures were approved by the University of Toronto and the University Health Network animal care committee.

Peptide analyses

Cells and tissues were homogenized in 1 N HCl containing 5% HCOOH, 1% trifluoroacetic acid, and 1% NaCl, and medium samples were made to 1% trifluoroacetic acid (TFA), as described previously (19, 20). Peptides were extracted by reverse phase adsorption onto cartridges of C_{18} silica (Sep-Pak, Waters Corp., Milford, MA). We previously reported that this methodology affords a greater than 88% recovery of peptides from tissue extracts (29). Tissue protein levels were determined using the Bradford method (Bio-Rad Laboratories, Inc., Richmond, CA) with bovine γ-globulin as the standard.

HPLC analysis of extracted peptides was carried out on a Waters Corp. liquid chromatography system, using a 72-min linear gradient of 30–80% solvent B (solvent A, 0.1% TFA; solvent B, 80% acetonitrile in 0.1% TFA), followed by a 10-min purge with 99% solvent B. The flow rate was 1.5 ml/min, and 1-min fractions were collected. [^{125}I]His^1-Ex-4 was generated using the chloramine-T method, as previously described (30). An antiserum (Ex18) directed against Ex-4 was prepared by immunization of rabbits with Ex-4 (Cocalico Biologicals, Inc., Reamstown, PA). Titer analysis revealed 35% binding of labeled Ex-4 at a final dilution of 1:50,000. Incubation of standard Ex-4 (5–2,000 pg/tube)/unknowns, tracer, and antiserum was carried out for 5 d at 4 C in 0.2 M glycine buffer (pH 8.8) containing 1% normal sheep serum and 1% human serum albumin, followed by separation of bound and free counts per minute by adsorption to dextran-coated Norit-A charcoal. Specificity studies were conducted using synthetic peptides (all from Bachem, Torrance, CA), at concentrations ranging from 5–200,000 pg/tube (27).

RNA isolation and RT-PCR analyses

Total RNA was isolated from tissues using the acid-guanidinium isothiocyanate method (31). For RT-PCR analyses, 10 μg of each RNA sample were treated with deoxyribonuclease I (Life Technologies, Inc., Gaithersburg, MD), and first strand cDNAs were generated using random hexamers (Life Technologies, Inc.) and RevertAid H$^-$ Moloney murine leukemia virus reverse transcriptase (MBI Fermentas, Flamborough, Ontario, Canada). To control for genomic DNA contamination of RNA preparations, first strand synthesis and PCR experiments were also carried out in the absence of reverse transcriptase. First strand cDNAs were treated with ribonuclease H (MBI Fermentas) to remove RNA, and PCR reactions were carried out using *Taq* DNA polymerase (MBI Fermentas) with first strand cDNAs as templates. For proEx-4-specific products, sense (5′-ATCATCCTGTGGCTGTGTGTT-3′) and antisense (5′-TCCGTTCTTAAGCCACTCAAT-3′) primers were used in 35 cycles of PCR performed at 94 C for 30 sec, 50 C for 30 sec, and 72 C for 1 min. For glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific products, sense (5′-GACCACAGTCCATGACATCACT-3′) and antisense (5′-TCCACCACCCTGTTGCTGTAG-3′) primers were used in 30 cycles of PCR performed at 94 C for 30 sec, 58 C for 30 sec, and 72 C for 1 min. PCR products were separated in 1.5% agarose gels and visualized by ethidium bromide staining. The predicted sizes for Ex-4- and GAPDH-specific PCR products are 221 and 450 bp, respectively.

Immunohistochemistry

Immunocytochemical stains were performed using the streptavidin-biotin-peroxidase technique. A primary antiserum directed against rat βLH was used at a dilution of 1:2500 (National Hormone and Pituitary Program, Rockville, MD), and the Ex-4 18 antiserum was used at a dilution of 1:5000.

