Severe experimental uraemia does not decrease the population of rat pituitary somatotrophs

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Abstract

Background. Growth hormone (GH) secretion by the anterior pituitary has been shown to be depressed in severely uraemic rats. Changes in the population of pituitary somatotrophs might be partially responsible for this decrease.

Methods. To analyse the population of pituitary somatotrophs in severe uraemia, immunocytochemical detection and quantification of GH-producing cells were carried out on paraffin sections from young rats either 5/6 nephrectomized, sham-operated fed ad libitum or sham-operated pair-fed with the nephrectomized animals.

Results. Nephrectomized rats were severely uraemic and growth retarded. The overall cell density (total pituitary cells/mm²) was higher in 5/6 nephrectomized animals in comparison with the two sham-operated groups. Thus, although the percentage of GH cells was slightly lower in nephrectomized than in control rats, no difference in either the density (cells/mm²) or the cross-sectional area of GH cells was found among groups.

Conclusions. These results suggest that severe experimental uraemia interferes with the maturation process of the pituitary gland and support the contention that differences in either the number or the size of pituitary somatotrophs cannot explain the reduced GH secretion previously reported in severely uraemic rats.

Key words: chronic renal failure; growth hormone; growth; immunohistochemistry; pituitary somatotrophs; rat

Introduction

Recent findings of our group have demonstrated impairment of growth hormone-releasing hormone (GHRH)-stimulated GH secretion in perfused pituitary cells from 5/6 nephrectomized rats with severe uraemia [1]. As GHRH exerts a trophic action on the population of pituitary somatotrophs [2] and as the hypothalamic input of GHRH has been shown to be decreased in uraemic animals [3], a relative reduction of pituitary somatotrophs might, at least in part, explain the reduction of GH secretion. Thus, to find out if the population of pituitary somatotrophs is altered in severe uraemia, the population of GH-producing cells was measured in 5/6 nephrectomized rats.

Subjects and methods

Male Sprague–Dawley rats, aged 30 ± 3 days, were classified in three groups of eight rats each: rats with chronic renal failure induced by 5/6 nephrectomy (Nx), and sham-operated rats with normal renal function, either fed ad libitum or pair-fed with Nx (SAL) or pair-fed with Nx (SPF).

All animals were housed in individual cages and had free access to tap water and rat chow. Surgical procedures, nephrectomy by excision of 5/6 of the renal mass or renal decapsulation without loss of renal mass (sham operation), were performed in two stages on days 0 and 4 under anaesthesia. On day 14, 10 days after either second-stage nephrectomy or second sham operation, the animals were sacrificed. Blood samples were centrifuged and serum was stored at −20°C until used.

From day 4 on, animals were daily weighed. Rats’ snout to tail-tip length was measured on days 0, 4, and 14. Growth was assessed by weight and length gained between days 4 and 14. Serum concentrations of urea nitrogen (SUN) were measured with an autoanalyser.

Pituitaries were fixed in Bouin’s solution, dehydrated and embedded in Paraplast. Two-micrometre-thick frontal sections were obtained using an ultramicrotome LKB and mounted on glass slides. From each pituitary two sections taken from the central part of the gland—between the anterior and the posterior poles—were processed for immunohistochemistry as previously described [4].

For morphometrical purposes a light-microscope (Zeiss Jena, Germany) connected to a MIP image analyzer (Microm, Spain) was used. A 50 × and a 100 × objective...
lenses were respectively used to calculate the percentage and the cross-sectional area of GH cells. Sections were evaluated by an independent observer (SC) unaware of the group the sections belonged to. To calculate the relative number of GH-cells (% of GH cells), the number of unlabelled cells and that of GH immunoreactive cells were recorded on 15 microscope fields (7497 μm² each) taken at random from a lateral wing towards the centre of the gland. The density of cells per surface unit (cells/mm²) was calculated both for the labelled cells—density of GH cells—and for the total (labelled and unlabelled) number of cells—overall cell density. To calculate the cross-sectional area of individual GH cells, the area of 100 immunostained cells was determined in two sections per animal. Results are given as mean ± SEM. Statistical analysis was carried out by one way ANOVA at a level of significance of 95%. Scheffe F test was used for comparisons between two groups.

Results

Nephrectomized rats were severely uraemic and growth retarded as shown by much higher SUN levels and lower weight and length gains than those found in sham-operated rats (Table 1).

