Differential Sorting of Human Parathyroid Hormone After Transduction of Mouse and Rat Salivary Glands

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Abstract

Gene transfer to salivary glands leads to abundant secretion of transgenic protein into either saliva or the bloodstream. This indicates significant clinical potential, depending on the route of sorting. The aim of this study was to probe the sorting characteristics of human parathyroid hormone (hPTH) in two animal models for salivary gland gene transfer. PTH is a key hormone regulating calcium levels in the blood. A recombinant serotype 5 adenoviral vector carrying the hPTH cDNA was administered to the submandibular glands of mice and rats. Two days after delivery, high levels of hPTH were found in the serum of mice, leading to elevated serum calcium levels. Only low amounts of hPTH were found in the saliva. Two days after vector infusion into rats, a massive secretion of hPTH was measured in saliva, with little secretion into serum. Confocal microscopy showed hPTH in the glands, localized basolaterally in mice and apically in rats. Submandibular gland transduction was effective and the produced hPTH was biologically active *in vivo*. Whereas hPTH sorted toward the basolateral side in mice, in rats hPTH was secreted mainly at the apical side. These results indicate that the interaction between hPTH and the cell sorting machinery is different between mouse and rat salivary glands. Detailed studies in these two species should result in a better understanding of cellular control of transgenic secretory protein sorting in this tissue.

Introduction

THE HOMEOSTASIS OF FREE CALCIUM in the blood is critically important for normal physiological functions throughout the body. Calcium serves as a structural element in bone, but is also an important cellular ionic messenger with many functions involving blood clotting, muscle contraction, and transmission of nerve impulses.

Parathyroid hormone (PTH) is an essential hormone for the regulation of serum calcium levels. PTH is secreted by the parathyroid glands as a polypeptide containing 84 amino acids in response to low serum calcium levels (Poole and Reeve, 2005). It acts to increase the concentration of calcium in the blood, primarily by mobilizing calcium from bone (Potts, 2005). To do this, PTH binds to its receptor on osteoblasts, which in turn upregulates RANKL (receptor activator for nuclear factor κ B ligand). RANKL binds to RANK on the surface of precursor osteoclasts, leading to their maturation and enabling the breakdown of bone and subsequent release of free calcium (Blair and Zaidi, 2006). PTH also enhances active calcium reabsorption in the kidney and, by converting vitamin D₃ to its active form, 1,25-dihydroxy vitamin D, in the intestine. When serum calcium levels are high, negative feedback inhibits PTH secretion by the

parathyroid glands (Jurutka *et al.*, 2007). In addition, calcitonin produced by the parafollicular cells (C cells) of the thyroid gland can decrease calcium concentration (Pondel, 2000).

Considering the tight regulation of serum calcium levels, hypoparathyroidism is a serious metabolic disorder. Untreated, hypoparathyroidism causes seizures, tetany, and death (Maeda *et al.*, 2006). Common causes of hypoparathyroidism are injury to the gland during thyroid or parathyroid surgery (Thomusch *et al.*, 2003) or autoimmune polyendocrine syndrome (Alimohammadi *et al.*, 2008). Conventional therapy involves vitamin D analogs and calcium supplementation. This costly treatment is not only inconvenient due to extensive monitoring, but these patients do not regain the calcium-retaining action in the kidney, resulting in hypercalciuria despite normal calcium levels. PTH replacement therapy has been shown to be clinically superior to conventional therapy (Winer *et al.*, 1996). However, two daily injections of PTH are required for effective treatment (Winer *et al.*, 1998). This regimen is expensive and is not physiologically ideal, providing a strong rationale for a more effective delivery system.

We propose to take advantage of the efficient protein-producing capacity of the salivary glands (Castle and Castle,

1998). Salivary glands are an attractive target tissue for gene transfer (Baum *et al.*, 2004). They are readily accessible by ductal cannulation and the vector does not need to be introduced systemically to achieve high levels of transgenic protein. This approach shows great potential for treating single-protein deficiencies and certain conditions of the upper gastrointestinal tract, depending on the route of sorting.

