Thyroid hormone modulation of VIP's induced salivary secretion in the submaxillary glands of rats

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ABSTRACT. The effect of changes in thyroid function upon vasoactive intestinal peptide (VIP) induced secretion of saliva were studied in male Wistar rats. Hyperthyroidism was induced by the sc administration every 12 h of 10 µg/100 g bw of l-triiodothyronine; hypothyroidism was induced by surgical thyroidectomy 2 weeks before the experiments. Progesteron intraperitoneal parasympathetic denervation was induced by sectioning the chorda tympani on the left side. The dose-response curves to increasing doses of VIP showed in the hypothyroid animals increased salivary secretion, while in the hyperthyroid ones the dose-response to the drug was reduced. This effect was seen on both sides, the denervated and the control ones. In the denervated glands there was a marked hypersensitivity to the administration of VIP producing greater responses with the same doses, in the 3 groups of animals. The negative modulation by thyroid hormones of the salivary response to VIP administration is compared with the positive modulation they induce in the salivary response to β-adrenergic and cholinergic drugs.

INTRODUCTION
In has been shown that thyroid hormones modulate the effect of adrenergic and cholinergic drugs upon the secretion of saliva (1). They also modulate the secretion of saliva induced by neurotransmitters, such as substance P (2). Their effect upon vasoactive intestinal polypeptide (VIP)-induced secretion of saliva is presented here. VIP has been found in neurons in the autonomic and central nervous system (3) and in glandular cells and nerves in the gastrointestinal system (4). The principal actions are vasodilation (5), relaxation of smooth muscle (6) and stimulation of the secretion of exocrine (7) and endocrine glands (8, 9). VIP containing nerves are found in salivary glands (10, 11) and role as neurotransmitter has been postulated (10-12).

MATERIALS AND METHODS
In these experiments 52 male Wistar rats, weighing 200 - 300 g, were used. In every animal parasympathetic decentralization, by sectioning the chorda tympani, was performed on the left side. The animals were divided into 3 groups: i) 18 control rats ( euthyroid rats), ii) 19 rats with sc administration every 12 h of 10 µg/100 g bw of sodium l-triiodothyronine (provided by Gloxo-Lab- oratories) during 14 days (hyperthyroid rats), iii) 16 surgically thyroidectomized rats, the operation being performed 2 weeks before the experiment (hypothyroid rats).

The animals were anesthetized with a-chloralose (Sigma Chemical Corporation) 100 mg/Kg bw given i.v. after previous induction with surgical ether; the trachea was intubated and both ducts of the submaxillary glands were exposed and cannulated with glass micromanipula (13). The secretory responses were quantified in weight by using fixed weight and a precision of 0.1 mg (14).

Dose-response curves to VIP (Sigma Chemical Corporation, V 3628) were obtained by sequential injections via the femoral vein of 0.1, 0.25, 0.5, 1.0, 3.0, 10.0, 30.0 and 100.0 µg/Kg bw of drugs. The dose-response was considered to be the total volume of saliva secreted. The next dose was injected 5 min after the response to the previous dose had finished. In all those experiments only the volume but not the chemical composition of saliva was measured.

When the experiments were finished, 4 ml of blood were removed by heart puncture in order to measure the serum levels of thyroxine (T4) and triiodothyronine (T3) in some animals. These determinations were made by radioimmunoassay by using the Upjohn Kits. The samples were taken 10 to 12 h after the last injection of triiodothyronine in the animals receiving the drug.

Immediately after the animals were killed the submaxillary glands were carefully dissected and weighed (wet wt). The dry weight was obtained after the glands were submitted to 105-110°C for 48 h. In every group, the
Table 1 - Serum levels of thyroxine (T4) and triiodothyronine (T3) in some experimental and control animals (means ± SE).

<table>
<thead>
<tr>
<th>Animals</th>
<th>n</th>
<th>T4 (ng/100 ml)</th>
<th>T3 (ng/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>6</td>
<td>3.29 ± 0.30</td>
<td>83.13 ± 8.10</td>
</tr>
<tr>
<td>T3-treated</td>
<td>6</td>
<td>2.50 ± 0.23</td>
<td>152.05 ± 7.78</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>6</td>
<td>1.0</td>
<td>28.86 ± 6.55</td>
</tr>
</tbody>
</table>

*p < 0.001 vs controls.

RESULTS

Blood levels of thyroid hormones

In the thyroidectomized animals there was a significant decrease of both the serum T3 and T4 levels (p < 0.001). In the T3-treated animals there was a significant increase in the serum T3 levels (p < 0.001), but the serum T4 levels were not changed (Table 1).

Effects upon glandular weight

In the glands with parasympathetic denervation the wet or dry weight was decreased (p < 0.001) in the 3 groups of animals. The wet and dry granular weights of either side were increased in the hypothyroid animals (p < 0.001). In hypothyroid animals the glandular weight was increased in the control side (wet weight, p < 0.05; dry weight, p < 0.001) but not in the denervated side (Table 2).

