Immunohistochemical detection of angiotensin receptors AT1 and AT2 in normal rat pituitary gland, estrogen-induced rat pituitary tumor and human pituitary adenomas

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Abstract: Male rat pituitary glands, diethylstilbestrol (DES)-induced rat pituitary tumors and 12 human pituitary adenomas were immunostained with antibodies raised against AT1 and AT2 angiotensin receptor proteins. Positive immunostaining of AT1 was observed in a subpopulation of anterior and intermediate pituitary lobe cells as well as in some nerve endings of the neurohypophysis. In the DES-induced rat pituitary tumors, the subpopulation of AT1-immunopositive cells was smaller than in the non-tumoral anterior pituitary. In human pituitary adenomas, weak AT1 immunostaining was found in 5 tumors. In the remaining adenomas, the AT1 immunostaining was trace (doubtful) or absent. The AT1 immunostaining in the peritumoral non-neoplastic pituitary tissue was stronger than that observed in the tumors. The normal rat pituitaries and rat tumors did not show immunostaining with anti-AT2 antibody. In human pituitary adenomas, the tumoral cells were AT2-negative but moderate to strong AT2 immunostaining was observed in intratumoral blood vessel walls. The data suggest that the experimental (in rat) and spontaneous (in man) pituitary tumorigenesis is associated with the down-regulation of AT1 receptors. The expression of AT2 receptors, in turn, may be connected with the process of tumoral neo-angiogenesis.

Key words: Angiotensin receptors - AT1 - AT2 - Pituitary gland - Pituitary adenomas - Immunohistochemistry

Introduction

Human and rat pituitary glands include local renin-angiotensin system (RAS). The renin-like activity was discovered in rat pituitary gland as early as in the seventies [4]. In the rat anterior pituitary gland, renin, angiotensinogen and angiotensin-converting enzyme (ACE) were localized in gonadotropes, which seem to be the main source of pituitary angiotensin II (Ang II) in this species [2, 17]. Further studies revealed the presence of renin in human pituitary specimens examined post mortem [6]. Renin, angiotensinogen and converting enzyme were also found by immunohistochemical techniques in normal and tumoral human lactotropes [15]. Ang II was shown to stimulate the prolactin (PRL), corticotropin (ACTH) and luteinizing hormone (LH) release from the rat anterior pituitary gland [18]. Ang II and its shorter fragment angiotensin IV (3-8 Ang II) stimulated the proliferation of lactotropes isolated from the estrogen-induced rat prolactinoma [10]. The stimulatory effect of Ang II was also observed in cells isolated from human prolactinoma [11]. Ang II and Ang IV were also shown to stimulate the anterior pituitary gland in ovariectomized female rats in vivo [13], whereas the administration of ACE blocker enalapril, which suppresses the biosynthesis of Ang II, exerted an opposite effect [7]. However, the role of RAS in the physiological control of the anterior pituitary secretion and cell proliferation and its involvement in the pituitary tumorigenesis remains still unclear. The binding studies with radiolabeled ligands and molecular biology techniques revealed in the rat anterior pituitary gland the presence of AT1 receptors belonging to the so-called AT1B subtype. The AT1B mRNA is expressed mainly in prolactin cells, and, to a lesser extent, on corticotropes [5]. The raising of polyclonal antibodies against the AT1 and AT2 receptor proteins created a possibility of the immunohistochemical detection of these receptors in different organs and tissues. The present study reports for the first time the immunohistochemical localization of
angiotensin receptors in the rat pituitary gland and human pituitary adenomas.

Material and methods

Normal rat pituitaries and estrogen-induced pituitary tumors. Three four-week old male Fisher 344 rats were subcutaneously implanted with silastic capsules containing diethylstilboestrol (DES, 10 mg/capsule). Six weeks after the implantation, the animals were sacrificed by decapitation. The pituitaries were removed and fixed in Bouin-Hollande solution. Three untreated animals of the same strain, sex and age were the donors of normal pituitaries.

Human pituitary adenomas. Twelve surgically excised adenomas were examined. The data concerning patients, clinical diagnosis and hormone immunostaining are presented in Table 1. Additionally, two samples of the non-tumoral anterior pituitary gland, adjacent to the excised tumors were also examined.

Immunohistochemistry. AT1 immunostaining was performed using the anti-AT1 polyclonal antibody (sc-1173). This antibody was raised against the N-terminal extracellular domain of AT1 receptor and recognizes human, rat and mouse receptor protein. AT2 receptors were revealed using the sc-9040 polyclonal antibody raised against the 221-303 fragment of the human AT2 receptor protein. The antibody detects human, rat, and mouse AT2 receptors. Both antibodies were purchased from Santa Cruz Biotechnology, CA, USA. The anti-AT1 and anti-AT2 antibodies were applied in working dilution 1:100.