Salivary glands are polarized epithelia having as a primary role the exocrine production of saliva. However, salivary glands can secrete exocrine and endocrine proteins to either the apical pole or the basolateral pole of the cells (Lawrence *et al.*, 1977; Leonora *et al.*, 1987; Baum *et al.*, 1999; Isenman *et al.*, 1999). Unfortunately, the direction of this secretion is not readily predictable for all transgenic proteins. In polarized epithelium, it is generally accepted that endogenous proteins have specific sorting signals that are recognized by the cell machinery directing the protein to the apical or basolateral cell surface. The endogenous sorting of membrane proteins and a few soluble proteins have been studied in polarized cell cultures, but the mechanisms described are complex and do not seem to involve universal signals that are similar for proteins or cell types (Arvan and Halban, 2004; Rodriguez-Boulan *et al.*, 2005). Several factors are known to affect the polarity of secretion of a transgenic protein in salivary glands cells, such as the pH of the secretory pathway (Venkatesh and Gorr, 2002; Venkatesh *et al.*, 2007), the composition of cargo proteins (Fasciotto *et al.*, 2008), and glycosylation (Potter *et al.*, 2006).

Before proposing a clinical gene transfer application for human PTH (hPTH), it is essential to study its sorting *in vivo*, in order to predict the secretion pathways followed in human salivary glands. In this study, for the first time, we examine the potential of hPTH gene transfer to the salivary glands. Specifically, we have studied hPTH secretion in mice and rats after transduction of salivary glands with a serotype 5 adenovirus (Ad5).

Materials and Methods

Animals

Both BALB/c mice (male and female, $n = 6$ per treatment group) and male Wistar rats ($n = 5$ per treatment group) were obtained from Harlan Sprague Dawley (Walkersville, MD) at 6 weeks of age. They were acclimated for 1 week before the start of experiments, and water and food were provided *ad libitum*. All animal experiments were approved by the Animal Care and Use Committee of the National Institute of Dental and Craniofacial Research and by the Biosafety Committee of the National Institutes of Health (Bethesda, MD).

Recombinant adenovirus construction

The hPTH carrier plasmid (GeneCopoeia, Germantown, MD) was digested with *XmnI* and *XhoI* and the obtained hPTH cDNA was directionally cloned into the adenoviral expression shuttle plasmid pACCMV-pLpA containing a cytomegalovirus (CMV) promoter and a simian virus 40 (SV40) polyadenylation signal. The resulting plasmid, pACCMV-hPTH, was confirmed by sequencing and hPTH expression was tested *in vitro* with 293 cells.

An E1-deleted recombinant Ad5 vector was generated as previously described (He *et al.*, 1998a). Briefly, pACCMV-hPTH and pJM17 were cotransfected into C7 cells by calcium

phosphate precipitation. The generated Ad.hPTH was amplified in 293 cells and the crude viral lysate was purified by two rounds of CsCl gradient centrifugation as previously reported (Delporte *et al.*, 1996). The control vector used in this study was an Ad5 expressing human erythropoietin (hEPO) driven by a CMV promoter (Ad.hEPO) (Voutetakis *et al.*, 2005), which was amplified and purified in parallel.

Determination of vector titer

Physical viral particle titer (VP) was measured by real-time quantitative polymerase chain reaction (Q-PCR) against a standard curve. SYBR green PCR master mix (Applied Biosystems, Foster City, CA) was used in an ABI PRISM 7700 sequence detector (Applied Biosystems). The conditions were as follows: 95°C for 2 min, 95°C for 8 min, 95°C for 15 sec, and 60°C for 1 min for 40 cycles. The primers were designed to amplify part of the CMV promoter region: forward, 5'-CAT-CTA-CGT-ATT-AGT-CAT-CGC-TAT-TAC-3'; reverse, 5'-TGG-AAA-TCC-CCG-TGA-GTC-A-3'. Q-PCR assays were performed in duplicate, with all samples and standards in triplicate.

In vitro cell transductions

In vitro studies were done with the SMIE cell line (He *et al.*, 1998b) and the A5 cell line (Brown *et al.*, 1989); both are rat submandibular cell lines. Cells were seeded at 80% confluence in 12-well plates in Dulbecco's modified Eagle's medium (DMEM)–10% fetal calf serum (FCS) and transduced with Ad.hPTH at multiplicities of infection (MOIs) ranging from 10^2 to 2×10^4 . Medium was harvested 48 hr later and stored at –80°C until assayed. All experiments were performed in duplicate.