Data analysis

All data are expressed as the mean ± SEM. Statistical analyses were performed by ANOVA using n-1 *post hoc* custom hypotheses tests on a

SAS system (SAS Institute, Inc., Cary, NC). Some data were $\log_{10}$-transformed before analysis to normalize variances.

Results

To assess the specificity of the Ex-4 antiserum Ex18, the ability of various Ex-4-related peptides to displace [^{125}I]His^1-Ex-4 from the antiserum was tested (Fig. 2). An N-terminally truncated form of Ex-4, Ex-4$^{9-39}$, fully displaced the labeled peptide, suggesting that the antigenic site was not contained within the first eight amino acids of Ex-4. In contrast, none of the GLP-1-related peptides (*e.g.* GLP-1$^{1-37}$, GLP-1$^{7-37}$, GLP-1$^{1-36NH_2}$, and GLP-1$^{7-36NH_2}$) nor any of the structurally related peptides, such as glucagon, glicentin, oxyntomodulin, GLP-2, glucose-dependent insulinotropic peptide, and vasoactive intestinal peptide, altered Ex18 antiserum binding of the tracer, indicating a high degree of specificity of this antiserum for Ex-4.

Sequence analysis of proEx-4 revealed a potential processing site for furin as well as for PC1 and/or PC2 (Fig. 1). Therefore, the full-length proEx-4 cDNA was transfected into the nonendocrine BHK fibroblast cell line that expresses furin (23), into AtT-20 corticotrope cells known to express PC1 (32), and into the islet InR1-G9 A cell line that expresses PC2 (20). Preliminary screening of transfected cell and medium extracts by RIA demonstrated the presence of immunoreactive Ex-4 in the medium from BHK cells, with less than 5% of the total Ex-4 immunoreactivity detected in BHK cell extracts (Fig. 3 and data not shown), consistent with the constitutive nature of the secretory pathway for these cells (19). In contrast, Ex-4-like immunoreactivity (Ex-4-LI) was found in both the medium and cells of transfected AtT-20 and InR1-G9 endocrine cell lines. No immunoreactivity was detected in the medium or cell extracts for any of the cell lines transfected with the control, antisense proEx-4 cDNA (data not shown).

HPLC analysis of BHK medium extracts revealed the presence of a peak of immunoreactive Ex-4 that eluted in the same position as synthetic, standard Ex-4 (Fig. 3). A second, larger peak was consistently detected that eluted with a shorter retention time than Ex-4, which, by analogy with studies of proglucagon processing in BHK cells (19, 33), may represent unprocessed proEx-4 (putative proEx-4). In contrast to the processing profile of proEx-4 detected in BHK cells, AtT-20 cells and medium contained a major peak of Ex-4-LI that eluted with the same retention time as Ex-4; only a very minor peak corresponding to putative proEx-4 was detectable. The profile detected in InR1-G9 cells differed from that in both BHK and AtT-20 cells, in that equivalent peaks of Ex-4 and the putative proEx-4 were found. However, an additional peak of intermediate hydrophobicity was detected, particularly in InR1-G9 medium. The identity of this peak and of the enzyme that liberated it remain unclear, as the PC cleavage sites for proEx-4 would not be expected to generate such a product.

