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ORIGINAL ARTICLE

Somatostatin receptor expression in thyroid disease

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SUMMARY

Somatostatin analogues are commercially available and used for the management of acromegaly and neuroendocrine tumours, but the expression of the receptors as a target in thyroid disease has not been explored. To assess somatostatin (SST) and somatostatin receptor (SSTR1-5) expression in both normal and thyroid disorders, as a potential target for somatostatin analogue therapy, 67 thyroid tissue specimens were reviewed: 12 differentiated thyroid carcinomas, 14 follicular adenomas, 17 multinodular goitres, 14 Graves disease, 10 Hashimotos thyroiditis specimens and five normal thyroids. Tissue was immunostained for SST and SSTR1-5. Positivity and the degree of positivity were recorded by double-blinded observers. Somatostatin receptor expression was highly expressed in normal tissue for SSTR1, 3, 4 and 5 (5 of 5, 4 of 5, 4 of 5 and 5 of 5 respectively) whilst SST and SSTR 2a and b were not expressed at all. The commonest receptor expressed for all pathological subtypes grouped together was SSTR2b (63 specimens). The commonest receptors expressed in differentiated thyroid cancer were SSTR5 (11 of 12 specimens) and SSTR2b (10 of 12 specimens). The commonest receptor expressed in benign disease was SSTR2b (53 of 55 specimens). SSTR5 was significantly under-expressed in Graves disease ($P < 0.05$). This study illustrates that SSTR 1, 3, 4 and 5 are highly expressed in normal, benign and malignant thyroid tissue. SSTR 2a and 2b appear absent in normal tissue and present in benign and malignant thyroid tissue ($P < 0.02$). This suggests that focussed SSTR2 treatment may be a potential therapeutic target.

Keywords
analogue, octreotide, receptor, somatostatin, thyroid

Somatostatin (SST) is a neuropeptide that is present in two biologically active forms: SST-14 and SST-28. It is widely distributed throughout the nervous system and peripheral endocrine tissues where they act as neurotransmitters and neurohormones.

Naturally occurring SST has a very short lifespan. Because of this synthetic analogues have been produced. These analogues have two key potential applications. The first is in imaging of disease within the neuroendocrine tissues. Here, SST analogues are tagged with radionucleotides to identify SST receptors (SSTRs) positivity. The resulting positron emission tomography scan images can be used for diagnosis and monitoring of malignant disease (Becker *et al.* 1995; Garancini *et al.* 1997). The second application is for treatment of disease where SST analogues have an inhibitory effect in neoplasia shown both *in vitro* and *in vivo* (Schally 1988; Weckbecker *et al.* 1999). This has been shown in a wide range of neuroendocrine tumours including thyroid Hurtle cell, papillary and medullary cell carcinomas and is licensed for the treatment of acromegaly and gastroenteropancreatic neuroendocrine tumours (Robbins *et al.* 2000; Ahlman *et al.* 1997; Reubi 1996). There have until recently been two main SST analogues widely available: octreotide and lanreotide. Both have been used in the diagnosis and treatment of neuroendocrine malignant disease (Mazzaferri 1999; Benali *et al.* 2000; Van Essen *et al.* 2007).

The action of SST is mediated by five receptor subtypes (SSTR1-5, including isoforms 2a and 2b) (Reisine & Bell 1995) These receptors are encoded by five genes on five different chromosomes (Scarpignato & Pelosini 2001) and are part of the G protein-coupled receptors by which they exert their biological effects (Hoffken 1993). The SSTRs have

been shown by polymerase chain reaction (PCR), immunohistochemistry staining and radionucleotide tagged SST analogues, and refinements of tagging techniques have resulted in SST analogues that are now able to demonstrate individual receptors (Van Essen *et al.* 2007; Decristoforo *et al.* 2000; Wester *et al.* 2003; Hofmann *et al.* 2001). The prevalence of specific SSTRs in a particular disease could guide a more targeted therapeutic use of SST analogues in treatment (Gabriel *et al.* 2010).

In malignant thyroid disease, SSTRs 1, 3 and 5 have been shown to be expressed and radiolabelled analogues have been shown to assess the presence and extent of malignant disease, being particularly useful at demonstrating subclinical lymph node that are involved. (Van Essen *et al.* 2007). The activated lymphocytes present in Graves ophthalmoplegia have also been shown to have an affinity for specific SSTRs. Radiolabelled analogues are being used to not only diagnose and monitor disease, but also as treatment in thyroid eye disease (Aguirre-Balsalobre *et al.* 2007; Cozma *et al.* 2007).

Thus far the focus of therapy has been on malignancy and there has been little attention paid to on the potential utility for SST analogues in benign thyroid disease. Only a few reports have discussed questions about benign disease (Pyronnet *et al.* 2008; Pisarek *et al.* 2009).

The aim of this study was to compare and contrast the distribution of SST and all five receptor subtypes in normal thyroid tissue, benign thyroid disease and differentiated thyroid cancer to determine if SST analogue therapy may have wider practical therapeutic potential.