In vivo gene transfer to submandibular glands

Animals were anesthetized by intramuscular injection of a mixture of ketamine chloride (60 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (6 mg/kg; Phoenix Scientific, St. Joseph, MO) before cannulation of both submandibular glands with modified PE-10 polyethylene tubing (BD Diagnostic Systems, Sparks, MD) as previously reported (Wang *et al.*, 2006; Zheng *et al.*, 2008). Atropine was delivered intramuscularly (0.5 mg/kg; Sigma-Aldrich, St. Louis, MO) 10 min before vector infusion to decrease salivary flow.

Vectors were suspended in 50 μ l (mice) or 200 μ l (rats) of saline and delivered to both submandibular ducts by retrograde instillation at doses of 10^9 and 10^{10} VP/gland (mice) or 5×10^9 and 5×10^{10} VP/gland (rats). After infusion, syringes were left in place for 10 min to prevent backflow of fluid.

Forty-eight hours later, animals were reanesthetized as described above with ketamine–xylazine and thereafter administered a subcutaneous injection of pilocarpine (0.5 mg/kg) to stimulate salivary flow. Saliva was collected as described (Wang *et al.*, 2006) and snap frozen immediately. Animals were killed in a CO₂ chamber and, after death was confirmed by bilateral thoracotomy, blood was collected via the vena cava and serum was separated and stored at –80°C. Submandibular glands were removed and cleaned, and each gland was cut in half longitudinally. One part was snap

frozen and the other part was fixed in buffered 4% formaldehyde solution for 24 hr at 4°C, and then stored in 70% ethanol at 4°C until paraffin embedding.

Analytical determinations

For Western blot detection, 30 μ l samples of medium were mixed with loading buffer containing 5% 2-mercaptoethanol and heated at 96°C for 4 min before electrophoresis in a 4–15% gradient Tris-HCl precast polyacrylamide gel (Bio-Rad, Hercules, CA). The proteins were then transferred onto nitrocellulose membranes, blocked in 5% nonfat dry milk, and incubated with rabbit anti-hPTH (diluted 1:500; Abcam, Cambridge, UK) for 1 hr at room temperature. Membranes were placed in a 1:5500 dilution of anti-rabbit IgG horse-radish peroxidase-conjugated antibody solution (Amersham Health, Arlington Heights, IL) for 1 hr. Finally, membranes were incubated for 3 min in chemiluminescent substrate (Pierce Biotechnology, Rockford, IL) and exposed to Kodak BioMax MR film (Carestream Health, Rochester, NY).

Human PTH was measured with an enzyme-linked immunosorbent assay (ELISA) kit from ALPCO Diagnostics (Salem, NH) in accordance with the manufacturer's instructions. Before samples were measured, a spike assay was done to reveal which dilutions showed no inhibition with saliva or serum. Each sample was measured in duplicate and compared with a simultaneously performed standard curve. The serum-to-saliva distribution ratio for hPTH was calculated as described previously (Baum *et al.*, 1999; Hoque *et al.*, 2001).

Calcium was determined by indirect potentiometry, using a calcium ion-selective electrode in conjunction with a sodium reference electrode. ISE electrolyte buffer reagent and ISE electrolyte reference reagent (Beckman Coulter, Brea, CA) were used according to the manufacturer's instructions and samples were processed on a Synchron LX-20 (Beckman Coulter).

Inorganic phosphorus was measured in a time-rated reaction with ammonium molybdate in an acidic solution to

form a colored phosphomolybdate complex. The change in absorbance of this yellow complex was measured at 365 nm.

Immunofluorescence confocal microscopy

Paraffin-embedded sections (thickness, 5 μ m) were dewaxed and rehydrated in a gradient of alcohols. Antigen retrieval was performed thereafter by heating the sections for 10 min at 121°C in 0.1 M citric acid, pH 6.0. After incubation with a blocking solution containing 10% goat serum in phosphate-buffered saline (PBS) for 30 min, slides were incubated with rabbit anti-hPTH (AnaSpec, San Jose, CA) at 20 μ g/ml in 0.5% bovine serum albumin in PBS for 1 hr at room temperature, washed with PBS, and incubated with the secondary antibody (diluted 1:200, Alexa 488-conjugated goat anti-rabbit; Invitrogen, Carlsbad, CA) together with phalloidin (diluted 1:200; Sigma-Aldrich) for 1 hr at room temperature. After extensive washing, sections were mounted in VECTASHIELD (Vector Laboratories, Burlingame, CA). Images were collected with a Leica confocal microscope and MetaMorph software (Molecular Devices, Sunnyvale, CA). As an internal control, an irrelevant rabbit IgG was used as primary antibody.