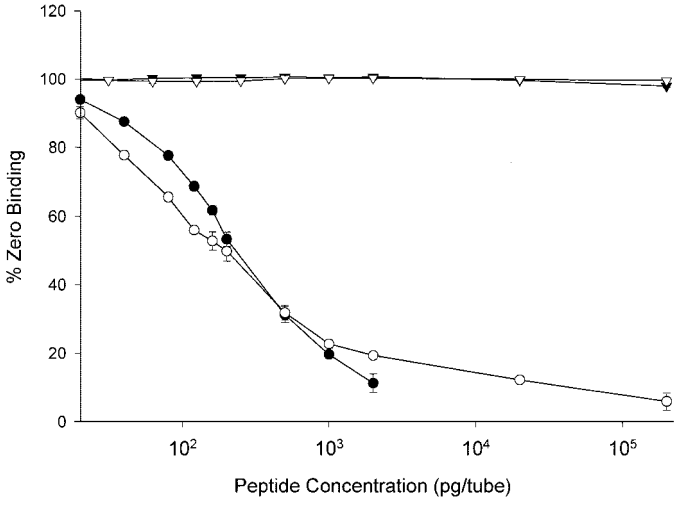

FIG. 2. Displacement curves for Ex-4 antiserum Ex18. Binding of [^{125}I]His^1-Ex-4 to Ex18 was tested in the presence or absence of Ex-4 (standard peptide; ●), Ex-4$^{9-39}$ (○), GLP-1$^{1-37}$, GLP-1$^{7-37}$, GLP-1$^{1-36NH_2}$, GLP-1$^{7-36NH_2}$ (▲), and glucagon, glicentin, oxyntomodulin, GLP-2, glucose-dependent insulinotropic peptide, and vasoactive intestinal peptide (△). Each peptide was tested in duplicate or triplicate in two or three independent assays. The SEMs are not visible for all data points.

To establish whether proEx-4 may be processed to Ex-4 in nontransformed mammalian cells, we assessed transgene expression and Ex-4-LI in tissues from MT-proEx-4 transgenic mice (27). Transgene mRNA transcripts were detected by RT-PCR in a broad range of tissues from MT-proEx-4 transgenic mice (Fig. 4A). Ex-4-LI was detected in multiple tissues from both male and female mice. Male mice exhibited relatively increased levels of Ex-4-LI ($P < 0.01$) in the testes and a trend toward elevated levels in fat, whereas females had increased adrenal Ex-4-LI ($P < 0.05$). No Ex-4-LI was detected in the ovaries (data not shown), whereas relatively high levels of Ex-4-LI ($P < 0.05$) were found in the pituitary (Fig. 4B). As previous studies with a preprosomatostatin construct under the control of the MT-1 promoter demonstrated expression in the gonadotropes of the anterior pituitary (34, 35), immunohistochemical analysis of the pituitary for Ex-4-LI and the β-subunit of LH was performed (Fig. 4C). Despite the ready detection of Ex-4-LI in cells of the *H. suspectum* salivary glands (positive control), little Ex-4-LI positivity was detected in sections of mouse anterior pituitary. Staining of adjacent sections for βLH revealed that the majority of gonadotropes did not contain detectable Ex-4-LI.

HPLC analysis of peptide extracts from pituitary, adrenals (from females), and testes and fat (from males) of MT-proEx-4 transgenic mice revealed marked differences in the profiles of Ex-4-LI between the tissues (Fig. 5). No peak eluting with the same retention time as Ex-4 could be detected in the pituitary, although a peak of putative proEx-4 was present. In contrast, equivalent peaks of Ex-4 and putative proEx-4 were observed in the testes and, to a lesser extent, adrenals. Finally, putative pro-Ex-4 was the major peak detected in fat, although a very small peak of Ex-4 could also be detected.

Discussion

The insulinotropic peptides, GLP-1 and Ex-4, are currently under consideration as therapeutic agents for the treatment of patients with type 2 diabetes. One possible approach to sustained enhancement of GLP-1 receptor signaling may involve a cell-based delivery system, by which cells that have