Each human tumor was also immunostained using the primary polyclonal or monoclonal antibodies against the pituitary hormones and alpha-subunit (alpha-SU). The data on the sources of antibodies were published in an earlier paper [9].

The visualization of primary anti-receptor and anti-hormone antibodies was done using the StreptABComplex/HRP Duet (Dako Cytomation) following the procedure recommended by the producer. In brief, the biotinylated goat antibody against rabbit and mouse immunoglobulin was applied as the secondary antibody, followed by streptavidin-biotinylated horseradish peroxidase complex and 3,3’-diaminobenzidine as chromogen.

Results

Rat anterior pituitary glands and estrogen-induced pituitary tumors

In the normal rat anterior pituitary lobe, approximatively 50% of irregularly scattered glandular cells expressed a strong positive immunostaining with anti-AT1 antibody. The reaction was localized in cytoplasm without visible enhancement in the cell membrane. The cell nuclei were negative (Fig. 1). A strong immunostaining could also be observed in some nerve endings in neurohypophysis, while the majority of the intermediate lobe cells showed moderate immunopositivity. (Fig. 2). In estrogen-induced pituitary tumors, the cells with strong positive immunostaining were less numerous in comparison with the normal pituitary gland (approx. 30%, Fig. 3). The cellular localization of AT-1 immunostaining was the same as in normal pituitaries. The remaining tumoral cells presented trace (doubtful) reaction.

Table 1. Data on human pituitary adenomas

<table>
<thead>
<tr>
<th>Patient’s initials</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Hormone</th>
<th>Immunostaining</th>
</tr>
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<tbody>
<tr>
<td>ZK</td>
<td>F</td>
<td>71</td>
<td>GH</td>
<td>+</td>
</tr>
<tr>
<td>HW</td>
<td>F</td>
<td>56</td>
<td>GH/PRL</td>
<td>+</td>
</tr>
<tr>
<td>MS</td>
<td>F</td>
<td>48</td>
<td>GH/PRL/alphaSU</td>
<td>+</td>
</tr>
<tr>
<td>MM</td>
<td>M</td>
<td>46</td>
<td>FSH/LH</td>
<td>+</td>
</tr>
<tr>
<td>KS</td>
<td>F</td>
<td>44</td>
<td>betaLH</td>
<td>+</td>
</tr>
<tr>
<td>JW</td>
<td>M</td>
<td>32</td>
<td>PRL/TSH</td>
<td>+/-</td>
</tr>
<tr>
<td>JG</td>
<td>F</td>
<td>65</td>
<td>GH/PRL/alphaSU</td>
<td>+/-</td>
</tr>
<tr>
<td>KJ</td>
<td>F</td>
<td>49</td>
<td>betaLH</td>
<td>+/-</td>
</tr>
<tr>
<td>KC</td>
<td>F</td>
<td>29</td>
<td>ACTH</td>
<td>+/-</td>
</tr>
<tr>
<td>HP</td>
<td>F</td>
<td>21</td>
<td>none</td>
<td>+/</td>
</tr>
<tr>
<td>KM</td>
<td>F</td>
<td>25</td>
<td>ACTH</td>
<td>0</td>
</tr>
<tr>
<td>ZKo</td>
<td>M</td>
<td>39</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>JM</td>
<td>F</td>
<td>47</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

F-women, M-men; ++ moderate positive immunostaining; + weak positive immunostaining; +/- trace (doubtful) immunostaining; 0 negative immunostaining.

Both normal rat pituitaries and pituitary tumors did not stain with anti-AT2 antibody.

Human pituitary adenomas

Weak positive immunostaining with anti-AT1 antibody was observed in 5 adenomas (patients ZK, HW, MS, MM, KS - Fig. 4). Like in the rat, the localization of reaction was mainly cytoplasmic. Trace (doubtful) reaction was found in 5 tumors (patients JW, JG, KJ, KC, HP). The remaining 2 adenomas (from patients ZKo and KM) did not stain with anti-AT1 antibody (Fig. 5). The AT1 immunostaining did not seem to depend on the patient’s age, sex and adenoma hormonal phenotype. In both samples of the non-tumoral anterior pituitary lobe, moderate immunostaining for AT1 receptor protein was found in a subpopulation of the glandular cells (approx. 30% of cells). In both cases, besides moderate immunopositivity in cell cytoplasm, a strong immunostaining of some cell nuclei could be observed.