Statistics

Differences in means between groups were determined by the Kruskal-Wallis test, followed by a Mann-Whitney *U* rank-sum test. $p < 0.05$ was considered statistically significant. All analyses were done with SPSS version 15.0 (SPSS, Chicago, IL). Data are expressed as means $\pm$ SEM.

Results

In vitro production of hPTH

After transduction of SMIE cells with Ad.hPTH, secretion of hPTH was considerable and vector dose dependent. This was evident by both Western blotting (Fig. 1, top) and ELISA (Fig. 1, bottom). With an MOI of 1000, the production of

FIG. 1. Secretion of hPTH from SMIE cells after transduction *in vitro* with Ad.hPTH. SMIE cells were incubated with the indicated MOIs of Ad.hPTH. After 48 hr, medium was harvested and assayed for hPTH by Western blot (*top*) and ELISA (*bottom*). The data shown are representative of two experiments performed in duplicate and are displayed as means $\pm$ SEM.

FIG. 2. Distribution and biological effect of hPTH in mice after administration of Ad.hPTH to both submandibular glands by retrograde ductal instillation. Two doses were given: 1×10^9 or 1×10^{10} VP/gland. Saline and an irrelevant vector, Ad.hEPO, were used as controls. Human PTH was measured by ELISA in serum (**A**, $p = 0.004$) and saliva (**B**) 2 days after vector infusion. The free calcium concentration in serum was determined by indirect potentiometry (**C**, $p = 0.002$) and thereafter was plotted against hPTH levels in serum to reveal a high correlation (**D**). Data are shown as means $\pm$ SEM (**A–C**) and as individual data points (**D**).

hPTH was ~ 12 ng/ml. Similar results were obtained with A5 cells (data not shown).

hPTH production after administration of Ad.hPTH to mouse salivary glands in vivo

We administered 10^9 or 10^{10} VP of Ad.hPTH to the submandibular glands of male and female mice by retrograde ductal infusion. In addition to treating mice with saline, dose-matched Ad.hEPO was used as vector control. Forty-eight hours after administering the vector, we measured the secretion of hPTH in serum and saliva. hPTH levels were unchanged in mice infused with 10^9 VP compared with controls (Fig. 2A). However, delivery of 10^{10} VP of Ad.hPTH led to significant ($p = 0.004$) production of hPTH detected in the serum of these mice. In contrast, only a slight increase ($p = 0.07$) above background in hPTH concentration was found in the saliva of mice receiving 10^{10} VP of Ad.hPTH (Fig. 2B). No differences between male and female mice were observed (data not shown) and their values are combined here.

Biological activity of hPTH after gene transfer to mouse salivary glands

We next assayed free calcium levels in the serum of Ad.hPTH-treated mice. PTH is known to increase serum calcium levels by mobilizing calcium from bone and enhancing

the absorption of calcium in kidney and intestine. Indeed, a significant (41% on average; $p = 0.002$) elevation of free calcium in the sera of mice treated with the highest dose of vector was observed (Fig. 2C). The concentrations of hPTH and calcium in the serum of individual mice were highly correlated ($R^2 = 0.93$), providing strong evidence of the biological activity of vector-mediated hPTH secreted from salivary glands (Fig. 2D). We observed no differences in phosphorus levels in serum; however, increased urinary phosphorus was found in treated animals (data not shown).

Vector-derived hPTH production after salivary gland transduction in rats

Similar to our experiments in mice, we delivered two doses (5×10^9 and 5×10^{10} VP/gland) to the submandibular glands of rats. Given that administration of Ad.hEPO was without effect on hPTH or calcium levels in mice, that is, similar to saline-treated mice, the rat treatment groups were compared with saline-infused animals only. Two days after vector delivery, serum and saliva were collected and assayed for hPTH.

