The TSH receptor in the pituitary and its clinical relevance
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Mouse Pituitary Folliculo-Stellate Cells Express Receptors for Many, but not All, Adenohypophyseal Hormones

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Submitted for publication
5.1 ABSTRACT

Adenohypophyseal hormone production is mainly regulated through stimulatory and inhibitory hypothalamic peptides and target-gland hormones. Additionally, paracrine regulatory feedback loops within the pituitary have been suggested, with folliculo-stellate (FS) cells as the potential intermediates. We recently showed TSH receptor (TSHR) expression in FS cells of the human anterior pituitary and speculated that receptors for other adenohypophyseal hormones might also be expressed by FS cells. Using RT-PCR, we therefore evaluated the expression of receptors for TSH, GH, ACTH, LH, FSH and PRL in a murine FS cell line, TtT/GF. Transcripts of TSHR, GHR and ACTHR were detected in this cell line. LHR, FSHR and PRLR expression, however, could not be demonstrated. We conclude that TtT/GF cells express some, but not all, receptors for anterior pituitary hormones. This indicates that FS might act as mediators in the paracrine regulation of at least some of the hormones secreted by the anterior pituitary.
5.2 INTRODUCTION

The anterior pituitary produces a variety of hormones that are under the control of regulatory peptides secreted from the hypothalamus. When released from the anterior pituitary, these hormones have their effects on specific target tissues which in turn release hormones that provide a feedback control on pituitary hormone secretion. In addition, paracrine regulatory mechanisms within the pituitary have been postulated and evidence is accumulating that it is the folliculo-stellate (FS) cells that provide this additional control. FS cells have first been identified by their star-like appearance and their ability to form follicles (1). Their long cytoplasmic processes engulfing cells in the vicinity and their secretion of cytokines and a variety of growth factors indeed suggest that FS might regulate hormone synthesis and release by neighbouring endocrine cells. We recently demonstrated the presence of the thyrotropin (TSH) receptor (TSHR) in human FS cells which suggests that FS cells are involved in the paracrine control of TSH secretion (2). We wondered whether FS cells would also express receptors for other adenohypophyseal hormones. If so, the FS cells might play a central role in the paracrine regulation of adenohypophyseal hormone secretion. We therefore evaluated the expression of adenohypophyseal hormone receptors in the murine folliculo-stellate cell line, TtT/GF. This cell line was established from a thyrotropic pituitary tumour and was characterised as folliculo-stellate cells by the presence of many lysosomes and numerous intermediate filaments in the cytoplasm, phagocytic activity, follicle formation, and glial fibrillar acidic protein and S-100 protein expression (3).

5.3 MATERIAL AND METHODS

Cell culture

TtT/GF cells (a gift from Dr K. Inoue) (3) were maintained in HAM’s F10 culture medium supplemented with 10% fetal calf serum, penicillin, streptavidine and fungizone (BioWhittaker, Vervier, Belgium). The cells were grown at 37°C in a humidified atmosphere with 5% CO₂.
RT-PCR

Total RNA was isolated from TrT/GF cells and mouse thyroid, liver, adrenal, and ovary tissue (RNeasy total RNA purification kit, Qiagen GmbH, Germany). Thyroid tissue was used as a positive control for thyrotropin receptor (TSHR) expression; liver tissue as a positive control for growth hormone receptor (GHR) expression; adrenal tissue for adrenocorticotropic receptor (MC2R, the type 2 receptor among the melanocortin receptor family) and prolactin receptor (PRLR) expression; and ovary tissue for follicle stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) expression. 1 µg of total RNA was used in a reverse transcription reaction (1st Strand cDNA synthesis kit for RT-PCR [AMV], Roche Molecular Biochemicals, Mannheim, Germany) and 1/10 of the resulting cDNA was subjected to PCR. FSHR, LHR, MC2R, GHR, PRLR and β-Actin cDNA fragments were amplified using LightCycler PCR (Roche Molecular Biochemicals, Mannheim, Germany). This technique allows amplification of fragments up to 1 kb. To demonstrate TSHR expression two different primer sets were designed to amplify the extracellular domain (TSHR-E, 1.2 kb) spanning exons 1-9 and the intracellular domain (TSHR-I, 1.3 kb) spanning exon 10 of the mouse TSHR gene (4). Due to the size of the fragments, these were amplified using conventional PCR (Biometra, Gottingen, Germany). The primer sets are described in Table 5.1.

