Effects of hypophysectomy and subsequent administrations of testosterone, triiodothyronine, and growth hormone on epidermal growth factor concentration in rat submandibular gland

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Introduction

Epidermal growth factor (EGF), a single-chain polypeptide comprising of 53 amino acids, was first isolated from the submandibular glands of male mice. A structurally and biologically similar polypeptide, β-urogastrone (human EGF), was later extracted from human urine. EGF is a potent mitogen for various types of mammalian cells, and its mode of action has been studied extensively and reviewed in detail. In mouse submandibular gland, the synthesis of EGF is largely stimulated by androgen, and thus the concentration in the gland is exceedingly higher in the male than in the female. In addition, it has been shown that thyroid hormone and glucocorticoid are also involved in the induction of this peptide.

EGF is likewise present in rat submandibular gland in a relatively high concentration. However, EGF in the rat has been shown to differ from the mouse one structurally and immunologically. We recently reported that EGF in rat submandibular gland is also androgen dependent. In this study, we investigated the effects of hypophysectomy and subsequent administrations of testosterone propionate (TP), triiodothyronine (T₃), and growth hormone (GH) on rat submandibular gland EGF.

Materials and Methods

Male Wistar rats were maintained under a 12-h light, 12-h dark cycle, and standard chow and tap water were available ad libitum. Hypophysectomy was carried out via the parapharyngeal route under ether anesthesia at 8 weeks of age, and then maintained throughout the experiments under the same condition as described above. For the hormone response study, hypophysectomized rats were administered the following hormones either singly or in combination: TP, T₃ (both purchased from Sigma Chemical Co.), and human GH(Nikken Chemical Co.) were injected subcutaneously for 14 days, beginning 4 weeks after the operation. TP (10 mg/kg) dissolved in sesame oil was injected every other day; and T₃ (0.15 mg/kg) in saline containing 5 mM NaOH, daily. Human GH (1 IU/kg) dissolved in saline was given twice a day at 08.00 and 20.00 h.

At the end of the treatments (14 weeks of
Table 1 Effects of hypophysectomy and hormone treatment on body weight, submandibular gland weight, and protein content in rat submandibular gland

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Gland weight (mg)</th>
<th>Protein (µg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>428 ± 19</td>
<td>620 ± 14</td>
<td>106.2 ± 4.7</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>258 ± 6***</td>
<td>204 ± 10***</td>
<td>66.2 ± 2.3***</td>
</tr>
<tr>
<td>+ TP</td>
<td>260 ± 6</td>
<td>296 ± 15**</td>
<td>86.7 ± 4.9**</td>
</tr>
<tr>
<td>+ T₃</td>
<td>232 ± 10</td>
<td>248 ± 11*</td>
<td>71.9 ± 4.5</td>
</tr>
<tr>
<td>+ GH</td>
<td>296 ± 11*</td>
<td>286 ± 14**</td>
<td>75.8 ± 3.1*</td>
</tr>
<tr>
<td>+ TP+T₃</td>
<td>256 ± 8</td>
<td>312 ± 16**</td>
<td>84.2 ± 4.4*</td>
</tr>
<tr>
<td>+ TP+GH</td>
<td>294 ± 11*</td>
<td>336 ± 13***</td>
<td>94.2 ± 8.8*</td>
</tr>
<tr>
<td>+ TP+T₃+GH</td>
<td>312 ± 21*</td>
<td>392 ± 20***</td>
<td>96.2 ± 8.2**</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E. of 5 to 7 animals. Values in parentheses show relative gland weight (mg/g body weight). ***p<0.001, compared with normal value. *p<0.05, **p<0.01, ***p<0.001, compared with hypophysectomized value.

age), the rats were killed by exsanguination under ether anesthesia, and the submandibular glands were excised. The gland from each animal was cut into small pieces and homogenized in 9 volumes of 20 mM sodium phosphate buffer, pH 7.0, in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 15,000×g for 30 min, and the resulting supernatant was used for the measurements of EGF and protein concentrations. EGF was measured by radioimmunoassay in a liquid-phase double-antibody system as previously reported. Protein content was determined by the method of Lowry et al.

Results and Discussion

The effects of hypophysectomy and hormone treatments on the body weight, submandibular gland weight, and protein concentration in the gland are shown in Table 1. The total and relative weights of the submandibular gland were markedly decreased by hypophysectomy; and the decrease was significantly, although not completely, restored by the administration of TP, T₃, GH, or any combination of these hormones. The restoration was the most prominent when the three hormones were given in combination. As shown in Figure 1, hypophysectomy caused a remarkable decrease in submandibular gland EGF to about 7% of the normal value. The reduction was significantly but not completely restored by the injection of each of the three hormones. The ratio of the recovery was higher for TP than for T₃ or GH. Moreover, the combined administration of TP with T₃ or GH, or with both had additive effects on the increase in submandibular gland EGF in hypophysectomized rats.

We previously reported an androgen-dependent sex difference in EGF content in rat submandibular gland. However, the sex difference in EGF observed in rats was far less than that reported in mouse submandibular gland, and EGF in female rats also increased with advancing age. These findings led us to speculate that multihormonal regulation is involved in the synthesis of rat submandibular gland EGF. In this study, we have found that not only androgen, but also T₃ and GH increase the weight of rat submandibular gland and for the induction of EGF in this gland. This finding suggests that the submandibular gland of rats is a receptor-possessing target organ of T₃ and GH as well as of androgen. It is unlikely that the increase in EGF content in the hormone-treated hypophysectomized rats is simply a consequence of the increase in the weight of submandibular gland, because the ratio of increase in EGF was higher than that in the weight of the gland.

In this study, however, the restoration of EGF by the three hormones and any combination of them was only partial, although the amounts of the hormones used were sufficient...
Fig. 1 Effects of hypophysectomy and hormone treatment on the EGF concentration in rat submandibular gland. Each column is the mean±S.E. of 5 to 7 animals. ***p<0.001, compared with normal value. **p<0.01, ***p<0.001, compared with hypophysectomized value.

for causing full biological activities\(^\text{13-15}\). Such incomplete restoration leads us to hypothesize that a hormone(s) other than TP, T\(_3\), and GH, which is associated with the pituitary directly or indirectly, might be involved in the induction of EGF in rat submandibular gland. Further study may resolve this question. Our results as to the suppressive effect of hypophysectomy on the weight of rat submandibular gland are inconsistent with those previously reported by Liu et al.\(^\text{16}\), who indicate that the relative weight of the gland in rats does not change after hypophysectomy. The reason for the discrepancy between their results and ours is not known, but one possible explanation may be the difference in the time of the operation: Liu et al.\(^\text{16}\) carried out the hypophysectomy at 3 weeks of age, whereas our rats were 8 weeks old at the time of surgery. In mice it is well-established that androgens and thyroid hormones induce EGF by a separate mechanism\(^\text{4,6}\). The results presented here provide evidence that EGF in rat submandibular gland is under multihormonal control including that of GH.

**Reference**