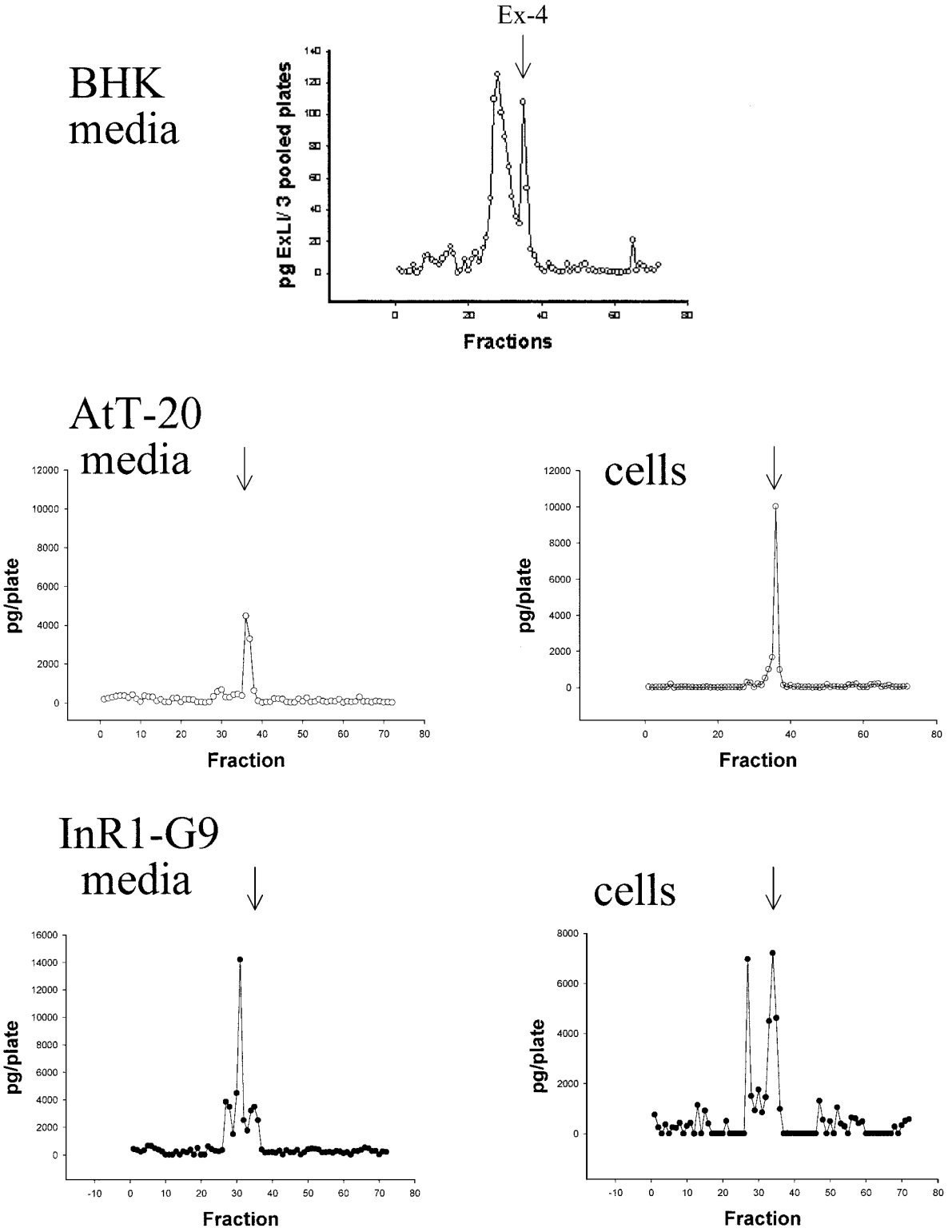

FIG. 3. HPLC analysis of Ex-4-like peptides in BHK medium and in AtT-20 and InR1-G9 media and cells. Peptides were extracted from cells and media by reversed-phase adsorption to C_{18} silica, followed by HPLC and RIA for Ex-LI in collected fractions. Ex-4 indicates the elution position of synthetic Ex-4. All profiles shown are representative of a minimum of three independent analyses.

been engineered to secrete either GLP-1 or Ex-4 are encapsulated and implanted into patients (26). Alternatively, gene transfer methodologies may be employed for the delivery of Ex-4-containing transgenes to specific targeted tissues *in vivo*. Both of these approaches would be expected to increase the circulating levels of GLP-1 or Ex-4 in patients with type 2

