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Incretin Receptors in Non-Neoplastic and Neoplastic Thyroid C Cells in Rodents and Humans: Relevance for Incretin-Based Diabetes Therapy

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Key Words

Diabetes therapy · GIP receptors · GLP-1 receptors · Incretin receptors · Medullary thyroid carcinomas · Thyroid C cells

Abstract

While incretins are of great interest for the therapy of diabetes 2, the focus has recently been brought to the thyroid, since rodents treated with glucagon-like peptide-1 (GLP-1) analogs were found to occasionally develop medullary thyroid carcinomas. Incretin receptors for GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) were therefore measured in various rodent and human thyroid conditions. In vitro GLP-1 and GIP receptor autoradiography were performed in normal thyroids, C-cell hyperplasia and medullary thyroid carcinomas in rodents. Receptor incidence and density were assessed and compared with the receptor expression in human thyroids, medullary thyroid carcinomas, and TT cells. GLP-1 receptors are expressed in C cells of normal rat and mice thyroids. Their density is markedly increased in rat C-cell hyperplasia and medullary thyroid carcinomas, where their incidence amounts to 100%. GIP receptors are neither detected in normal rodent thyroids nor in C-cell hyperplasia, but are present in all rat medullary thyroid carcinomas. No GLP-1 or GIP receptors are detected in normal human thyroids. Whereas only 27% of all human medullary thyroid carcinomas express GLP-1 receptors, up to 89% express GIP receptors in a high density. TT cells lack GLP-1 receptors but express GIP receptors. GLP-1 receptors are frequently expressed in non-neoplastic and neoplastic C cells in rodents while they are rarely detected in human C-cell neoplasia, suggesting species differences. Conversely, GIP receptors appear to be massively overexpressed in neoplastic C cells in both species. The presence of incretin receptors in thyroid C cell lesions suggests that this organ should be monitored before and during incretin-based therapy of diabetes.

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Introduction

Incretins such as glucagon-like peptide-1 (GLP-1) or glucose-dependent insulinotropic polypeptide (GIP) are important glucose-dependent insulin secretagogues released primarily from the gastrointestinal tract in re-

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sponse to nutrient intake [1–3]. In addition to their regulation of glucose-dependent insulin secretion, those peptides have other common actions on β cells, including stimulation of cell proliferation and reduction of β-cell apoptosis [3–5]. The concordant incretin effects of GLP-1, including stimulation of insulin, suppression of glucagon, delaying gastric emptying and increasing β-cell mass, have suggested its possible use for diabetes 2 treatment. Therefore, stable synthetic GLP-1 analogs such as exenatide, liraglutide or taspoglutide have been designed and developed for that indication [6–9]. During preclinical development, it was observed in animal studies evaluating the long-term effect of GLP-1 analogs that a significant number of animals developed medullary thyroid carcinomas [10, 11]. This observation is puzzling. In a study on GLP-1 receptors in cancer tissue [12], we previously found species differences for GLP-1 receptors in thyroids and lungs, with a much higher expression in the thyroids and lungs of rat and mouse than in the corresponding human organs. Since the observation of a species difference was not along the main aim of that study, it was not followed in depth.

Because of the considerable impact that thyroid side effects of new GLP-1- and GIP-related drugs may have on diabetes 2 treatment strategies, the aim of the present study was to investigate in more details the expression and localization of GLP-1 and GIP receptors in various conditions of thyroid tissue in rodents and humans. The aim was to investigate under which thyroid conditions the GLP-1 and GIP receptors are expressed, whether they are specifically located in C cells and whether species differences can be observed.

As method of choice, we used in vitro GLP-1 and GIP receptor autoradiography in combination with immunohistochemistry for calcitonin and synaptophysin on adjacent sections, in order to identify the C cells in the thyroid tissues used for the incretin receptor detection. First, we investigated normal thyroids of rat, mouse and humans. Since these normal tissue samples may contain very few C cells, we also determined the incretin receptors in two animal models with C-cell hyperplasia and with subsequent medullary thyroid carcinoma development, namely the familial WAG/Rij rat medullary thyroid carcinoma model [13] and a MENx rat model [14]. For comparison, we analyzed a large number of human medullary thyroid carcinoma samples and, where available, of non-neoplastic thyroid tissue adjacent to the tumor. Finally, we also analyzed incretin receptors in TT cells and in animal xenografts of TT cells that represent human medullary thyroid carcinomas [15, 16]. We had to limit our study to in vitro receptor autoradiography methods, since there are no adequate GLP-1 or GIP receptor antibodies commercially available at the moment that would allow a comparative receptor immunohistochemistry approach.

Material and Methods

Tissues

Fresh frozen samples of normal, hyperplastic and neoplastic thyroid tissues from rats, mice and humans were used in this study. Normal thyroids were obtained from Wistar and Sprague-Dawley rats, BALB/C and C57BL/6 mice. TT cells (ATCC-CRL-1803; LGC Standards, Teddington, UK), as well as nude mice xenografts from human TT cells, thyroids with C-cell hyperplasia or medullary thyroid carcinomas from old WAG/Rij rats (n = 11) or old MENx heterozygous mutant rats (n = 5) were taken from studies in which the tissues were used for other receptors determinations [14, 16, 17] (table 1). 36 human medullary thyroid carcinomas and 6 normal thyroids were surgical resection specimens characterized for peptide receptors in previous studies [18, 19] that conformed to the ethical guidelines of the Institute of Pathology, University of Berne, and were reviewed by the Institutional Review Board.

