Effects of Experimental Thyroid Dysfunction on the Reactivity of Isolated Tracheal Smooth Muscle in the Guinea Pig

Saeideh Naeimi, Goudarz Sadeghi-Hashjin, Herman Meurs, Mohammad Vojgani, Samad Muhammadnejad, Alireza Bahonar and Mostafa Moin

Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Department of Molecular Pharmacology, Groningen University Medical Centre (UMCG), Groningen, The Netherlands
Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences and Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Abstract: Thyroid disease and asthma, both being common conditions, occasionally occur simultaneously. The aim of the present study was to determine the effects of experimental thyroid dysfunction on the reactivity of isolated trachea. Male, adult guinea pigs were placed into three groups of euthyroid (EUT), hyperthyroid (HRT) and hypothyroid animals (HOT). HOT was induced by methimazole in drinking water (50 mg/kg b.w. daily for 7 weeks). HRT was induced by i.p. injections of L-thyroxine (100 µg/kg once daily for 10 days). Animals were anesthetized with CO₂, blood samples were taken for T₃, T₄ and TSH measurements. Thereafter, they were sacrificed with an overdose of CO₂, tracheal rings from all groups were removed and mounted in an organ bath system for isometric measurement of smooth muscle contractions. Cumulative concentration/response curves (CRCs) were made with acetylcholine (ACh) and histamine. An increased airway reactivity to histamine was observed in HRT animals, shifting the CRC upwards significantly (P<0.05). In the HOT group, airway reactivity to ACh was suppressed partially, leading to a 40% reduction of the efficacy of the agonist. Findings of this research work showed that thyroid dysfunction can cause changes in the airway responsiveness. The change can be stimulus-specific as it was different in response to histamine compared to ACh.

Key words: Guinea pig · Hyperthyroidism · Hypothyroidism · Asthma · Airway Responsiveness

INTRODUCTION

The pathways involved in the control of airway tone in man are complex. Airway smooth muscle can be activated by hormones and vasoactive peptides reaching the lungs from the blood stream, by neurotransmitters released from nerve endings and by molecules released locally from other cells within the airways [1-2].

The relationship between asthma and thyroid disease provides indirect evidence for a role of thyroid hormones in maintaining airway function. Thyroid disease and asthma, both being common conditions, occasionally occur together. Hyperthyroidism (HRT) has been noted to increase the severity of asthma [3]. Control of HRT may relieve asthma symptoms and the development of hypothyroidism (HOT) may ameliorate coexistent asthma [4-5]. Conversely, treatment of the HOT state may result in worsening airway obstruction [6].

The first documented cases of asthma and HRT together appeared in 1972 concerning five cases in a study that was initiated in 1956 by Settipane [7]. These patients were found to have severe, intractable asthma concomitant with HRT. Treatment of the HRT provided prompt and dramatic improvement of asthma. Before that time, there was a brief impression by Feinberg in his textbook that the HRT state may maintain an asthmatic state [8]. No supporting data were given. In 1968, Kasperlik-Zaluska reported on two cases of bronchospastic state imitating bronchial asthma and associated with HRT [9].
Although the pathophysiologic basis of a relationship between asthma and thyroid dysfunction is not known, understanding this interaction is necessary for the proper management of both disorders. The present study is a solid experimental approach to verify the relationship in an animal model, a work that should have been accomplished much earlier.

**MATERIALS AND METHODS**

**Animals:** Twenty-seven male Dunkin-Hartly guinea pigs (400-600g) were acclimatized in the animal house (12 h light/dark periods, temperature of 22-25°C) for 10 days and received a regular chow diet. After acclimation period, guinea pigs were randomly categorized into three groups: control euthyroid (EUT), hyperthyroid (HRT) and hypothyroid (HOT).

Thereafter, they were sacrificed with an overdose of CO₂, tracheal rings from all groups were removed and mounted in an organ bath system for isometric measurement of smooth muscle contractions. Cumulative concentration/ response curves (CRCs) were made with acetylcholine (ACh) and histamine.

**Induction of Hyper- and Thypo- Thyroidism:** HOT was induced by methimazole in drinking water (50 mg/kg b.w. daily for 7 weeks) [10]. HRT was induced by i.p. injections of L-thyroxine (100 µg/kg once daily for 10 days) [11]. EUT guinea-pigs received saline solution. Animals were anesthetized with CO₂, blood samples were taken through heart puncture for T₃, T₄ and TSH measurements. Thyroid dysfunction was confirmed by ELISA measurement of serum concentrations of thyroid hormones using a commercial laboratory kit specific for the guinea-pig (Bojan Teb Company, Tehran, Iran).