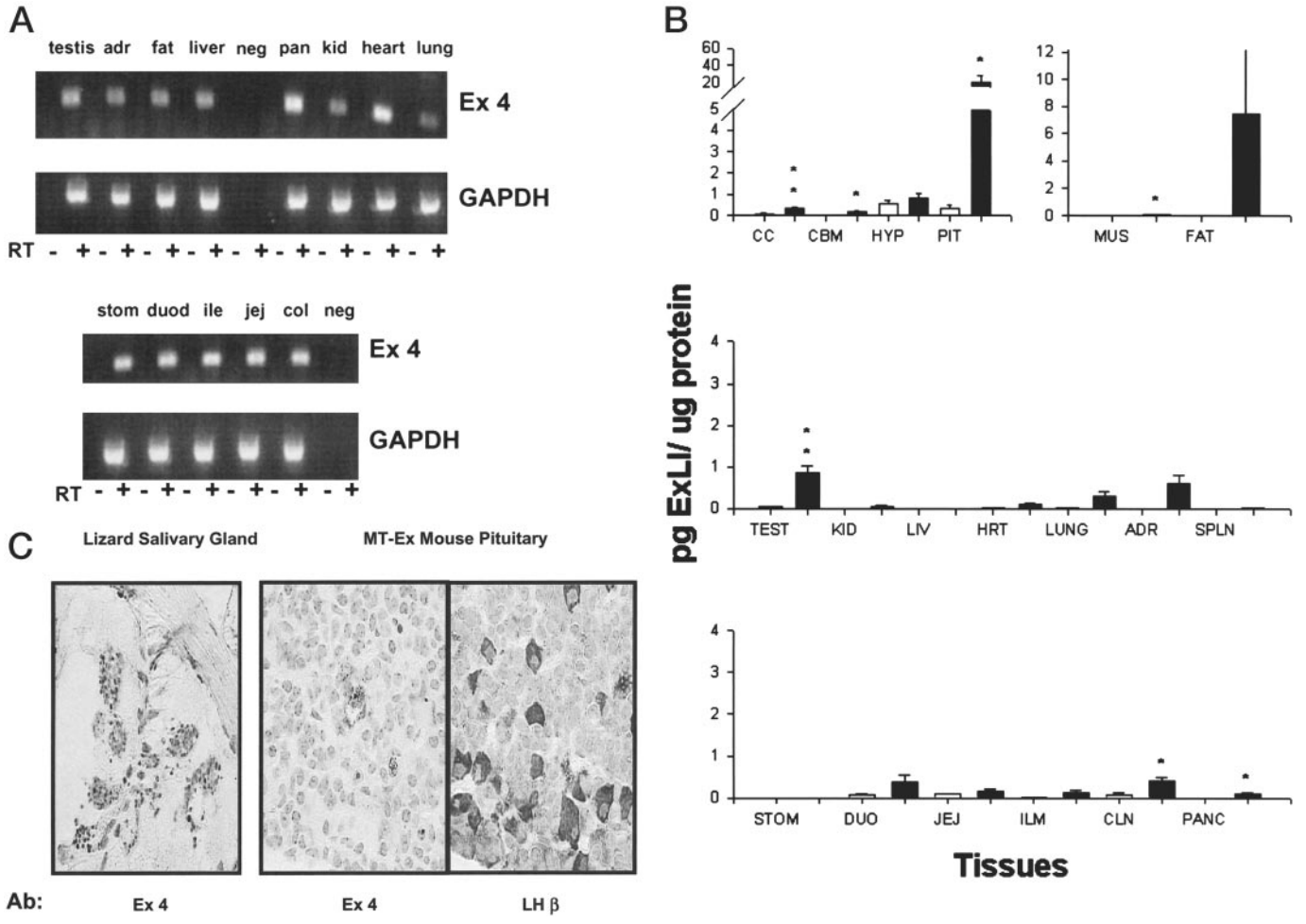

FIG. 4. Expression of Ex-4 in MT-proEx-4 transgenic mice. A, RT-PCR analysis of proEx-4 and GAPDH mRNA transcripts in MT-proEx-4 transgenic mouse tissues. RT-PCR products were visualized on 1.5% agarose gels containing ethidium bromide. RT − and + denote the absence or presence of reverse transcriptase in the first strand cDNA synthesis reaction, respectively. Neg, RT-PCR reactions carried out in the absence of RNA; adren, adrenal gland; panc, pancreas; stom, stomach; duod, duodenum; jej, jejunum. B, Ex-4-LI in extracts of tissues from male MT-proEx-4 transgenic (■) and control male (□) mice. All mice were treated with $ZnSO_4$ before death. Peptides were extracted from tissues by reversed-phase adsorption to C_{18} silica, followed by RIA for Ex-4-LI and Bradford assay for protein content (n = 3–6 for all groups of mice). Results are not shown for female mice, but were not different from those observed in male mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (*vs.* nontransgenic mice). C, Immunohistochemical localization of Ex-4-LI in salivary glands from *H. suspectum* (*left panel*) as well as in mouse anterior pituitary (*middle panel*); the panel on the *right* shows a serial section of mouse anterior pituitary stained for the β-subunit of LH.

diabetes, leading to reductions in glycemia and improved metabolic control.

Previous studies have demonstrated that PC1 is both sufficient and necessary for the liberation of GLP-1 from its precursor prohormone, proglucagon (19–22). However, the very short-half life of native, unmodified GLP-1 currently limits its clinical utility (8, 9). By contrast, lizard Ex-4 