In vitro GLP-1 Receptor Autoradiography

The in vitro GLP-1 receptor autoradiography was carried out as described previously [12]. 20-μm-thick frozen tissue sections were incubated for 2 h at room temperature in the incubation solution containing 170 mM Tris-HCl buffer (pH 8.2), 1% bovine serum albumin (BSA), 40 μg/ml bacitracin, 10 mM $MgCl_2$, and 15,000 cpm/100 μl ^{125}I-GLP-1(7–36)amide (2,000 Ci/mmol; Anawa, Wangen, Switzerland). Nonspecific binding was determined by incubating tissue sections in the incubation solution additionally containing 100 nM unlabeled GLP-1(7–36)amide (Bachem, Bubendorf, Switzerland) which at this concentration completely and specifically displaces ^{125}I-GLP-1(7–36)amide at the receptors. Further pharmacological displacement experiments were performed in order to differentiate GLP-1 receptors from other members of the glucagon receptor family. For this purpose, serial tissue sections were incubated with ^{125}I-GLP-1(7–36)amide together with increasing concentrations of one of the following analogs: the GLP-1 receptor-selective analog GLP-1(7–36)amide (Bachem) [20], the GLP-2 receptor-selective hormone GLP-2 (Bachem) or the glucagon receptor-selective hormone glucagon(1–29) (Bachem). After incubation, the slides were washed 5 times in ice-cold Tris-HCl buffer (170 mM; pH 8.2) containing 0.25% BSA and twice in ice-cold Tris-HCl buffer without BSA. The slides were dried for 15 min under a stream of cold air and then exposed to Kodak Biomax MR® films for 7 days at 4°C. The signals were analyzed in correlation with morphology using corresponding HE stained tissue slides. The receptor density was quantitatively assessed using tissue standards for iodinated compounds (Amersham, Aylesbury, UK) and a computer-assisted image processing system (Analysis Imaging System; Interfocus, Mering, Germany).

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Table 1. GLP-1 and GIP receptor incidence and density in various thyroid tissues (dpm/mg tissue, mean ± SEM)

Tissue	Age months	Normal thyroid		C-cell hyperplasia		C-cell neoplasia (MTC)	
		GLP-1 R	GIP-R	GLP-1 R	GIP-R	GLP-1 R	GIP-R
Thyroids from normal rats							
Wistar	<3	823 ± 133 focal	0				
Wistar	<3	1,150 ± 641 focal	0				
Sprague/Dawley	<3	2,017 ± 568 focal	0				
Sprague/Dawley	<3	0	0				
Sprague/Dawley	<3	2,472 ± 234 focal	0				
Sprague/Dawley	<3	0	0				
Mean		1,616 ± 381 (n = 4)					
Thyroids from normal mice							
BALB-C	<3	3,870 ± 365 focal	0				
C57BL/6	<3	694 ± 167 focal	0				
C57BL/6	<3	1,091 ± 145 focal	0				
C57BL/6	<3	1,362 ± 362 focal	0				
C57BL/6	<3	2,062 ± 547 focal	0				
Mean		1,816 ± 560 (n = 5)					
Thyroids from old WAG/Rij rats							
WAG/Rij	20–24			2,677	0		
WAG/Rij	20–24			4,352 ± 864	0		
WAG/Rij	20–24			3,248 ± 22	0		
WAG/Rij	20–24			3,487 ± 337	0	10,452 ± 52	0
WAG/Rij	20–24			3,572 ± 1,624	0	8,966	633 ± 89
WAG/Rij	20–24			3,951 ± 479	0	11,111 ± 259	3,349 ± 381
WAG/Rij	20–24			4,467 ± 249	0	4,862	
WAG/Rij	20–24			2,445 ± 177	0		
WAG/Rij	20–24			3,554 ± 270	0		
WAG/Rij	20–24			3,652 ± 7	0	10,339	
WAG/Rij	20–24			3,564 ± 313	0		
Mean				3,543 ± 184 (n = 11)		9,146 ± 1,126 (n = 5)	1,991 ± 1,358 (n = 2)
Thyroids from old MENx heterozygous mutant rats							
MENx	23			2,701 ± 82	0	7,778 ± 214	6,699 ± 258
MENx	20			5,134 ± 314	0		1,201
MENx	19			1,733 ± 111	0	8,195 ± 97	804 ± 39
MENx	21			4,837 ± 349	0	4,401 ± 152	1,957 ± 74
MENx	18			4,978 ± 477	0	11,493 ± 650	8,330 ± 11
Mean				3,877 ± 696 (n = 5)		7,967 ± 1,450 (n = 4)	3,798 ± 1,550 (n = 5)

Absence of a density value in a sample indicates lack of the respective tissue of that sample. Single value means single receptor measurement due to tissue shortage.

In vitro GIP Receptor Autoradiography

GIP receptor autoradiography was performed by the same method than GLP-1 receptor autoradiography described above. The peptide analog used as radioligand was human GIP(1–30). It was radiolabeled by the lactoperoxidase method and purified by HPLC (Anawa). The peak (2,000 Ci/mmol) representing ^{125}I-[Tyr^{10}]-GIP(1–30) was used in all experiments. 20,000 cpm/100 μl of radioligand were added to the incubation solution. Nonspecific binding was determined by incubating sections in an incubation solution additionally containing 100 nM unlabeled human