Materials and methods

Sixty-seven formalin-fixed and paraffin-embedded thyroid pathologies were selected from the histopathology archive at Hull and East Yorkshire Hospital NHS Trust, Kingston Upon Hull, UK. These pathologies consisted of 12 differentiated thyroid carcinomas, 14 follicular adenomas, 17 multinodular goitres, 14 Graves disease and 10 Hashimotos thyroiditis specimens. A further five samples of normal thyroid tissue were also selected from the archive to act as a control.

Antibodies for SSTRs 1-5 and rabbit monoclonal primary antibodies were purchased from Gramsch Laboratories, Schwabhausen, Germany, and used at a concentration of 1:10,000. Sections were cut to 3 μm thickness and floated on positively charged slides (SuperFrost*Plus; Menzel-Glaser, Braunschweig, Germany). The slides were dewaxed and rehydrated in xylene and ethanol respectively. Somatostatin receptor staining was carried using DAKO Catalyzed Signal Amplification Peroxidase System. Slides underwent heat epitope retrieval in citrate buffer pH6 with an initial 3 min at 1000 W and then 20 min at 150 W. This was followed by blocking of nonspecific binding with Avidin/Biotin Blocking Kit (SP 2002; Vector, Burlingame, CA, USA), hydrogen peroxidase and non-specific serum before incubation in SSTR primary antibodies raised against the C-terminal part of the human receptor proteins, at 1:10,000 overnight at 4 °C. Slides were then incubated in streptavidin-biotin complex followed by amplification reagent and streptavidin-peroxidase complex.

Sixty-one of these slides were also stained using SST antibodies (11 MNG, 14 Graves, 8 Hashimotos, 14 follicular adenoma, 9 differentiated thyroid cancers and 5 control tissues). Six of the MNG tissue specimens were not stained.

All slides were visualized with peroxidase substrate diaminobenzidine (DAB), and nuclei counterstained with Harris's haematoxylin before rehydration and mounting with DPX. Samples of anterior pituitary and normal pancreas known to express the relevant antigen were used as positive controls. Negative controls omitted the primary antibody and replaced it with non-specific serum.

Each slide was assessed for positive or negative SST and SSTR expression in thyrocytes. The degree of thyrocyte staining was also assessed for SSTR expression in thyrocytes. This was carried out by two independent observers blinded to pathological subtype. Three high-powered fields were selected at random on each slide, and an average score calculated from the total scores of the three fields. The scoring system was from 0 to 3 where 0 represented no visible cytoplasmic staining within thyrocytes and 3 represented dense staining. Once scoring was completed for both observers, kappa scores were calculated for interobserver agreement for staining compared to no staining in thyrocytes. Weighted kappa scores were then calculated for interobserver agreement for degree of staining in thyrocyte cytoplasm.

Following assessment of the slides, the results were analysed using SPSS version 16 (SPSS Inc., SPSS for Windows, Chicago, IL, USA) to calculate *P* values.

Ethical approval

The study, and use of the specimens, was prospectively approved by the Research and Ethics Committee at the Hull and East Yorkshire Hospital NHS Trust.

Results

Kappa scores revealed that interobserver agreement for positive staining for SSTRs against negative was good ($\kappa = 0.66$). Weighted kappa scores for degree of staining indicated moderate interobserver agreement ($\kappa = 0.44$).

Table 1 Table of proportion of specimens with positively immunostaining thyrocyte and vascular endothelial cells for somatostatin

	Thyrocyte staining
MNG	3/11
Graves	2/14
Hashimotos	1/8
Follicular adenoma	6/14
Differentiated thyroid cancer	3/9
Control tissue	0/5

Table 2 Table of proportion of specimens with positively staining thyrocytes and mean score of intensity of staining in positively staining specimens (in brackets)

	SSTR-1	SSTR-2a	SSTR-2b	SSTR-3	SSTR-4	SSTR-5
MNG	16/17 (2.1)	12/17 (2)	16/17 (1.75)	12/17 (1.6)	11/17 (1.5)	16/17 (1.5)
Graves	4/14 (1)	12/14 (1.3)	14/14 (1.7)	9/14 (1.2)	12/14 (1.1)	4/14 (1)
Hashimotos	6/10 (1.6)	6/10 (2)	10/10 (1.75)	6/10 (1.5)	10/10 (1.25)	7/10 (2)
Follicular adenoma	9/14 (2)	11/14 (1.8)	13/14 (1.4)	12/14 (1.5)	11/14 (1.5)	11/14 (1.7)
Differentiated thyroid cancer	7/12 (2)	7/12 (1.4)	10/12 (1.25)	8/12 (1.7)	9/12 (1.6)	11/12 (1.5)
Control	5/5 (1.4)	0/5 (0)	0/5 (0)	4/5 (1.2)	4/5 (1.8)	5/5 (1.4)

The differences in SST and SSTR expression in thyrocyte cytoplasm are shown in Tables 1 and 2. In thyrocytes, SST was expressed most commonly in follicular adenoma (43%). If benign and malignant pathologies were separated and compared with control, it was evident in positively staining specimens that the staining pattern was heterogenous with areas of positivity dispersed in small sections throughout areas of negativity.

