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Original article

Acute effect of TSH on oxygenation state and volume of erythrocytes from subjects thyroidectomized for differentiated thyroid carcinoma

Silvana Balzan [a,*], Angelo Carpi [c], Monica Evangelista [a], Giuseppina Nicolini [a], Alberto Pollastri [a], Antonio Bottoni [b], Giorgio Iervasi [a]

[a] Institute of Clinical Physiology, CNR, via Moruzzi 1, Pisa 56124, Italy
[b] Gabriele Monasterio Fondation, FGM, via Moruzzi 1, Pisa 56124, Italy
[c] Department of Reproduction and Ageing, University of Pisa, Pisa, Italy

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ABSTRACT

We previously reported the presence in the membrane erythrocyte of a TSH receptor (TSHR), a G-protein coupled receptor, which responds to TSH with increased cAMP level. Since there is evidence for a role of G protein receptors as oxygen sensor(s) implicated in cell volume regulation, we hypothesized that erythrocyte TSHR, by TSH stimulation, could modify the erythrocyte volume and the oxygenation state of erythrocytes. We determined the effect of TSH on the gas analysis in 35 thyroidectomized patients for stage I differentiated thyroid cancer enrolled for recombinant human thyroid-stimulating hormone (rhTSH) test during chronic treatment with synthetic l-thyroxine. Moreover, we explored the influence of TSH on the shape of erythrocytes. Venous blood-gas analysis before and after TSH were determined with a pH/blood gas electrolyte and 682 CO-Oxymeter. In a subgroup of subjects ($n = 10$), the isolated red blood cells (RBC) were analyzed by flow cytometry for morphological changes. After TSH stimulation, we found a significant decrease in PCO_2 ($P < 0.001$), an increase in pH ($P < 0.01$) and an increase of % O_2-Hb ($P < 0.05$) and pO_2 ($P < 0.05$). By flow cytometry, the erythrocytes after TSH showed a significant enrichment on the mean number in the selected region R1 corresponding to bigger volumes ($P < 0.05$, $n = 10$). Finally, by contrast phase microscopy, when the cell area was measured, a mean increased volume was observed in erythrocytes after TSH compared to the basal before TSH ($P < 0.05$). In conclusion, our results indicate that acute stimulation of TSH by rhTSH modifies the oxygenation state and volume of erythrocyte.

1. Introduction

Erythrocytes carry oxygen to the tissues and interact with different substances and signalling mechanisms, thus participating actively as a sensor of oxygen requirements. They transport protons, CO_2, chloride ions, organic phosphates and nitric oxide (NO) that modulate tissue O_2 supply, either directly through transition between low affinity and high affinity states of hemoglobin (Hb), or indirectly by influencing cellular metabolism, erythrocyte volume and tissue perfusion. In blood, oxygenation-deoxygenation cycles are accompanied by changes in parameters such as PO_2, PCO_2, pH, inorganic cation such as Mg_2+ or the 2,3-dyphosphoglycerate (DPG) [1–6]. Several effectors, including hormones neurotransmitters, growth factor and G protein coupled receptors are considered as volume sensor and can also induce cell swelling or shrinkage [7,8]. These effects on cell volume are often mediated by cAMP [9] as it has been reported by thyroid stimulating hormone (TSH) in thyroid cells [10].

In the thyroid gland, TSH binds to its receptor, a G-protein coupled receptor, activating adenylate cyclase and increasing cAMP levels [11–13]. The regulation of cell volume by TSH via cAMP involves several cation transporters, such as Na/K-ATPase activity, Na, K, CL symporter and Na/I symporter [14–16].

In erythrocytes, we recently reported the presence of a functional TSHR which responds to TSH increasing intracellular cAMP levels and interacting with Na/K-ATPase [17,18]. Since there is evidence for a role of G protein receptors as volume sensor in the cells [19,20], in the present work, we hypothesized that in erythrocyte TSH could act through its receptor modifying oxygenation and erythrocyte volume.

Therefore, we investigated the acute effect of TSH on hemogas analysis parameters in a group of thyroid cancer patients enrolled for the rhTSH test after thyroidectomy. The present study explored also the effect of TSH on the volume of erythrocytes.

* Corresponding author. Tel.: +39 050 3152652; fax: +39 050 3152166.
E-mail address: balzan@ifc.cnr.it (S. Balzan).

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2. Patients and methods

2.1. Subjects

Clinical investigation was performed according to the Declaration of Helsinky and approved by our Institutional Ethics Committees. Informed consents were obtained from all subjects.

We studied 35 subjects (26 females and nine men, aged, 52 ± 4 years, mean ± SEM) that they had undergone total thyroidectomy and radioiodine ablation for primary treatment of stage I differentiated thyroid cancer 6.50 ± 1.46 years before the study. All patients underwent routine follow-up at the time of the study and they were not affected by other known disease or received pharmacological treatment except L-T_4 and they had serum thyroglobulin (Tg) levels less than 0.1 μg/ml measured by the ultrasensitive immuno-chemiluminescent (ICMA) Access method [21], suggesting the absence of cancer recurrences. The patients received two consecutive injections of rhTSH (0.9 mg/days), 24 hours apart [22]. At baseline and the day after the second administration of rhTSH, blood samples were collected at 08 h 00 in the morning for the determination of serum Tg, FT4, FT3,TSH and for gas analysis. Ten healthy euthyroid subjects, matched to the patient group for sex and age, were recruited among the staff and served as the control group.