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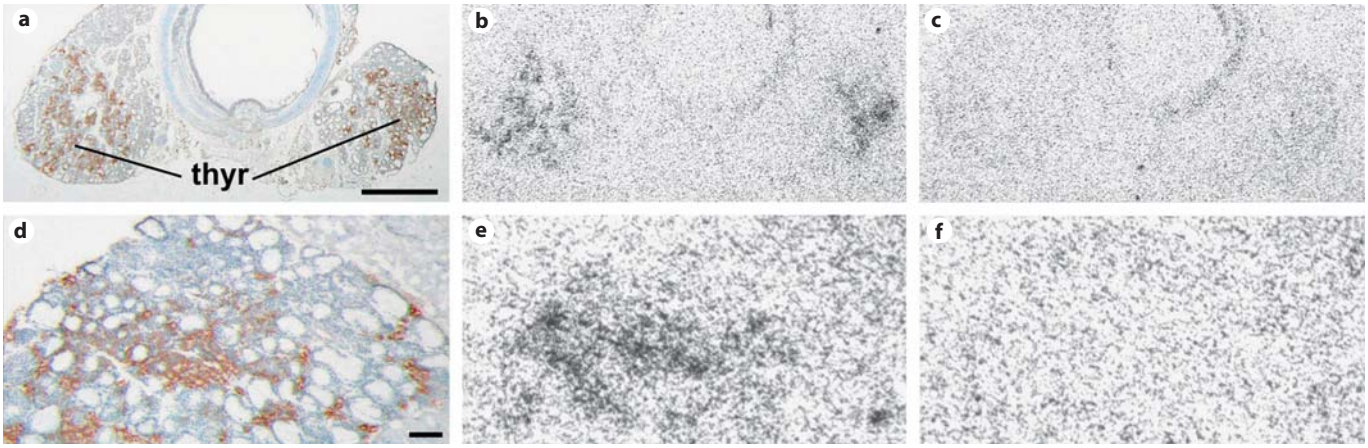

Fig. 1. GLP-1 receptor autoradiography of rat (**a–c**) and mouse (**d–f**) thyroids. **a**, **d** Calcitonin immunohistochemistry identifies C cells within the thyroid (thyr). Bar in **a** = 1 mm; bar in **d** = 0.1 mm. **b**, **e** Autoradiograms with total binding of ^{125}I-GLP-1(7–36)amide show focal expression of GLP-1 receptors in the C cell-rich areas of the thyroid in both species. **c**, **f** Autoradiograms with non-specific binding of ^{125}I-GLP-1(7–36)amide (in the presence of 100 nM unlabeled GLP-1).

GIP (Bachem), a concentration that completely and specifically displaces ^{125}I-GIP(1–30) at the receptors. Further pharmacological displacement experiments were performed in order to differentiate GIP receptors from GLP-1 and glucagon receptors. For this purpose, serial tissue sections were incubated with ^{125}I-GIP(1–30) together with increasing concentrations of GLP-1 or glucagon (Bachem). Quantification was performed as mentioned above for GLP-1 receptors.

Immunohistochemistry

Immunohistochemistry for calcitonin and synaptophysin was performed on cryostat sections adjacent to the sections used for GLP-1 or GIP receptor autoradiography as previously described [12]. Immunohistochemistry on cryostat sections, suboptimal for morphology and resolution compared to formalin-fixed, paraffin-embedded sections, was necessary in this study to allow direct comparison with receptor autoradiography, a method requiring cryostat sections. The antibody for calcitonin (NCL CALP) was purchased from Leica (Newcastle, UK) and the antibody for synaptophysin (MO 776) was from DAKO (Baar, Switzerland).

Results

GLP-1 receptors are identified by in vitro receptor autoradiography in normal mouse and rat thyroids (table 1). These receptors are usually expressed focally in restricted areas of the thyroid. Their density in these areas can be considered moderate. Density values, measured at locations with the highest density (mean density: 1,616 ± 381 dpm/mg tissue (n = 4) for rats, 1,816 ± 560 dpm/mg tissue (n = 5) for mice), can be compared with the density values of GLP-1 receptors (1,322 ± 143 dpm/mg tissue) found in human pancreatic islets, the main physiological target of GLP-1 [12]. Because GLP-1 receptors are distributed in such a highly heterogeneous way, not every tested tissue sample is found to express GLP-1 receptors (table 1). Importantly, the receptors colocalize immunohistochemically with synaptophysin and calcitonin, two specific markers of C cells in the thyroid, therefore strongly suggesting that the GLP-1 receptors are localized on C cells. These immunohistochemical results are in agreement with the previous observation that C cells are relatively numerous in rodent thyroids. This is illustrated in figure 1 for thyroids of normal rats and mice. No GIP receptors are identified in normal mouse and rat thyroids (table 1).

As observed previously, more C cells are present in thyroids of aged rats [11]. This is confirmed by immunohistochemical data obtained with the old WAG/Rij and the old MENx rats (table 1). As a consequence, more GLP-1 receptors are found to be expressed in these thyroids, as seen in the in vitro autoradiography data (table 1). The receptor status can be precisely evaluated in aged rat models with C-cell hyperplasia or medullary thyroid carcinoma, not only because of the higher proportion of C cells in the tissues but also because of a more homogeneous distribution of these cells (fig. 2). In all these tissues, the GLP-1 receptor colocalizes with calcitonin or synaptophysin as seen in figure 2. The incidence of GLP-1 receptors in rat medullary thyroid carcinomas is 100% (table 1). This is the case in both mod-