exhibits a significantly prolonged half-life *in vivo* compared with mammalian GLP-1 (15, 16), but nothing is known about the factors that determine Ex-4 biosynthesis in either endogenous lizard salivary gland or heterologous mammalian tissues. Therefore, to understand the mechanisms underlying the processing of the lizard prohormone encoding proEx-4, we assessed the liberation of Ex-4 in both transfected immortalized cells and murine tissues expressing the lizard proEx-4 transgene.

Transfection experiments using cell lines that differentially express the prohormone convertases demonstrated that proEx-4 is processed to Ex-4 in cells expressing PC1 (*e.g.* AtT-20 cells), but is only partially processed in cells that express furin (*e.g.* BHK cells) or PC2 (*e.g.* InR1-G9 cells), but not PC1. These findings clearly implicate mammalian PC1 as a regulator of lizard Ex-4 biosynthesis, at least in transfected heterologous cells *in vitro*. ProEx-4 is normally expressed in the salivary gland of the lizard *H. suspectum* (Gila monster) (25). However, although conservation of the mammalian prohormone convertase family during evolution has been well established (23, 24), nothing has been reported to date about the processing enzymes expressed by *H. suspectum*. Nonetheless, as *H. suspectum* also expresses the proglucagon gene encoding the sequence of GLP-1 (25), the presence of a lizard prohormone convertase similar or identical to PC1 in its specificity and activity appears likely.

In contrast to the results of the *in vitro* studies, analysis of proEx-4 processing *in vivo* in MT-proEx-4 transgenic mice revealed only small amounts of Ex-4-LI in most tissues and