2.2. Endocrine tests

Serum FT4, FT3, TSH and Ab Tg levels were measured by a completely automated AIA 1800 system (Tosho Corporation, Tokyo, Japan). rhTSH (Thyrogene) was purchased by Genzyme Ltd. (Oxford, UK).

2.3. Venous blood-gas analysis

At baseline and the day after the second administration of rhTSH, were determined with a IL-1650 pH/blood gas electrolyte and 682 CO Oxymeter, (Instrumentation Lab, Ma, USA). For study, the in vitro effect of TSH on gas analysis values, aliquots (1 ml) of venous blood sample from three health donors were incubated in absence and presence of two different concentrations of TSH (1–100 μg/ml) for 30 minutes at 37 °C and then assayed for pCO_2, pH and $\%O_2Hb$.

2.4. Flow cytometric analyses and microscopic study

For flow cytometric analysis and microscopic study, after gas analysis determination, the remaining blood sample of a subgroup of patients ($n = 10$) before and after rhTSH was separated by 5 minutes centrifugation at 350 g, plasma, platelet and leukocytes were carefully removed and erythrocytes were resuspended at 0.4 and 2% hematocrit in PBS buffer respectively.

Erythrocyte suspensions were analyzed for morphological changes with a Beckton Dickinson FACScan flow cytometer (San Jose, CA, USA) using the forward scatter (abscissa) versus side scatter (ordinates) dot-plot and at least 10,000 cells were counted.

By contrast phase microscopy before and after TSH (40 Carl Zeiss vision GMBH), the erythrocyte suspensions placed on glass slides were observed and images was captured using Axiovision 3.0. The area of the cells were measured (15 cells per slides) by the Scion Image program (Frederick, MA, USA).

2.5. Statistical analysis

Statistical analysis was performed using Student *t*-test; unpaired *t*-test to compare gas analysis data of control group against the thyroidectomized patients at baseline and paired *t*-test to evaluate the difference before and after TSH treatment in the thyroidectomized patients. Comparison of flow cytometry analysis was carried out by the Kolgomorov-Smirnov testing, using the Becton-Dickinson Cell Quest software. All reported in the text represent mean ± standard deviation (SD). The level of significance in all tests was set at $P < 0.05$.

3. Results

3.1. Effect of rhTSH on hemogas analysis

Hormonal and hemogas analysis values are reported in Table 1. Concerning the thyroid state, all patients were negative to serum Tg before and after treatment and no differences were observed for FT3 and FT4 that were in the normal range. When we compared the baseline gas analysis values of the thyroidectomized patients to the control group, PCO_2, pO_2, $\%O_2Hb$ and pH were different but not significantly. Inside the patient group, after TSH treatment the analysis of blood gases revealed a significant decrease in PCO_2 (44.5 ± 6.7 vs 48.3 ± 7.2 before TSH, $P < 0.001$), an increase in pO_2 and $\%O_2Hb$ (respectively 34.1 ± 17 vs 29.4 ± 12 before TSH, $P < 0.05$ and 57.7 ± 20.9 vs 50.7 ± 21.2 before TSH, $P < 0.05$) and an increase in pH (7.37 ± 0.04 vs 7.34 ± 0.05 before TSH, $P < 0.01$). A similar trend was observed "in vitro" when two different doses of TSH was added to three samples for analysis of blood gases (Fig. 1).

3.2. Effect of rhTSH on cell volume

Fig. 2 shows a typical dot-plot analysis of a patient erythrocyte shape and the three regions selected for the analysis before and after the TSH treatment. The left region (R2) corresponds to smaller erythrocyte that can be considered as senescent cells, whereas the right (R1) and upper (R3) respectively correspond to bigger volumes and to higher density. When a subgroup of subjects was analyzed, a significant enrichment on mean cell number was observed in R1 after TSH treatment compared to R1 before treatment (3581 ± 433 vs 3365 ± 388 cells, $P < 0.05$, $n = 10$; Table 1). No significant changes were observed for the two other regions (R2, R3) (data not shown).

Finally, when the cell area was measured, a mean increased area was observed in erythrocytes after TSH compared to the basal (0.14 ± 0.02 vs 0.12 ± 0.01 (square inches) $n = 10$, $P < 0.05$; Table 1).

4. Discussion

The principal finding of this study is that rhTSH is able to modify the oxygenation state of erythrocytes in patients thyroidectomized for differentiated thyroid carcinoma under treatment with L-T4. In fact, following TSH administration the analysis of blood gases revealed a significant decrease in PCO_2, an increase in pO_2, $\%O_2Hb$ and an increase in pH. Moreover, by flow cytometry, we observed an enrichment in the portion of bigger volume erythrocyte. This effect on the erythrocyte volume was also confirmed by phase contrast microscopy.

A wide range of substances has been found to interact with the erythrocyte modulating tissue O_2 either through the states of hemoglobin molecules or through O_2 sensors implicated in cell volume regulation [2,19] but until now, to our knowledge, no data has been reported in literature about a similar action for TSH in erythrocytes. An increment in cell volume by TSH has been observed in thyroid cells via TSHR/cAMP [10] and the change in cell volume was correlated with the regulation of ion exchanges like as Na/K-ATPase activity, Na, K, CL symporter and Na/I symporter [14–16].

Concerning the erythrocytes, it has been also postulated that G protein coupled receptors could be putative volume sensors [19,20]. In a previous work, we found in erythrocytes the presence of a functional TSHR (G protein coupled receptor), which responds