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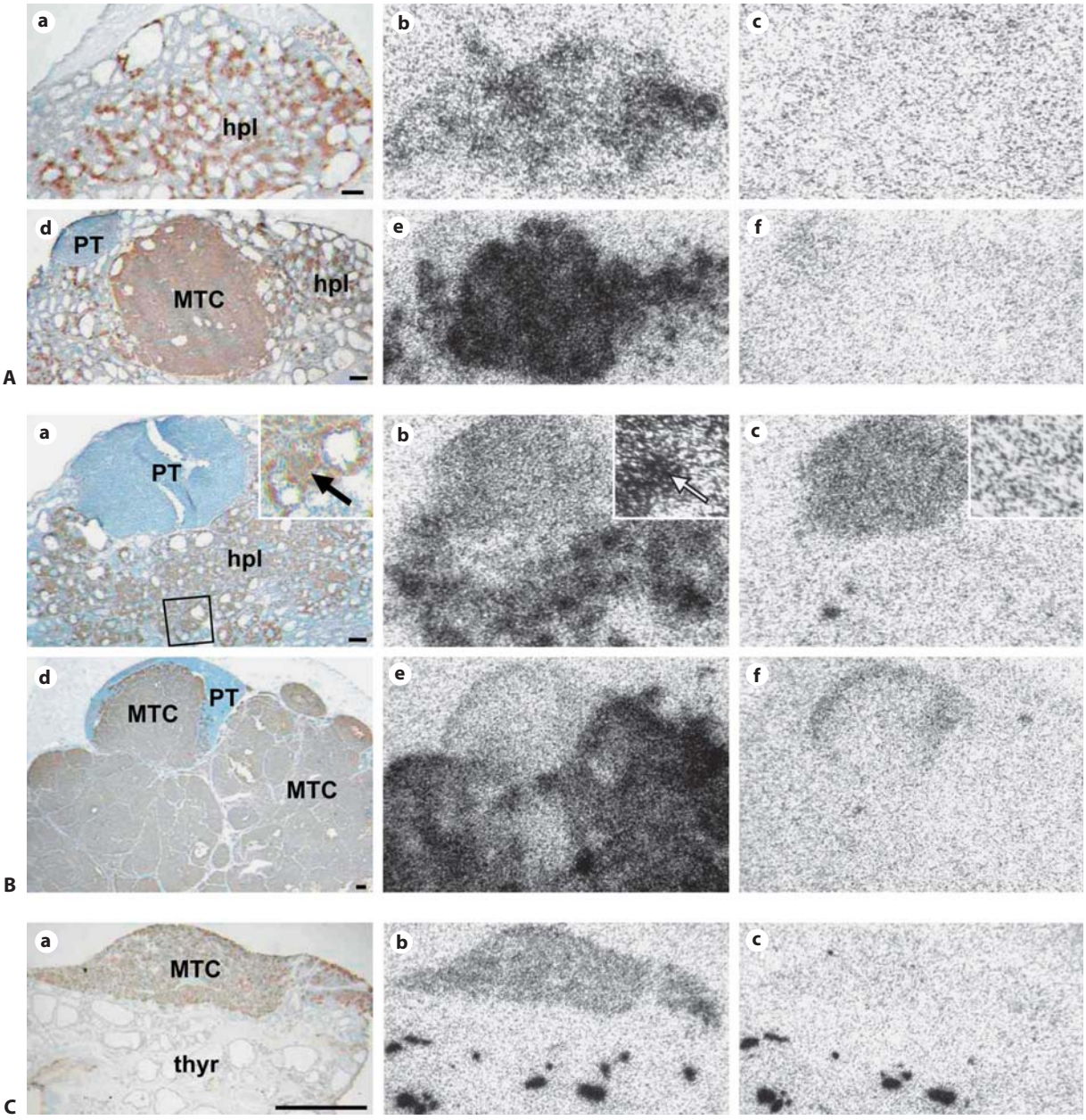

Fig. 2. GLP-1 receptor autoradiography of hyperplastic and neoplastic thyroid tissues in old WAG/Rij rats (**A**) and in MENx rats (**B**), and in a human MTC within a thyroid (**C**). **Aa**, **d** Immunohistochemistry for calcitonin (**a**) or synaptophysin (**d**) identifies hyperplastic C cells and MTC in WAG/Rij rats. Bars = 0.1 mm. **Ab**, **e** Autoradiograms with total binding of ^{125}I-GLP-1(7–36) amide showing GLP-1 receptors in the C-cell hyperplasia of the thyroid and in the MTC. Parathyroid gland and non-C cell areas are not labeled. **Ac**, **f** Autoradiograms showing nonspecific binding of ^{125}I-GLP-1(7–36)amide. **Ba**, **d** Immunohistochemistry for synaptophysin identifies hyperplastic C cells and MTC in MENx rats. Bars = 0.1 mm. **Bb**, **e** Autoradiograms with total binding of ^{125}I-GLP-1(7–36)amide showing GLP-1 receptors in the C cell-rich hyperplastic areas of the thyroid and in the MTC. Note in **Be** the heterogeneous distribution of GLP-1 receptors in the MTC that partly invades the parathyroid gland. The parathyroid gland itself is GLP-1 receptor-negative. **Bc**, **f** Autoradiograms showing nonspecific binding of ^{125}I-GLP-1(7–36)amide. **Insets** in **Ba–c** show details at higher magnification. Arrows = Synaptophysin-positive C cells (**a**) expressing GLP-1 receptors (**b**). **Ca** Immunohistochemistry for synaptophysin identifies the neoplastic C cells of the human MTC. Bar = 1 mm. **Cb** Autoradiogram with total binding of ^{125}I-GLP-1(7–36)amide shows GLP-1 receptor expression in the MTC but not in the surrounding thyroid. **Cc** Autoradiogram with nonspecific binding. Note the high nonspecific binding of the colloid. PT = Parathyroid gland; MTC = medullary thyroid carcinoma; hpl = hyperplastic C cells; thyr = adjacent thyroid tissue.