In short, 45 cycles of PCR were used with 50°C annealing temperature for LHR, FSHR, GHR, MC2R and PRLR. For TSHR PCR the cDNA was subjected to touchdown PCR, i.e. an annealing temperature of 60°C was used for the first 10 cycles, which was then decreased during the following 30 cycles by 0.33°C every second cycle, to a ‘touchdown’ annealing temperature of 51°C. PCR products were resolved by 1% agarose gel electrophoresis, stained with ethidium bromide and visualised using LumilImager software (Roche Molecular Biochemicals, Mannheim, Germany).

Southern Blotting

Since the TSHR PCR showed multiple bands on gel, the specificity of these bands was assessed by southern hybridisation. The PCR products were first resolved on 1% agarose gel in 1x TRIS-borate + EDTA. The gel was denatured in 0.5 M NaOH/1.5 M NaCl for 2x 15 min and neutralised in 0.5 M Tris pH 7.0/1.5 M NaCl for 2x 15 min. DNA was transferred
Table 5.1. Primer sequences used for RT-PCR with the calculated fragment lengths.

<table>
<thead>
<tr>
<th>Target</th>
<th>fragment length (bp)</th>
<th>primer sequence</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSHR-E</td>
<td>1185</td>
<td>F: 5' ATT GTT GGG TAC AAG GAA 3'</td>
<td>(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5' GTA GTG TTA ACT AAC ACC GT 3'</td>
<td></td>
</tr>
<tr>
<td>TSHR-I</td>
<td>1305</td>
<td>F: 5' GGC GGA ATG GGG TGT T 3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5' GAA CTT GTA GGC CAT TAT CTG CTT 3'</td>
<td></td>
</tr>
<tr>
<td>GHR</td>
<td>242</td>
<td>F: 5' GAA TGG AAA GAA TGC CCT GA 3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5' GGT TGC CAA CTC ACT TGG AT 3'</td>
<td></td>
</tr>
<tr>
<td>MC2R</td>
<td>298</td>
<td>F: 5' TTC AGC CTG TCT GTC ATT GC 3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5' GCA CCC TTC ATG TTG GTT CT 3'</td>
<td></td>
</tr>
<tr>
<td>FSHR</td>
<td>239</td>
<td>F: 5' TTA TTC TTT GCC ATT TCC GC 3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5' CTG GAG TGG AAG TTG TGG GT 3'</td>
<td></td>
</tr>
<tr>
<td>LHR</td>
<td>516</td>
<td>F: 5' CTT ATA CAT AAC CAC CAT ACC AG 3'</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5' ATC CCA GCC ACT GAG TTC ATT C 3'</td>
<td></td>
</tr>
<tr>
<td>PRLR</td>
<td>309</td>
<td>F: 5' GAA GCA GAA GAG TGG GAG ATC CAT TTT 3'</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5' TCC TTT TAT TTT TGG CCC CGG AAC TGG TGG 3'</td>
<td></td>
</tr>
</tbody>
</table>

* These primer sets were designed at our department.

onto nitrocellulose membrane (Nitran, Schleicher and Schuell GmbH, Dassel, Germany) by semi-dry electroblotting using 20x SSC and cross-linked by UV irradiation. The membrane was hybridised to a digoxigenin-labelled RNA probe complementary to a unique sequence within the TSHR (corresponding to nucleotides 1033-1168). The probe was in vitro transcribed as described earlier (2). Hybridisation took place overnight at 45°C in hybridisation buffer (DIG EasyHyb, Roche Molecular Biochemicals, Germany). Hybridisation was detected with anti-DIG-Fab conjugated to alkaline phosphate and visualised using the chemiluminescent substrate CDP-Star and LumiImager software according to the manufacturer (Roche Molecular Biochemicals, Mannheim, Germany).

5.4 RESULTS

TSHR expression was detected by touchdown PCR in the TtT/GF cell line and in mouse thyroid tissue as a 1.2 kb ECD and a 1.3 kb ICD fragment (Figure 5.1a). Southern hybridisation with a probe overlapping part of the ICD as well as the ECD nucleotides

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sequence confirmed the identity of the amplified products (Fig. 5.1b). GHR and MC2R expression were also detected in TtT/GF cells as 242 bp and 298 bp fragments (Figure 5.2). Similar GHR and MC2R transcripts were detected in positive control tissues (mouse liver and adrenal, respectively). MC2R expression was further confirmed by performing a ‘minus-RT’ control that showed no band.

LHR, FSHR and PRLR expression was not detected in the cell line. Positive control tissues, however, did show fragments of the expected sizes.