**Measurement of Airway Responsiveness In vitro:** Animals were sacrificed with an overdose of CO₂ and tracheae were removed, prepared free of serosal connective tissue and cut into small segments of three cartilage rings. They were mounted for isometric recording of smooth muscle contractions (Semi Automatic Organ bath LST Lecta, Spain). The Krebs-bicarbonate solution used as medium in 30 ml organ bath contained NaCl 118.1 Mm, KCL 4.70 Mm, Mgso₄ 1.18 Mm, cacl₂ 2.50 Mm, KH₂PO₄ 1.28 Mm, NaHCO₃ 25.0 Mm and D-glucose 8.30 Mm. The buffer was continuously gassed with a mixture of 5% CO₂ and 95% O₂ maintaining the pH at 7.4. The medium temperature was fixed at 37°C. Each side of the trachea rings was tied to a stainless steel hook and connected to a force transducer for measurement of isometric tension (Power Lab/ 4SP AD Instrument Company, Australia). During a 45 min equilibration period, with wash-outs every 15 min, resting tension was adjusted to 1 g. After equilibration, cumulative concentration response curves (CRCs) to histamine and acetylcholine (ACh) were constructed ranging from 10⁻⁸ to 10⁻⁵ M. The following parameters were derived from the average CRCs: maximum contractions (effects) obtained (Eₘₐₓ), concentrations leading to 10%, 50% and 90% of the maximum effect, i.e., EC₁₀, EC₅₀ and EC₉₀ respectively.

**Drugs and Chemicals:** Histamine, L-thyroxin and methimazole were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetylcholine chloride was obtained from Novartis Pharma Stein AG (Stein, Switzerland).

**Data Expression and Analysis:** Data in this experimental work are reported as means ± SEM. Average values of EC₁₀, EC₅₀ and EC₉₀ as well as the Eₘₐₓ depicted from the CRCs, were statistically evaluated first with one-way ANOVA and, thereafter, group by group with Bonferroni’s t test. To compare the whole CRCs, Kruskal-Wallis and Man-Whitney tests were applied. A P value smaller than 0.05 represented a startistically significant difference.

**RESULTS**

**Thyroid Dysfunction:** In HOT animals the level of T₃ and T₄ were decreased by 41% and 57%, respectively (P<0.05, Table 1). In the HRT group, T₃ and T₄ levels were increased by 41% and 66%, respectively (Table 1). Blood TSH level, presumably as a feedback mechanism, was increased by 100% in HOT group while it was decreased by 66% (P<0.05, Table 1).

**Histamine:** HRT increased the total contraction induced by histamine significantly, however, HOT did not affect the C/R curve (P=0.03, Fig. 1A). Values demonstrating the efficacy and potency of histamine in 3 groups are shown in Table 2.

**Ach:** In contrast with histamine C/R curves, HOT suppressed the ACh C/R curve (profound although not reaching the level of significance), whereas HRT had no effect on the curve (Fig. 1B). Table 1 shows the values for efficacy and potency of ACh in the experimental groups.
Table 1: Blood levels of thyroid hormones in the guinea-pigs with either normal thyroid function or iatrogenic hypo- or hyperthyroidism

<table>
<thead>
<tr>
<th></th>
<th>T3 (ng/dL)</th>
<th>T4 (µg/dL)</th>
<th>TSH (mIU/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>42±15</td>
<td>3.50±0.90</td>
<td>0.35±0.05</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>25±4.5</td>
<td>1.50±0.06*</td>
<td>0.70±0.05*</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>59±17</td>
<td>5.20±0.07</td>
<td>0.12±0.07*</td>
</tr>
</tbody>
</table>

* P <0.05 in comparison to the hormone levels in the euthyroid, control animals

Table 2: Efficacy and potency of histamine and ACh in the induction of tracheal contractions in the guinea pig in vitro

<table>
<thead>
<tr>
<th></th>
<th>Efficacy</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g contraction)</td>
<td>EC10</td>
</tr>
<tr>
<td>HISTAMINE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthyroid</td>
<td>0.44±0.08</td>
<td>7.63±0.16</td>
</tr>
</tbody>
</table>
| Hypothyroid      | 0.38±0.13 | 7.66±0.23    | 5.92±0.30 | 4.08±0.29 *
| Hyperthyroid     | 0.67±0.19 | 7.75±0.14    | 5.19±0.14 | 3.44±0.19 |
| ACETYLCHOLINE    |           |              |     |      |      |      |      |
| Euthyroid        | 0.86±0.16 | 7.63±0.16    | 5.63±0.23 | 3.53±0.09 |
| Hypothyroid      | 0.52±0.19 | 7.83±0.11    | 5.25±0.23 | 3.25±0.12 |
| Hyperthyroid     | 0.89±0.22 | 7.81±0.09    | 5.94±0.21 | 3.94±0.16 |