On average, a small, statistically insignificant increase was seen in serum hPTH levels in the highest dose group (Fig. 3A; $p = 0.15$). Despite this lack of statistical significance, there was a highly significant correlation between serum

FIG. 3. Production of hPTH in rats after transduction of both submandibular glands with Ad.hPTH. Ad.hPTH at either 5×10^9 or 5×10^{10} VP/gland was introduced into glands by cannulating the ducts. Saline was used as a control. Two days after vector delivery, serum (**A**) and saliva (**B**, $p = 0.016$ for 5×10^9 VP and $p = 0.008$ for 5×10^{10} VP, compared with control) were assayed for hPTH by ELISA.

hPTH and calcium levels ($R^2 = 0.97$). Conversely, in saliva an enormous secretion of hPTH (up to ~ 15 ng/ml) was detected at both vector doses ($p = 0.016$ and $p = 0.008$, respectively). This hPTH production was dose dependent (Fig. 3B). As an additional test to determine whether vector-encoded hPTH mediated biological activity in rats, four rats were injected systemically (intravenously, same dose as delivered to the glands: 5×10^{10} VP) with Ad.hPTH. After 2 days, the animals showed significant hPTH levels in serum and, consequently, elevated calcium levels compared with saline-treated controls (data not shown).

Species-specific hPTH sorting difference in salivary glands

The contrast in hPTH sorting and secretion between rats and mice is striking. Table 1 shows the serum-to-saliva ratio of total hPTH secreted. These ratios clearly demonstrate this dramatic difference in sorting of the same protein expressed from the same vector in these two commonly studied rodent

species. Whereas after transduction of salivary glands in mice hPTH is secreted primarily at the basolateral side into the bloodstream, in rats hPTH was secreted predominantly at the apical side into saliva. In mice, the lower vector dose used was apparently too low to result in significant production of hPTH. In rats, however, the lower dose resulted primarily in hPTH secretion into saliva.

Cellular localization of hPTH in transduced salivary glands by immunofluorescence

Two days after vector infusion, salivary glands were excised and fixed in formaldehyde. After being embedded in paraffin, glands were sectioned and fluorescently labeled with an antibody directed against hPTH, using phalloidin to visualize cell morphology.

Even after stimulation with pilocarpine, hPTH was clearly visible in all treated glands, albeit more abundant in mouse glands (Fig. 4A and C). The cellular localization of hPTH was different between species (Fig. 4A and C, arrows). Whereas in mice hPTH was found mainly on the basolateral side of the cells, in rats hPTH was located preferentially on the apical side. Neither control mice (Fig. 4B) nor control rats (Fig. 4D) demonstrated a significant positive signal after staining with the anti-hPTH antibody. The irrelevant IgG control also did not exhibit staining (data not shown).

Discussion

Herein, we tested the hypothesis that biologically active hPTH can be produced by salivary glands *in vivo* after gene transfer. Previously, we demonstrated that salivary gland cells can distinguish and sort transgenic proteins to distinct

TABLE 1. DISTRIBUTION RATIO OF HUMAN PARATHYROID HORMONE AFTER TRANSDUCTION OF MOUSE AND RAT SUBMANDIBULAR GLANDS^a

	Mouse	Rat
High dose	613 ± 527	0.93 ± 0.47
Low dose	ND	<0.01

^aRatios are shown as total production in serum:total production in saliva $\pm$ SEM. High dose represents 1×10^{10} VP/gland in mice and 5×10^{10} VP/gland in rats. Low doses are 1 log lower in both species. ND, none detected.

FIG. 4. Detection and localization of hPTH in transduced salivary glands. Two days after vector infusion, submandibular glands were removed, fixed in formaldehyde, and embedded in paraffin. Subsequent to sectioning, slides were immunofluorescently stained for hPTH (green), and phalloidin was used to visualize gland morphology (red). A transduced mouse gland is shown in (A) and a control mouse gland is shown for comparison (B). A rat gland after transduction is displayed in (C), and a control rat gland is shown for comparison (D). Arrows indicate the cellular localization of hPTH. Original magnification: 100 $\times$. Insets in (A) and (C): Enlargements of boxed areas.

extracellular locales *in vivo* (Baum *et al.*, 1999). Thus, understanding the sorting of hPTH in salivary glands is essential for any eventual clinical application with these tissues. Protein sorting can differ between cells growing *in vitro* and in an *in vivo*, native situation. For example, secretion of human growth hormone is nonpolarized in cultured MDCK cells (Gottlieb *et al.*, 1986), basolateral in cultured colon epithelial cells (Rindler and Traber, 1988), and apical in salivary glands *in vivo* (Baum *et al.*, 1999). PTH, under normal physiological conditions, is stored in secretory granules and secreted in a regulated manner by the parathyroid gland into the bloodstream.