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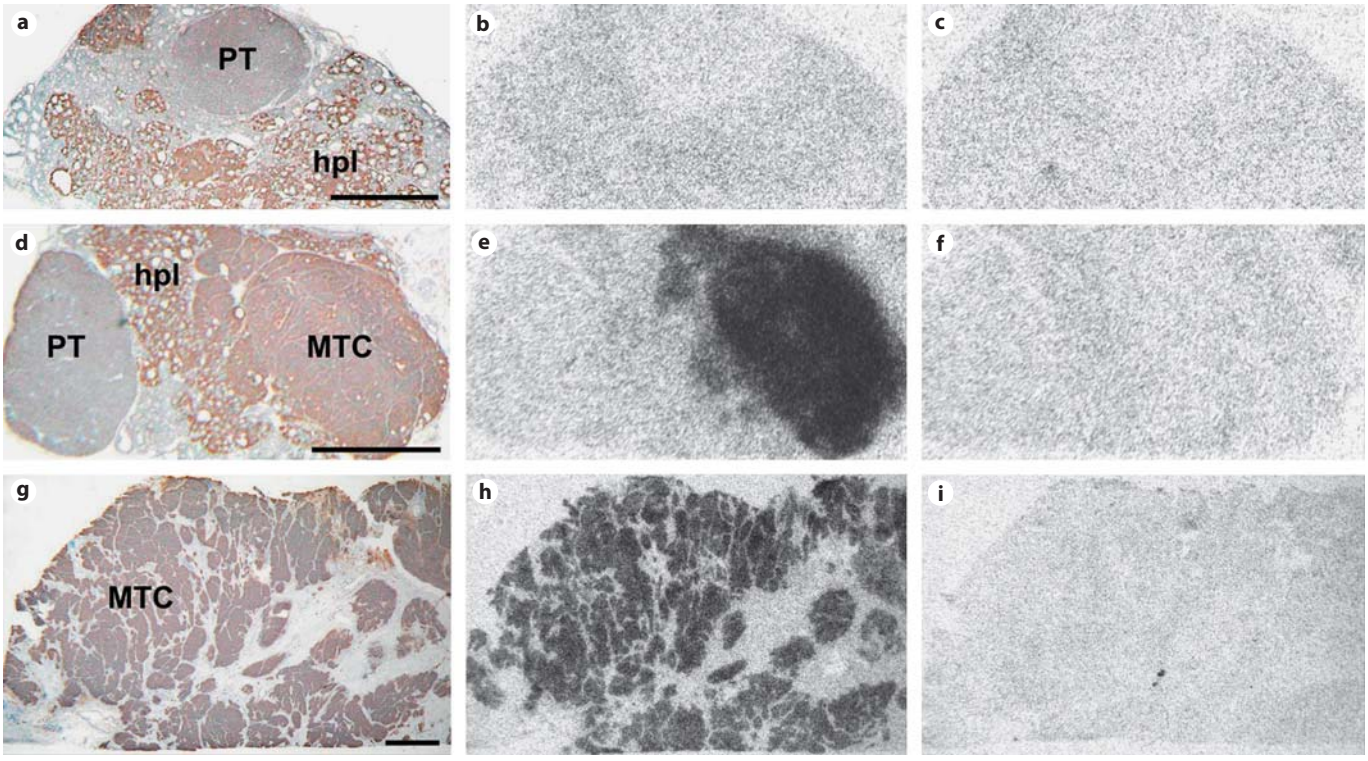

Fig. 3. GIP receptor autoradiography in WAG/Rij rat (**a–f**) thyroids and human MTC (**g–i**). **a**, **d**, **g** Synaptophysin immunohistochemical staining identifying C-cell hyperplasia (hpl) and MTC. MTC = Medullary thyroid carcinoma. Bars = 1 mm. **b**, **e**, **h** Autoradiograms of total binding of ^{125}I-GIP(1–30) showing the absence of GIP receptors in the rat hyperplastic C cells, but their presence in the rat (**e**) and human (**h**) MTC. **c**, **f**, **i** Autoradiograms showing nonspecific binding (in the presence of 100 nM GIP).

els of medullary thyroid carcinoma (table 1). The density of receptors is high in rat C-cell hyperplasia and even higher in rat medullary thyroid carcinomas (table 1; fig. 2A, B). The mean receptor density in the WAG/Rij rats is 3,543 ± 184 dpm/mg tissue (n = 11) in the thyroid hyperplasia and 9,146 ± 1,126 dpm/mg tissue (n = 5) in the medullary thyroid carcinoma cases. The mean receptor density in the MENx rats is 3,877 ± 696 dpm/mg tissue (n = 5) in the hyperplasia and 7,967 ± 1,450 dpm/mg tissue (n = 4) in the medullary thyroid carcinoma cases. Interestingly, we have noticed a heterogeneous GLP-1 receptor expression in some medullary thyroid carcinomas despite a homogenous calcitonin or synaptophysin expression. This was observed much more frequently in the MENx model than in the WAG/Rij model of medullary thyroid carcinomas. One example of heterogeneity in the GLP-1 receptor distribution is shown in the thyroid of the MENx rat in figure 2Bd–f.

Conversely, no GIP receptors were detected in C-cell hyperplasia of either the WAG/Rij or the MENx model (table 1). However, the GIP receptors appear in high concentrations in the medullary thyroid carcinomas of both models. The mean density is 1,991 ± 1,358 dpm/mg tissue in the WAG/Rij rats and 3,798 ± 1,550 dpm/mg tissue in the MENx rats. A representative example is given in figure 3 showing GIP receptors in the medullary thyroid carcinoma but not in the adjacent C-cell hyperplasia of a MENx rat thyroid.