Results are presented as means±s.e.mean for three experiments. In each case dose response curve was obtained with cumulative increases in histamine or ACh from $10^{-8}$ to $10^{-6}$ mmol/L. Airway reactivity to histamine and ACh was evaluated as p EC10, p EC50 and p EC90 values

DISCUSSION

This study was designed to explore the effects of thyroid dysfunctions on the airway responsiveness. Protocols used to induce HOT and HRT guinea-pigs were successful as thyroid hormones were decreased and increased, respectively. Normal average of blood T₃ and T₄ levels in the guinea-pigs have been reported to be between 39-44 ng/dL and 2.9-3.2 µg/dL, respectively [16]. Interestingly, TSH levels were found to be affected oppositely, suggesting a negative feedback effect of exogenous hormones on its production and/or release.

In the present study, 10 successive daily treatments with L-thyroxin affected the airway responsiveness; response to histamine was increased in HRT animals. On the other hand, response to ACh was declined in HOT animals. Our findings are in agreement with case reports suggesting that excess thyroid function might increase airway responsiveness [4, 5, 12, 17] and that depressed thyroid function might decrease airway responsiveness [6, 13].

The relationship between the respiratory system and thyroid glands has been well-documented in the literature. Walezynski found a greater incidence of goiter in asthmatic children (28%) when compared with the control group (10%) [14]. In 1917, Peabody and Wentworth [15] first noted a reduction of vital capacity (VC) in HRT patients. They found that some of their patients had a VC <80% of what predicted normal. This reduction, however, was attributed to respiratory muscle weakness. Harrison and Tattersfied reported an inverse relationship between the airway adrenergic responsiveness and the level of thyroid function [17]; the airway response to sulbutamol was reduced in thyrotoxic subjects before treatment. Cockcroft and colleagues noted a decrease in the nonspecific bronchial reactivity in an asthmatic patient after treatment for hyperthyroidism [18].

Several factors may contribute to increased airway reactivity in hyperthyroid patients. Changes in respiratory muscle strength, work capacity, or sensation of dyspnea due to thyrotoxicosis might have been interpreted as worsening of the asthma. Siafakas and colleagues [20] reported decreased respiratory muscle strength in hyperthyroid subjects and demonstrated improvement with return to the euthyroid state. Similarly, Mier and colleagues [20] studied thyrotoxic patients with dyspnea and proximal muscle weakness and observed small decreases in vital capacity and in mouth and transdiaphragmatic pressures that improved with treatment of the thyrotoxicosis. There is evidence

Fig. 1: CRCs of histamine (A) and ACh (B) on isolated tracheal rings in 3 groups of guinea-pigs: euthyroid (open circle), hypothyroid (closed circles) and hyperthyroid (open squares) *P=0.03 compared to the control group
showing that the excess thyroid hormones might have influence on asthma by either of the following mechanisms: altering cellular levels of cyclic nucleotides [21], altering the sympathetic nervous system activity [22], increasing the rate of corticostreoid metabolism [23], or potentiating the direct effects of prostaglandin on the airways [24].

Thyroid hormones play important roles in metabolism, growth and differentiation of virtually every cell and organ [25]. Giuriato and colleagues [26] administered L-Thyroxine to rabbits for as long as 26 days. They demonstrated that the hormone can induce a marked intimal thickening, which is paralleled by a change of the myosin isoform expression in the aortic wall. However, Hollingsworth and colleagues [27] showed that mild T3-induced thyrotoxicosis of 4-wk duration had no effect on lung function, airway responsiveness, or exercise capacity with mild asthma. Observations of Behera and his colleagues [28] indicated that bronchodilator response is impaired in the presence of excess thyroid hormones and improves when euthyroid state is achieved. Study of Carrillo-Sepulveda and his colleagues [29] indicated that T3 causes NO-dependent rapid relaxation of vascular smooth muscle cells and that this effect is mediated by the PI3K/AKT signaling pathway. This suggests that our findings are likely to be related to a specific effect of thyroid hormones on airway smooth muscle.

CONCLUSION

A relationship exists between the levels thyroid hormones and airway responsiveness. The change can be stimulus-specific as it was different in response to histamine compared to acetylcholine. Further studies will be required to determine the precise mechanisms involved in thyroid hormone modulation of airway responsiveness.

REFERENCES