In mice, we found that after salivary gland transduction the levels of hPTH in serum were quite significant. Conversely, only a modest amount of hPTH was found in the saliva of these mice, that is, hPTH secretion was primarily in an endocrine direction in mouse salivary glands. Although less commonly considered, secretion into the bloodstream from the salivary glands is well described and occurs via ill-defined basolateral secretory pathways (Kagami *et al.*, 1996; Baum *et al.*, 1999; Isenman *et al.*, 1999; Gorr *et al.*, 2005). The immunofluorescent staining of transduced mouse glands revealed a significant accumulation of hPTH on the basolateral side of parenchymal cells, consistent with the hypothe-

sis that hPTH was sorted to the basolateral pole after entry to the trans-Golgi network.

Once in the bloodstream, transgenic hPTH was biologically active, and we showed that hPTH clearly elevated serum calcium levels in mice. Indeed, there was a strong correlation between hPTH and calcium levels in serum, and the observed increase was considerable in view of the strong feedback regulation by both hPTH itself and by calcitonin in these physiologically intact mice.

After infusion of Ad.hPTH into rat salivary glands, there was minimal secretion of hPTH into serum, and only at the highest vector dose. However, there was still a highly significant correlation between serum hPTH and calcium levels in these animals ($R^2 = 0.97$). In contrast, saliva contained extremely high amounts of hPTH. The difference between mice and rats was striking, both in absolute levels and in the serum-to-saliva ratio of total hPTH secreted. Clearly, in rats hPTH sorted primarily toward the apical membrane and on immunofluorescent staining hPTH was found in the apical region of salivary epithelial cells. The exact pathways used to achieve this apical secretion of hPTH remain to be elucidated, and could involve the major regulated secretory pathway (dense-core granules) (Castle and Castle, 1998), as well as the minor regulated and constitutive-like secretory pathways, both of which originate in immature secretory granules (Castle and Castle, 1996). Given the amount of hPTH produced by the transduced rat submandibular glands, the hPTH secretion found in serum may in part result from a spillover effect due to saturation of the apical pathway (Marmorstein *et al.*, 2000).

Although the specific mechanism(s) responsible for the species differences in hPTH sorting are still unclear, there are several possible reasons that can be experimentally tested. For example, it is possible that a specific amino acid sequence found in hPTH can facilitate its secretion to an exocrine pathway in rats, but is not recognized by the sorting machinery in mouse salivary cells. Indeed, in the biologically active part of the hPTH molecule, there are five amino acids that differ between human and mouse or rat PTH (Potts, 2005). Also, it is possible that hPTH propeptide processing is different in mouse and rat salivary glands, which could impair entry or storage of hPTH protein in murine secretory granules, resulting in its secretion into the constitutive pathway (bloodstream). It is widely recognized that the intracellular pathways followed by secretory proteins in epithelial cells are directed by a large repertoire of carefully regulated molecules (Rodriguez-Boulan *et al.*, 2005). Thus, there are numerous intracellular steps wherein a genetic or metabolic change could lead to a difference in the final destination of the transgenic protein (Folsch *et al.*, 1999; Cosen-Binker *et al.*, 2007, 2008).

Extrapolating our results to eventual hPTH gene transfer to human salivary glands remains difficult. If hPTH gene transfer to human glands were to lead primarily to exocrine secretion of the transgene product, as it does in rats, secretion into the bloodstream would need to be increased for a clinical application. This might be achieved, for example, by concurrent administration of a weak base such as hydroxychloroquine (Hoque *et al.*, 2001). Regardless, longer lasting hPTH expression than seen with an Ad5 vector also needs to be achieved. Serotype 2 adeno-associated viral (AAV2) vectors lead to long-term stable transgene expression in sali-

vary glands (Voutetakis *et al.*, 2004), and seem well suited to addressing this need.

In conclusion, this study demonstrates that biologically active hPTH is readily produced after transduction of salivary glands. Theoretically, our findings suggest the potential for hPTH gene therapy for the treatment of hypothyroidism. However, the differential sorting of hPTH after transduction of mouse and rat salivary glands indicates that the interaction between hPTH and the cell sorting machinery in different salivary glands needs to be clarified. Comprehensive studies in these species should result in a better understanding of cellular sorting mechanisms operative in salivary glands and could eventually lead to an innovative therapy for patients with hypoparathyroidism and other systemic disorders.

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Author Disclosure Statement

No competing financial interests exist.

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