In non-neoplastic human thyroid tissues, obtained either from normal thyroids or from thyroids adjacent to medullary thyroid carcinoma, we have not been able to detect measurable amounts of GLP-1 or GIP receptors (table 2). Of notice, compared to rodents, the amount of C cells in normal thyroids is very scarce; the method may not be able to detect receptors in single C cells if the receptors are not expressed in a high enough density. It is more favorable and conclusive to study human thyroid conditions characterized by a proliferation of C cells

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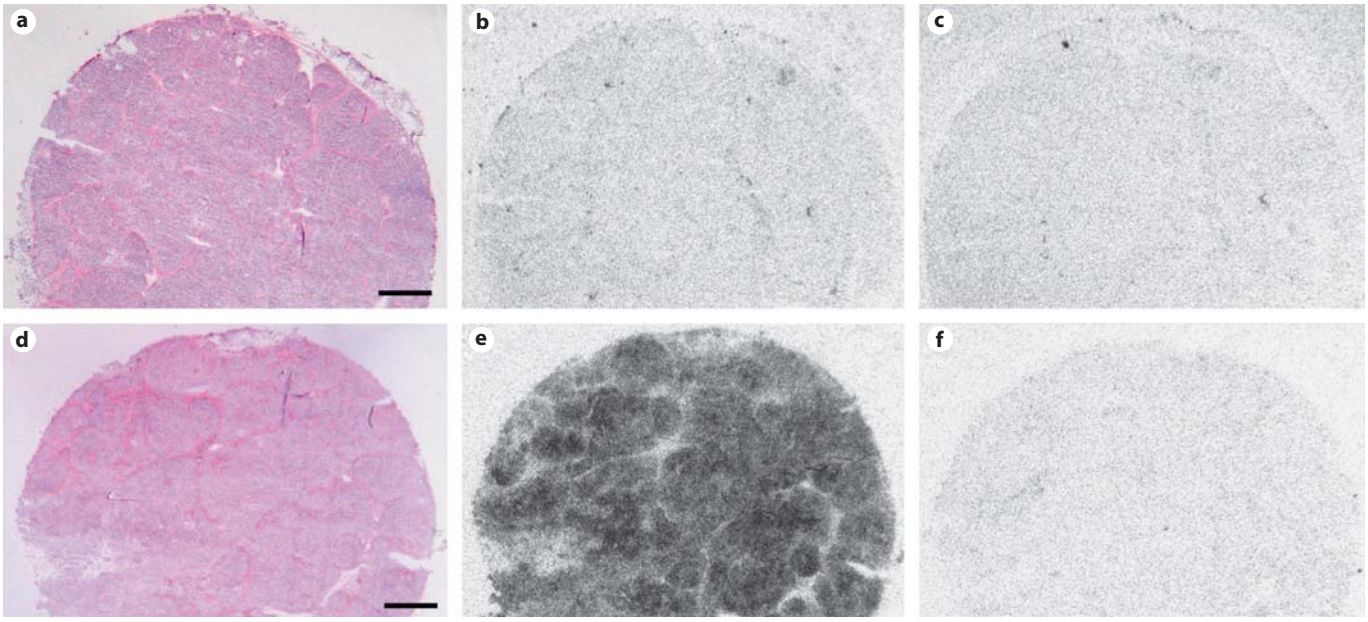

Fig. 4. GLP-1 receptor (**a–c**) and GIP receptor (**d–f**) autoradiography of TT xenografts in nude mice. **a**, **d** Hematoxylin eosin-stained sections showing the tumor. Bars = 1 mm. **b**, **e** Autoradiograms of total binding of ^{125}I-GLP-1 (**b**) or ^{125}I-GIP(1–30) (**e**). The TT xenografts massively express GIP receptors but not GLP-1 receptors. **c**, **f** Autoradiograms showing the respective nonspecific binding.

such as medullary thyroid carcinoma; interestingly, as opposed to the situation found in rat medullary thyroid carcinoma models, only a restricted number of human medullary thyroid carcinoma tumors express GLP-1 receptors, namely 27% of the cases (table 2). Moreover, their density, remaining generally only low to moderate, never reaches the levels found in the rat medullary thyroid carcinoma. Indeed, the mean density values for the 10 human medullary thyroid carcinoma expressing GLP-1 receptors is 2,007 ± 401 dpm/mg tissue only. Figure 2C is an illustration of a GLP-1 receptor-positive human medullary thyroid carcinoma. For comparison, a normal, receptor-negative thyroid is shown. At difference, the GIP receptor incidence in medullary thyroid carcinomas is much higher than the GLP-1 receptor incidence, reaching 89% (table 2). The GIP receptor density is high and reaches 3,578 ± 429 dpm/mg tissue. Figure 3 shows an example of a GIP receptor-positive human medullary thyroid carcinomas, with receptor-negative adjacent thyroid.

The human TT cell pellets and the human TT thyroid tumor xenografted in nude mice were found to completely lack GLP-1 receptors, a further indication that human medullary thyroid carcinomas may differ from rodent medullary thyroid carcinomas in their GLP-1 receptor expression (table 2; fig. 4). Conversely, the same TT cells and tumors were found to express high levels of GIP receptors (fig. 4). The GIP receptor density in the TT tumors reached 1,903 ± 707 dpm/mg tissue.

Table 2. GLP-1 and GIP receptor expression in human thyroid tissues

	GLP-1 R	GIP R
Incidence		
Human MTC		
Incidence in tumor	10/36 (27%)	24/27 (89%)
Incidence in surrounding thyroid	0/6	0/2
Non-neoplastic normal human thyroids		
Incidence in thyroid	0/6	0/6
Human TT thyroid tumors		
Mice xenographs	0/2	2/2
Cell pellets	0/2	2/2
Receptor density in the positive MTC cases		
Mean ± SEM (dpm/mg tissue)	2,007 ± 401 (n = 10)	3,578 ± 429 (n = 24)

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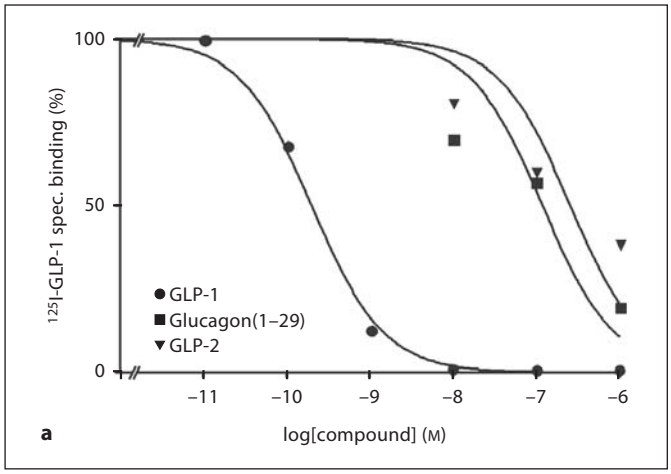