The commonest receptor expressed for all pathological subtypes grouped together was SSTR2b that was not seen in the normal thyroid tissue ($P < 0.01$). If benign and malignant pathologies were separated and compared with normal thyroid, the commonest receptor expressed in differentiated thyroid cancer was SSTR5, but in benign disease was SSTR2b ($P < 0.01$). In control tissue, SSTR1 and SSTR5 were seen equally as commonly. Interestingly, SSTR5 was seen significantly less in Graves disease than in any other benign disease or in differentiated thyroid cancer ($P < 0.05$). It was also seen in Graves significantly less than normal tissue. Intensity of expression within thyrocytes was then studied for positively staining specimens. It was evident that in benign disease, SSTR1 was the most strongly expressed receptor in positively staining thyrocytes, and this pattern was repeated in malignant pathologies.

Discussion

In the study, control tissue specimens demonstrated receptor subtypes 1, 3, 4 and 5. In keeping with other studies SSTR2 was not found in normal tissue where RT-PCR and Northern blotting respectively had been examined (Ain *et al.* 1997; Forsell-Aronsson *et al.* 2000). High affinity for SSTR 3 and 5 in normal tissue had also been described, in accord with our findings (4/5 and 5/5 specimens respectively). Conversely, we report that SSTR4 was the strongest receptor expressed in normal tissue (1.8/3 intensity). This expression using RT-PCR was not found, although this was not statistically significant. Our findings suggest that SST analogues, such as octreotide or lanreotide, directed against SSTR2 in thyroid disease may have therapeutic potential (Druckenthaner *et al.* 2007).

The most frequently expressed SSTR in differentiated thyroid cancer was SSTR 5 (5/5 specimens) although the strongest expressed was SSTR1 (2/3 intensity). Given that SSTR 1, 2 and 5 are known to exert an antiproliferative effect (Law *et al.* 1995), our study further suggests that analogues specific to SSTR 1, 2 and 5 may be beneficial as therapeutic agents This accords with the views of others (Ain *et al.* 1997; Weckbecker *et al.* 2003), although SSTR2 a and b subtypes were the only receptors expressed to a significant degree when compared to control normal tissue ($P < 0.02$).

Of the 55 benign thyroid disease specimens, SSTR2 was seen in 82% with a greater number expressing type SSTR2b than SSTR2a (93% and 71% respectively). This receptor was also demonstrated most intensely (1.75/3). Interestingly, the intensity was higher in SSTR2a than 2b. The least frequently expressed SSTR was 1 (64%), and the least intensity was seen for SSTR 4 (1.3/3). This is in accord with a study that focussed on SSTR2 expression where enhanced expression in adenomatous thyroid disease was reported (Druckenthaner *et al.* 2007). In that study, this pattern was also seen in malignant disease, which does not hold with the findings reported here and in other studies (Pisarek *et al.* 2009; Ain *et al.* 1997; Forsell-Aronsson *et al.* 2000; Klagge *et al.* 2010).

Whilst SSTR1 and 5 were present in 100% of the control samples, they were two of the lowest expressed in benign disease (64% and 71%) suggesting preferential receptor loss. This was seen to the most significant extent in Graves disease. This has been described previously when assessing cold and hot thyroid nodules: both demonstrated a reduced expression of SSTR5 (Klagge *et al.* 2010). SSTR4 was seen in 79% of specimens. Previous studies have not recorded SSTR4 in benign disease(Pisarek *et al.* 2009; Ain *et al.* 1997). SSTR expression in follicular adenoma has been reported previously where it was suggested that this could be a precursor to follicular carcinoma (Hofmann *et al.* 2001).

The results from this study add significantly to what is known about the expression of SSTRs in benign thyroid disease. There are little data on the presence of SSTR receptors in benign thyroid disease with three reports focussed on follicular adenomas and a fourth with a very small sample size (four patients) (Pisarek *et al.* 2009; Ain *et al.* 1997; Forsell-Aronsson *et al.* 2000; Druckenthaner *et al.* 2007). The variation between the results of the studies examined may reflect the different analysis methods used, including immunohistochemistry, Northern blot testing and PCR (Pisarek *et al.* 2009; Ain *et al.* 1997; Forsell-Aronsson *et al.* 2000).

The limitations of this study include possible interobserver bias, although this was limited as much as possible by

applying blinding to the assessors and by using kappa scoring.

Conclusion and future investigation

There are still limited data in the literature regarding the use of SSTR receptor analogues in thyroid disease. Diseased thyroid tissue shows a higher degree of expression for both subtypes of SSTR2 that would be preferentially targeted by the current somatostatin analogues. We have shown that SSTR1 and 5 were present in all normal thyroid tissues and were the dominant receptors in malignant tissues, whilst being limited in benign tissue, particularly Graves disease. Somatostatin receptor 2 receptor analogues are likely to have an application in all thyroid disease classes. Further investigation into the use of SSTR 1 and 5 analogues such as pasireotide for malignant disease may have merit.

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Conflict of interests

The authors have no competing interests to declare.

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