Dose-response curves to VIP

The dose-response curves to VIP of glands in the unoperated side for the 3 groups of animals are presented in Figure 1. The curve of the hypothyroid animals is displaced to the left (increased sensitivity) (p < 0.001 with every dose). The same peak was reached with the hypothyroid animals (10 µg/Kg dose) and the control ones (30 µg/Kg dose) which suggests changes in the affinity of the receptors. The curve of the hypothyroid animals was displaced to the right (decreased sensitivity) (10, 30 and 60 µg/Kg; p < 0.001; 3 µg/Kg; p < 0.02; 1 µg/Kg bw, ns). As the peak was reached with the same dose (30 µg/Kg), the decreased sensitivity of the hypothyroid animals could be due to a decrease in the number of receptors.

In Figure 2 the results obtained in the denervated glands of the 3 groups of animals were compared. The curve of hypothyroid animals was also displaced to the left (increased in sensitivity) (p < 0.001 with every dose). The curve of the hypothyroid animals was displaced to the right (decrease in sensitivity) (p < 0.001).

Table 2 - Body weight and submaxillary gland weight after 2 weeks of T3 treatment or 2 weeks after surgical thyroidectomy (means ± SE).

<table>
<thead>
<tr>
<th>Animals</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Glandular weight (mg/100 gr bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Wet Denervated</td>
</tr>
<tr>
<td>Controls</td>
<td>18</td>
<td>216 ± 9</td>
<td>50.94 ± 1.23</td>
</tr>
<tr>
<td>T3-treated</td>
<td>18</td>
<td>211 ± 6</td>
<td>74.63 ± 0.662</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>18</td>
<td>219 ± 9</td>
<td>59.30 ± 1.082</td>
</tr>
</tbody>
</table>

1p < 0.05 vs controls; 2p < 0.001 vs control.
Thyroid function on VIP's induced saliva

excepting 3 μg/Kg dose which was not significant and 60 μg/Kg dose, p < 0.05). The peak of the curves was reached in different doses being higher in the hypothyroid ones and lower in the hyperthyroid ones. Both a change in the number of receptors and a change in affinity may be involved.

In Figure 3 the curve dose-response of the 2 sides denervated and normal are compared in the 3 groups of animals. The curves of the denervated side compared to the control side were displaced to the left either in the hypothyroid animals (0.3 and 1 μg/Kg dose, p < 0.001; 3 μg/Kg dose, p < 0.01; 10 μg/Kg dose, NS) in the control ones (p < 0.001 except 3 μg/Kg dose, p < 0.01) or in the hyperthyroid ones (p < 0.001 except the 3 μg/Kg dose, p < 0.05). As the peak in both sides was obtained with the same dose, that would suggest that parasympathetic denervation increases the number of receptors in the 3 groups of animals.

DISCUSSION

The administration of VIP produces vasodilatation in the salivary glands (15, 16), it does not induce salivary secretion in dogs and cats (15, 16), but it modifies the composition of saliva in rabbits and rats (17). The above presented experiments have shown that VIP induce a marked secretion of saliva in the rat, where it is as potent as metacholine (1).

VIP and acetylcholine have a complementary role for cat submaxillary gland blood flow and secretion (18). VIP is found in submaxillary gland cholinergic nerves together with acetylcholine and induce c-AMP formation in these glands which is potentiated by carbacol (19).

The interactions between VIP and acetylcholine in the salivary glands could explain why the preganglionic parasympathetic denervation would produce such a
marked desensitization hypersensitivity to the administration of VIP, as we have shown. The structure of VIP is related to that of secretin and its effects upon exocrine pancreatic secretion are similar (7). VIP receptors have been described in pancreatic acinar cells (7). There is a potential for the vasodilating effects of acetylcholine, secretin and VIP in dog submaxillary glands, but VIP and secretin do not elicit salivary secretion in these animals (15).

It has been shown that thyroid hormones produce a positive modulation of the effects of β-adrenergic and cholinergic drugs upon salivary secretion, the effects being greater in the hyperthyroid animals and lessor in the hypothyroid ones (1). They also produce a positive modulation of substance P effects (2). But it is shown in our experiments that thyroid hormones exert a negative modulation of VIP effects upon the secretion of saliva, which was greater in hyperthyroid animals and lesser in the hypothyroid ones. This negative modulation was seen in both the operated and the denervated submaxillary glands. Moreover, the VIP hypersensitivity of the denervated animals was greater in the hypothyroid ones. Our T3-treated animals looked hyperthyroid and had high T3 serum levels, but T4 levels were not modified. Perhaps the T3 dose and the time interval used was not enough the depression T4.

We do not know if the chloralose anesthesia used in these experiments may have modified the response to VIP in hyperthyroid or hypothyroid animals. When the dose-response curves were compared the changes in the respective peaks suggested changes in the affinity, rather than on the number of receptors. These are only suggestions as VIP receptor analysis was not performed in any of the experiments.

ACKNOWLEDGMENTS

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