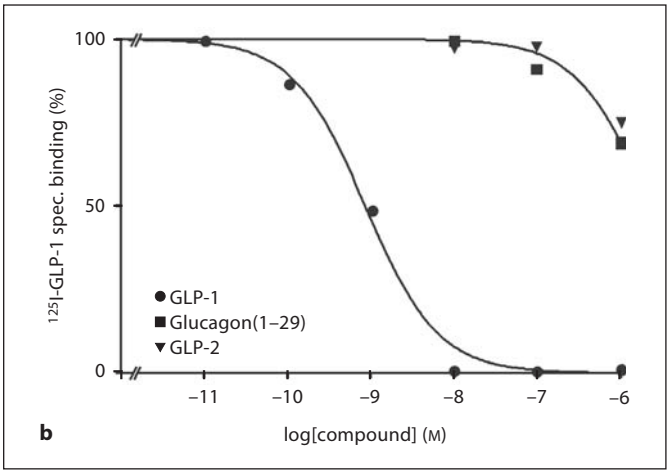

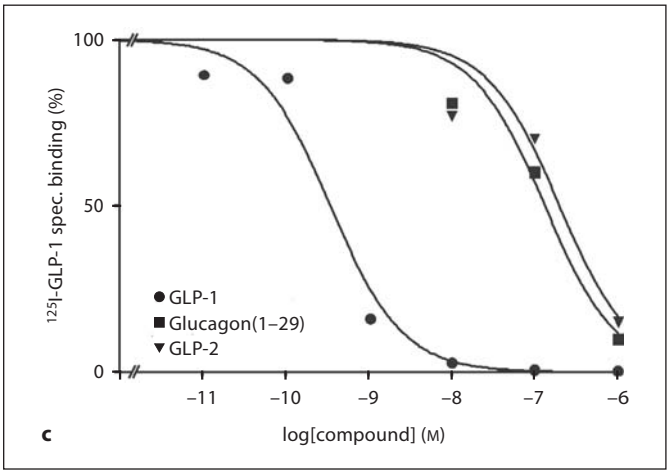

Fig. 5. Representative competition experiments in rat thyroid C cells (**a**), rat MTC (**b**) and human MTC (**c**). In all examples, high-affinity displacement of ^{125}I-GLP-1(7–36)amide by the GLP-1 receptor-selective agonist GLP-1(7–36)amide (GLP-1) and low-affinity displacement by the GLP-2 receptor-selective agonist GLP-2 and the glucagon receptor-selective agonist glucagon(1–29) was evident.

The GLP-1-receptors identified in the present receptor autoradiography experiments are of high affinity and selective for GLP-1. This is illustrated in figure 5. In all 3 examples, namely thyroid hyperplasia in the rat, medullary thyroid carcinoma in the rat and medullary thyroid carcinoma in the human, the GLP-1 receptors identified in competition experiments reveal a high-affinity binding for GLP-1 in the nanomolar range, but only a very low affinity for GLP-2 or glucagon. This high affinity for GLP-1 and the rank order of potency of the other peptides is characteristic for GLP-1 receptors. The GIP receptors detected in the present study are also of high affinity and selective for GIP. As shown in figure 6, the GIP receptors identified in competition experiments in rat and human medullary thyroid carcinomas reveal a high-affinity binding for GIP and GIP(1–30) in the nanomolar range, but only a very low affinity for GLP-1, GLP-2 or glucagon.

Discussion

The present study unequivocally shows that in rodents the thyroid C cells express GLP-1 receptors. The receptors were identified in all thyroid conditions tested. In the normal thyroid of young rats and mice, the focal expression of the GLP-1 receptors reflects the focal distribution of the C cells. In the C-cell hyperplasia and medullary thyroid carcinomas observed in the 2 rat models, the receptors are more homogenously distributed. While there is a great variability in individual receptor densities in the normal thyroids due to variable C cell distribution, we observe an increased GLP-1 receptor expression in the 2 types of cell lesions, with a homogenous high receptor density in C-cell hyperplasia and an even higher density in medullary thyroid carcinomas. Furthermore, all cases of C-cell hyperplasia and medullary thyroid carcinomas in the rat strongly ex-

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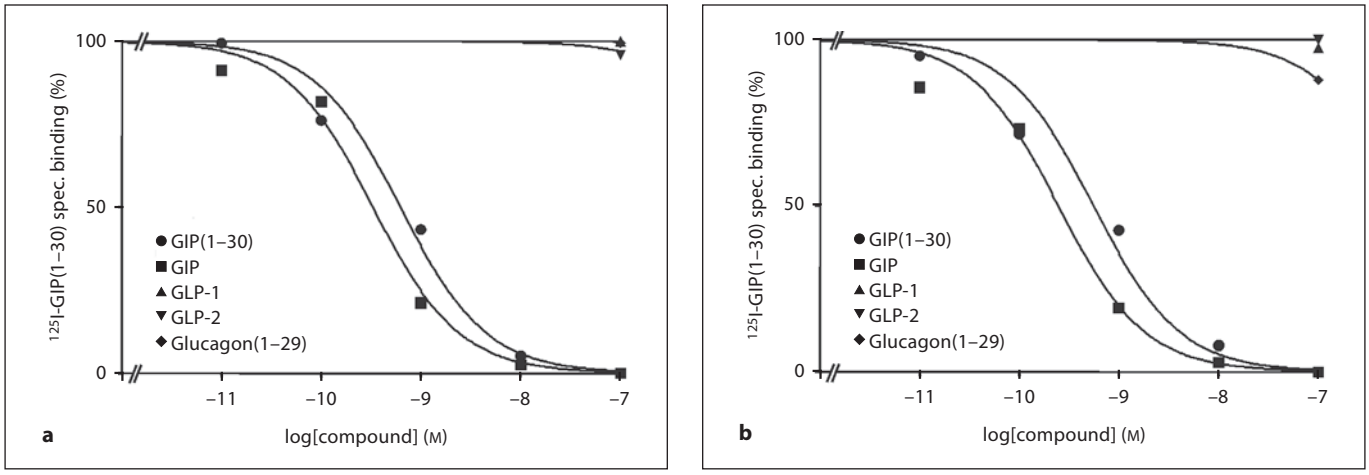