Immunostaining with antibody to GH provided a clear identification of GH cells (Figure 1). No difference in the immunostaining pattern was found among groups. As shown in Table 2, the overall cell density was higher in Nx animals than in the other two groups. Thus, although the percentage of GH cells was lower in nephrectomized rats than in the other groups, no difference in the density of GH cells or in the cross-sectional area of these cells was found among groups. Overall cell density and percentage of GH-positive cells were not different between SAL and SPF groups.

Discussion

The findings presented here provide some new information on the effect of chronic renal failure on GH metabolism. The anterior pituitaries of young uraemic rats had greater cell density and lower relative number of GH secreting cells than the two groups of rats with normal renal function. This effect was due to the uraemic state and not to the associated malnutrition.

Table 1. Growth data and serum urea nitrogen concentration (SUN) of 5/6 nephrectomized rats (Nx), sham-operated rats fed ad libitum (SAL), and sham-operated rats pair-fed with the Nx group (SPF).

<table>
<thead>
<tr>
<th>Group</th>
<th>SUN (mg/dl)</th>
<th>Weight gain days 4–14 (g)</th>
<th>Length gain days 4–14 (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>13.5 ± 0.6</td>
<td>71.6 ± 3.9</td>
<td>6.7 ± 1.8</td>
</tr>
<tr>
<td>Nx</td>
<td>*87.3 ± 5.3</td>
<td>*14.5 ± 5.4</td>
<td>*2.5 ± 0.2</td>
</tr>
<tr>
<td>SPF</td>
<td>10.7 ± 0.5</td>
<td>*28.9 ± 1.7</td>
<td>*3.5 ± 0.1</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=8 each group). *Significantly different from SAL animals; #significantly different from SPF animals.

since it was not observed in the diet-restricted sham-operated group pair-fed to the nephrectomized rats.

The increased cell density found in the pituitaries of young uraemic rats can be interpreted as a disordered process of maturation of the anterior pituitary gland. The increase of weight and size of the anterior pituitary during normal postnatal growth has been shown to be mostly determined by a sustained increase of total cell number [5]. However, not only hyperplasia but also hypertrophy plays an important role in pituitary growth. Noticeably, while cellular proliferation has little effect on cell density, cell hypertrophy is closely related to the number of cells per surface unit in the anterior pituitary, so that, as rat grows, there is a progressive decline of the overall cell density [6]. Thus, the increased cell density found in the pituitaries of uraemic rats suggests that uraemia itself interferes with the maturative process of the anterior pituitary gland. Unfortunately, our study does not provide any insight into the mechanisms leading to this potential alteration of the maturation process.

The percentage of GH cells found in nephrectomized rats was slightly but significantly lower than that of control rats. Therefore, in spite of having a greater number of total pituitary cells/mm², the absolute number of GH cells/mm² was not different in uraemic animals in comparison with the two groups of rats with normal renal function. Moreover, there was no significant change in the size of individual somatotrophs, as assessed by the cross-sectional area, among
Table 2. Morphometric analysis of GH cells and total pituitary cells in 5/6 nephrectomized rats (Nx), sham-operated rats fed ad libitum (SAL), and sham-operated rats pair-fed with the Nx group (SPF)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total pituitary cells</th>
<th>GH cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Density (10^4 cells/mm^2)</td>
<td>Percentage (% of total cells)</td>
</tr>
<tr>
<td>SAL</td>
<td>1.2 ± 0.4</td>
<td>38.9 ± 0.7</td>
</tr>
<tr>
<td>Nx</td>
<td>*1.4 ± 0.6</td>
<td>*35.0 ± 1.9</td>
</tr>
<tr>
<td>SPF</td>
<td>1.2 ± 0.3</td>
<td>37.3 ± 1.6</td>
</tr>
<tr>
<td>P value</td>
<td>0.02</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 8 each group). *Significantly different from any other group.

the three groups of animals. Thus our results support the contention that differences in either the number or the size of GH-producing cells cannot explain the reduced GH secretion recently reported in severely uremic rats [7]. This is important because on the basis of both the reduced hypothalamic levels of GHRH mRNA found in uremic rats [3], and the trophic action that GHRH exerts on the population of pituitary somatotrophs [2], the hypothesis of a low number of somatotrophs as pathogenic factor of the reduced GH secretion in uremia might be considered. However, from a methodological point of view, it is important to recognize that when GH secreted by a given number of pituitary cells is analysed, such as in perfusion systems [1,8], the relative number of somatotrophs is slightly lower than normal in rats with severe renal failure. In any case, the magnitude of the differences found in the present study in the percentage of GH cells cannot explain the marked differences in the GHRH stimulated GH secretion found in deeply uremic rats [1].

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