Tumoral cells in all the investigated pituitary adenomas and glandular cells in fragments of non-tumoral anterior pituitary lobes did not stain with AT2 antibody. However, a strong reaction with AT2 antibody was found in the vascular walls of the intratumoral blood microvessels (Fig. 6). Positive immunostaining included not only endothelium, but also other elements of the...
Fig. 1. Normal rat anterior pituitary gland. Immunostaining with anti-AT1 antibody (× 400).

Fig. 2. Intermediate (IL) and neural lobe (NL) of the normal rat pituitary gland. Immunostaining with anti-AT1 antibody. Arrows indicate the immunopositive nerve endings (× 400).

Fig. 3. Diethylstilbestrol-induced rat pituitary tumor. Immunostaining with anti-AT1 antibody (× 400).

Fig. 4. Somatoprolactinoma in 56-years old woman (H.W.). Weak immunostaining of tumoral cells with AT1 antibody. Arrow indicate the immunopositive cell nucleus. V - blood vessel lumen filled with (unspecifically?) immunostained material (× 400).

Fig. 5. ACTH-secreting adenoma (corticotropinoma) in 25-years old woman (K.M.), negatively immunostained with AT1 antibody (A). The non-tumoral anterior pituitary lobe (NP) immunostained positively (× 100).

Fig. 6. Plurihormonal pituitary adenoma (GH/PRL/alfaSU) in 65-years old woman (J.G.). Immunostaining with anti-AT2 antibody; strong staining of blood vessel walls (× 400).
vascular wall. In contrast, microvessels within the non-tumoral fragments of the gland did not show immunostaining.

Discussion

Positive AT1 immunostaining of a subpopulation of the anterior pituitary gland cells in the rat remains in concordance with the earlier studies performed using the radiolabeled ligand binding and molecular biology techniques. The identification of cell category expressing the AT1 receptors is not possible because we did not investigate the co-localization of AT1 receptors with pituitary hormones. However, on the basis of earlier molecular studies [5] it could be hypothesized that they mostly represent lactotropes. The presence of the strong reaction in neurohypophysial nerve endings is compatible with the well known role of Ang II and/or Ang III in stimulating vasopressin release [14]. The distribution of AT1 receptors in human non-tumoral anterior pituitary gland is very similar to that observed in the rat. The reaction is mainly cytoplasmic, but some cell nuclei are also stained. The latter finding is concordant with earlier observations indicating the presence of a nuclear binding site for Ang II and its AT1-like properties [19].

The most interesting finding in the present study is the low expression of AT1 receptors in pituitary adenomas, both experimental in the rat and spontaneous in man. This expression seems to be lower than in normal, non-tumoral anterior pituitary gland. Although this observation is based on semiquantitative evaluation, the differences were impressive. The possible mechanism and pathogenetic role of the discussed finding remains unclear. Estrogens were shown to down-regulate AT1 receptors [16] and this observation might explain the lower AT1 expression in the rat estrogen-induced pituitary tumor. However, such an explanation is unlikely when the human pituitary adenomas are concerned. The investigated female patients were in majority in the post-menopausal age and the female younger patients suffered from the secondary ovarian deficiency evoked by pituitary tumor (see Table 1). Thus, the presumed levels of estrogens in these patients were low. The male patients also presented rather low levels of estrogens. Another possible cause of the down-regulation of AT1 receptors could be the increased local level of the endogenous Ang II. Such a mechanism has been found as responsible for the down-regulation of AT1 receptors in benign prostate hyperplasia [3]. Another finding worth to be underlined is the over-expression of vascular AT2 in human pituitary adenomas. The AT2 block has been recently shown to inhibit angiogenesis via suppression of the crucial factor involved in this process - vascular endothelial growth factor (VEGF) [20]. Interestingly, the vascular expression of AT2 receptors can be stimulated in rats by Ang II [1]. The hypothesis that the observed alterations of AT1 and AT2 receptors in pituitary adenomas depend on local overproduction of Ang II needs to be confirmed in further studies but corroborates with our earlier observations on the role of Ang II in the control of pituitary cell proliferation [7, 10, 11] and in pituitary angiogenesis [8]. The down-regulation of AT1 receptors does not exclude the involvement of the excessive Ang II production in stimulation of cell proliferation in pituitary adenomas, since proliferative effect of this peptide may be mediated, at least in part, by non-AT1 receptors [13]. It is also worth to underline that both AT1 and AT2 receptors may be involved not only in the control of cell proliferation but also in the induction of apoptosis [12]. To conclude, our findings - taken together with earlier observations from our and other laboratories - suggest the involvement of local RAS in the pathogenesis of pituitary adenomas.

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