Fig. 6. Representative competition experiments for GIP receptors in rat MTC (**a**) and human MTC (**b**). High affinity displacement of ^{125}I-GIP(1–30) by GIP(1–30) or GIP is observed, whereas GLP-1, GLP-2 and glucagon(1–29) have no effect.

press GLP-1 receptors, reaching an expression incidence of 100% in both tissues. Interestingly, while the medullary thyroid carcinomas in the WAG/Rij model usually has a homogenous GLP-1 receptor distribution in the whole tumor, the GLP-1 receptors in the MENx model are differently expressed in various areas of medullary thyroid carcinomas, suggesting a differential regulation of GLP-1 receptor expression by the neoplastic cells within this tissue.

The situation is completely different with the other tested incretin receptor, the GIP receptor. No GIP receptors can be detected, neither in the normal rat and mouse thyroids nor in the C-cell hyperplasia in both rat models. Interestingly, however, all rat medullary thyroid carcinomas investigated strongly overexpress GIP receptors. The absence of GIP receptors in non-neoplastic C cells suggest that it is the neoplastic transformation of C cells that triggers the GIP receptor overexpression.

The GLP-1 receptor expression in human thyroid neoplasia is very different to the one observed in rodent thyroids, as reflected by the medullary thyroid carcinomas data: although there are some human medullary thyroid carcinomas expressing GLP-1 receptors, their incidence is, however, only 27%, indicating that the majority of human medullary thyroid carcinomas lack GLP-1 receptors. Moreover, the GLP-1 receptor density in the receptor-positive human medullary thyroid carcinomas is usually much lower than in the rodent tissues. The limited GLP-1 receptor expression in human medullary thyroid carcinomas is further supported by the absence of GLP-1 receptors in the human TT cells and TT thyroid tumor model xenografted in nude mice. The expression of GLP-1 receptors in medullary thyroid carcinomas is therefore largely species dependent. The highly heterogeneous expression of GLP-1 receptors in human medullary thyroid carcinomas suggests a complex regulation of the GLP-1 receptor expression in these tissues. Could the same complex regulation be at the basis of the heterogeneous GLP-1 receptor expression in some of the MENx medullary thyroid carcinomas?

Conversely, the GIP receptor expression in the human thyroid C-cell neoplasia compares very well with the expression in rat C-cell neoplasia: there is no evidence of species differences. The great majority, namely 89%, of human medullary thyroid carcinomas massively overexpress GIP receptors and, since no GIP receptors are found in the normal thyroid, it is likely that this GIP receptor expression is directly linked to the neoplastic transformation. Further support is given by the strong expression of GIP receptors in the TT cells and tumors.

Whether the absence of GLP-1 receptors in normal human thyroids, as opposed to rat and mice thyroids, is also based on a species specificity, cannot be concluded definitively, as the number of C cells in normal human thyroid (compared to rodent thyroid) is too low and may prevent a receptor detection in those cells. Indeed, the resolution of the in vitro receptor autoradiography meth-

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od may not be sufficient to identify receptors at low density in scattered single C cells. This limitation is, however, largely compensated by the advantages of the method to be morphological, quantitative, of excellent sensitivity and of high selectivity. It permits indeed to conclusively identify in this study high-affinity GLP-1 and GIP binding sites having a very high selectivity for the respective peptides, a strong proof that true GLP-1 and GIP receptors have been detected.

Whether the observed GLP-1 receptors in normal and hyperplastic thyroid C cells may mediate the development of C cell tumors in rodents treated with GLP-1 analogs, perhaps through stimulatory effects of GLP-1 on calcitonin release [10] and subsequent proliferative action of calcitonin, will require further investigations. Also the finding of a species-related difference, namely a higher GLP-1 receptor expression in C cell related lesions in rodents than in humans, may require further investigations before discussing a possible extrapolation of the rodent in vivo effects of GLP-1 to humans. Our data, nevertheless, suggest that at present time, caution, namely thyroid monitoring, should be necessary in human trials with long-acting GLP-1 analogs.

The situation is different for GIP receptors. First, we observe no species difference in their expression between rodents and humans. Second, neoplastic C cells, and only neoplastic C cells, appear to express GIP receptors, at a very high incidence, in both rodents and humans. This suggests, here as well, that caution in terms of thyroid monitoring may be necessary in human trials with long-acting GIP analogs [2, 21]. On the other hand, the unexpectedly high GIP receptor expression in human medullary thyroid carcinomas might be the molecular basis for GIP receptor targeting in vivo in those patients. The development of GIP-derived radioligands for preclinical studies may be greatly facilitated by our findings of a high GIP receptor expression in the TT cell line. Future studies hopefully may tell us to which extent incretins such as GLP-1 and GIP may play a significant role in an organ such as the thyroid, topographically and functionally so much far away from the pancreas.

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Disclosure Statement

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