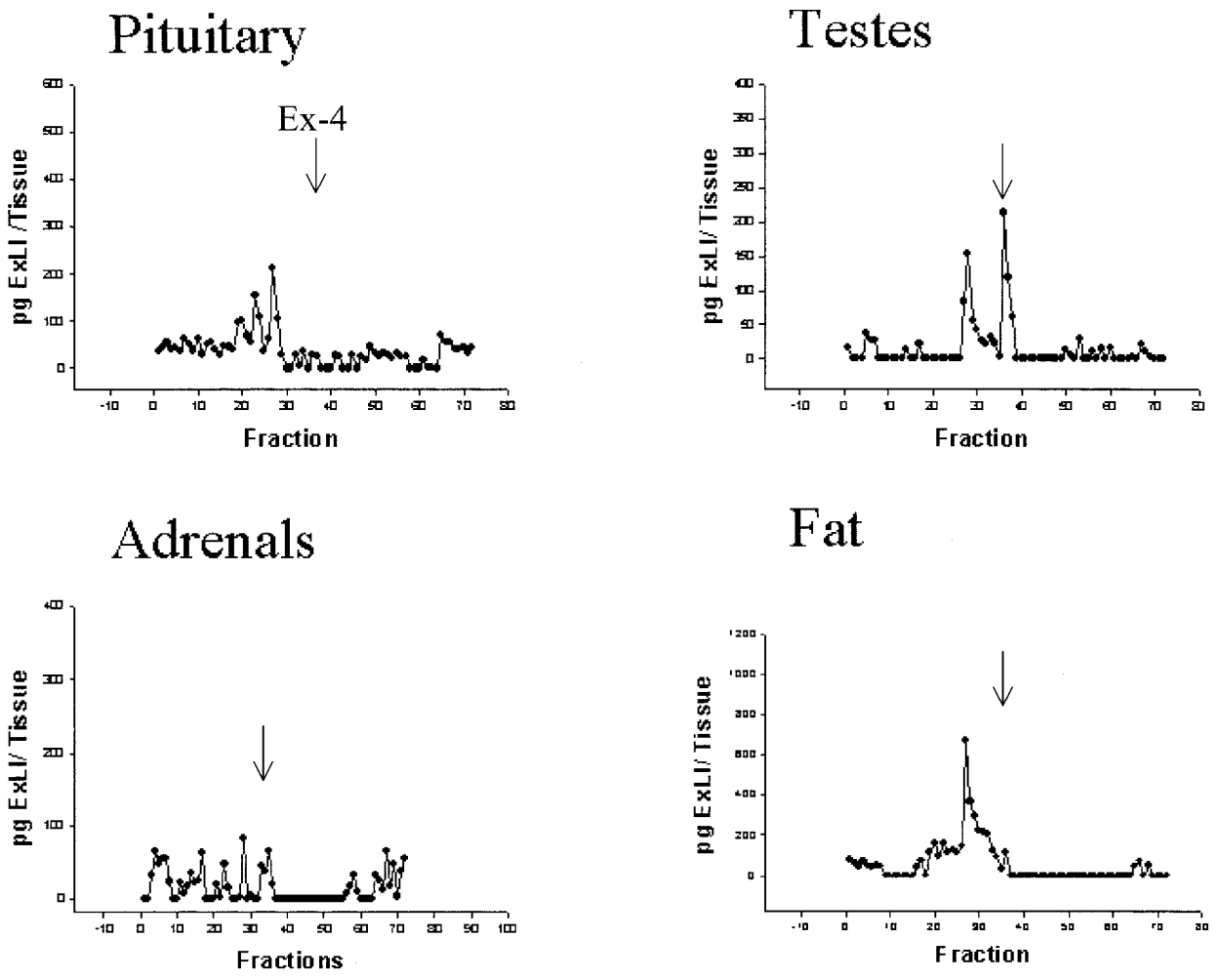

FIG. 5. HPLC analysis of Ex-4-like peptides in pituitary, adrenals (from females), and testes and fat (from males) from MT-proEx-4 transgenic mice. Mice were treated with $ZnSO_4$ for 3 d, after which tissue peptides were collected by reversed-phase adsorption to C_{18} silica. Extracts were analyzed by HPLC, and RIA was used to determine Ex-4-LI in collected fractions. Ex-4 indicates the elution position of synthetic Ex-4. Profiles shown are representative of a minimum of three independent analyses.