5.5 DISCUSSION

In this study we characterised the mouse folliculo-stellate (FS) cell line, TtT/GF, in terms of adenohypophyseal-hormone receptor expression. We found that TtT/GF cells express TSHR, GHR and MC2R mRNA, but not mRNA encoding for FSHR, LHR and PRLR.

TSHR expression by folliculo-stellate cells in the human anterior pituitary has been reported before (2,5) and it was suggested that TSH secretion might not only be regulated by hypothalamic TRH and thyroid hormones, but also directly at the level of the pituitary via an
ultra-short loop mechanism with FS cells as the paracrine mediators. Recent results obtained in a rat model indeed supported this view. Rats that were unable to mount a thyroid response because of treatment with methimazole, showed decreased TSH plasma levels after injection of TSHR autoantibodies-containing IgG as compared to control IgG (6). This indicates that TSH levels are also regulated via an extrathyroidal TSHR, irrespective of circulating thyroid hormone levels which remained unaffected in both groups.

GHR expression in the anterior pituitary has been reported before. Mertani et al. (7) showed GHR immunoreactivity in somatotrophs, lactotrophs and gonadotrophs of the human anterior pituitary. These authors did not perform immunohistochemistry with an antiserum against FS-specific proteins (e.g. S-100 or MHC-class II) in order to identify GHR expression in FS cells. Thus, to our knowledge, this is the first demonstration of GHR expression in FS cells of the anterior pituitary, which also holds true for MC2R expression.

PRLR, LHR and FSHR mRNA expression was not detected in the TtT/Gf cell line. Jin et al. (8) used combined in situ hybridisation and immunohistochemistry to demonstrate PRLR mRNA expression in lactotrophs and gonadotrophs of the normal human pituitary. In another study, PRLR mRNA expression was observed in whole pituitary homogenates. However, neither of these authors specifically addressed FS cells. Gonadotropin receptors have not been described with respect to the anterior pituitary yet.

For now we can only speculate about the biological significance of the observed receptor-expression pattern. On the one hand, they might play a role in metabolic processes
within the FS cells. On the other hand, the expression of only some, but not all, of the adenohypophyseal hormone receptors in TtT/GF cells suggests that FS cells are directly involved in regulating the secretion of TSH, GH and ACTH. Remarkably, the receptors for hormones that are involved in reproduction (i.e. LH, FSH and PRL) are not expressed by TtT/GF cells. This might indicate that reproductive functions are not regulated directly by FS cells at the level of the pituitary. However, Baes et al. (9) did show that coaggregating FS cell-enriched populations from adult female rats with other pituitary cell populations resulted not only in an inhibition of the GH response to GH-releasing factor and β-adrenergic agents, but also in a decreased PRL response to TRH and angiotensin II, as well as a decreased LH response to LHRH. Similar results were described by Allaerts et al. (10) who demonstrated that co-culturing rat gonadotroph-enriched cell aggregates with a FS cell-enriched population resulted in the attenuation of LH response to GnRH. This suggests that FS cells are nevertheless involved in the paracrine regulation of PRL and LH, but the mechanism behind this regulation would clearly differ from that of TSH, GH or ACTH. Another possibility is that different populations of FS cells within the anterior pituitary, not represented by the TtT/GF cells, express different subsets of hormone receptors.

Folliculo-stellate cells make up 10% of the pituitary cell population but their function has long been elusive. Now, it appears that they are capable of regulating hormone secretion. They produce several growth factors, such as basic fibroblast growth factor (FGF) (11), vascular endothelial growth factor (VEGF) and follistatin (12). The TtT/GF cell line may help to elucidate the function of FS cells, and further characterisation of this cell line is thus important. In addition to our findings, this cell line produces VEGF (13). Basic FGF, however, appears not to be secreted by this cell line. FS cells also produce nitric oxide (14) which is a known modulator of pituitary hormone secretion. The production of cytokines by FS cells is even more interesting, especially the production of interleukin-6 (IL-6). It is reported that TtT/GF cells express TNF receptors, and that TNF stimulates IL-6 secretion in vitro (15). The possible paracrine functions of IL-6 have been reviewed by Renner et al (16).

In conclusion, we have demonstrated that the murine FS cell line (TtT/GF) expresses receptors for TSH, GH and ACTH. This not only elaborates on our previous finding of TSHR expression in the human anterior pituitary (2), but it also supports the putative role of the FS cell as a paracrine mediator within the anterior pituitary (6). The expression of these receptors in the TtT/GF cell line offers the possibility to clarify this role in an in vitro model.
5.6 REFERENCES