only limited processing of proEx-4 to Ex-4 in several tissues. These findings were quite unexpected in view of our previous findings that significant amounts of processed Ex-4 are present in the plasma of both male and female MT-proEx-4 transgenic mice (~1000 pg/ml circulating Ex-4 *vs.* <30 pg/ml in control mice) (27). However, because of autocrine, paracrine, and endocrine peptide secretion, the tissue peptide content does not necessarily correlate with the levels of these peptides in the circulation. Thus, the widespread distribution of transgene mRNA transcripts taken together with the limitations inherent in extrapolating the results of studies on tissue peptide content preclude an accurate assessment of precisely which murine tissue(s) gives rise to the majority of correctly processed, circulating Ex-4 detected in the plasma *in vivo*.

Interestingly, the testes were found to contain high levels of total Ex-4-like immunoreactivity as well as significant amounts of correctly processed Ex-4 relative to the levels of putative proEx-4. These findings are consistent with the results of widespread transgene expression under the control of the mouse MT-1 promoter, including the testes, as described in related transgenic experiments (34, 36–38). Most notably, the testes express high levels of a third prohormone convertase, PC4, but do not express either PC1 or PC2 (39, 40). Within the testes, PC4 is believed to contribute to the posttranslational processing of propituitary adenylate cyclase-activating polypeptide, which appears to play an important role in male fertility (40, 41). These findings, therefore, implicate PC4 and PC1 in the posttranslational processing of transgene-derived proEx-4 *in vivo*.

Although relatively high concentrations of total Ex-4-LI were found in the pituitary of MT-proEx-4 transgenic mice, HPLC analysis revealed a major peak consistent with the putative proEx-4, with little processed Ex-4 peptide detectable. Previous studies using the same MT-1 promoter as that in the present study linked to the preprosomatostatin-coding sequence have demonstrated high levels of expression in the gonadotropes of the anterior pituitary (34, 35). The presence of both PC1 and PC2 in the gonadotropes (42) is consistent with the demonstration of fully processed prosomatostatin in the pituitaries of MT-prosomatostatin mice (43, 44) as well as with *in vivo* and *in vitro* findings that prosomatostatin can be

fully processed by either of these enzymes (45, 46). Unexpectedly, however, immunohistochemical localization of Ex-4-LI did not reveal immunopositivity in the majority of gonadotropes, and indeed, few immunopositive cells were detected in the pituitary. Furthermore, processed Ex-4 was not detected in pituitary extracts from MT-proEx-4 mice. These findings, therefore, suggest that the expression and/or processing of lizard proEx-4 in the pituitary differ markedly from those of a mammalian prosomatostatin transgene. Whether this is related to the fact that proEx-4 is not normally expressed by mammalian cells *in vivo* remains to be determined.

Finally, proEx-4 was found to be expressed in the adipose tissue of the MT-proEx-4 mice. Although the adipocyte is now known to produce a significant number of biologically active peptides and proteins, including leptin and TNFα, the expression of the prohormone convertases in this tissue has not been widely reported. It therefore remains to be determined whether the potential absence of proEx-4 processing observed in this tissue is consequent to a lack of the appropriate enzymatic machinery.

In summary, the results of the present study provide strong evidence that mammalian cells express the molecular machinery for processing of a lizard prohormone. Integrating the known expression profiles of prohormone convertases in cells and murine tissues together with the profiles of proEx-4 processing obtained in transfected cells and transgenic tissue, it seems reasonable to postulate a role for PC1 and potentially for PC4 in the posttranslational processing of proEx-4. As Ex-4 exhibits a prolonged insulinotropic effect compared with GLP-1, these findings have implications for the design of transgenes and the selection of cell and tissue transduction strategies for the development of transgenic Ex-4 delivery systems to reduce glycemia in patients with type 2 diabetes.

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